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EFFECT OF SOIL CONTAMINATION WITH ANTHRACENE AND PYRENE ON YIELD AND ACCUMULATION OF MACRONUTRIENTS IN BUTTER LETTUCE (*LACTUCA SATIVA* L.)

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Abstract

Toxic compounds which belong to PAHs are generated during all types of combustion of fuels and other substances as well as a result of natural processes (mineralisation). Products which appear during the above processes eventually reach soil, where they accumulate. The objective of this study has been to evaluate the effect of anthracene and pyrene accumulated in soil on yield, concentration of macronutrients (N, P, K, Mg, Ca, Na) and their uptake by cv. Vilmorin butter lettuce (*Lactuca sativa* L.) grown under the minimum and 3-fold enriched abundance of substrate. A pot experiment in four replicates was carried out twice in a greenhouse at the University of Warmia and Mazury in Olsztyn, in the spring of 2007 and 2008. Supplementary fertilisation was applied before planting butter lettuce. At the first rate of fertilisation, nitorgen was introduced in a full dose before planting lettuce, but at the triple rate of nutrients in substrate, it was divided into 2/3 of the dose before planting and the remaining 1/3 applied to soil 10 days after planting. Soil contamination with anthracene (ANT) and pyrene (PYR) or their mixture started 10 days after planting lettuce. Soil application of the two PAHs was performed 5 times at five-day intervals until the end of the growing season of lettuce. Determination of macronutrients (N, P, K, Mg, Ca, Na) was accomplished with standard methods after mineralisation (H_2SO_4 + H_2O_2) of the plant material dried at 60°C. The determinations were peformed in comparison to certified material (CTA-VTL-2).

Increasing the abundance of substrate in nutrients (N, P, K, Mg, Na, Cl) by three-fold led to a 13.7% increase in yield of lettuce heads. The PAH compounds present in the substrate depressed lettuce yield. This tendency was more evident when anthracene rather than pyrene contaminated soil. ANT and PYR depressed the concentration of nitrogen but raised the concentration of calcium in substrate less abundant in nutrients.

Key words: *Lactuca sativa* L., *macronutrients*, anthracene, pyrene, soil, fertilizer rates.

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WPŁYW SKAŻENIA GLEBY ANTRACENEM I PIRENEM NA PLONOWANIE I KUMULACJĘ MAKROSKŁADNIKÓW W SAŁACIE MASŁOWEJ (*LACTUCA SATIVA* L.)

Abstrakt

Toksyczne związki z grupy WWA powstają nie tylko w wyniku szeroko pojętych procesów spalania paliw, lecz również wskutek procesów naturalnych (mineralizacji). Produkty powstałe w trakcie tych procesów ostatecznie trafiają do gleby i ulegają kumulacji. Celem badań była ocena wpływu antracenu oraz pirenu zakumulowanych w glebie na plonowanie oraz zawartość makroskładników (N, P, K, Mg, Ca, Na) i ich pobranie przez sałatę masłową (*Lactuca sativa* L.) odmiany Vilmorin uprawianą w warunkach minimalnej i 3-krotnie zwiększonej zasobności podłoża. Doświadczenie wazonowe w 4 powtórzeniach prowadzono 2-krotnie wiosną w hali wegetacyjnej UWM w Olsztynie, w latach 2007-2008. Uzupełniające nawożenie zastosowano przed posadzeniem sałaty masłowej. W przypadku minimalnego poziomu nawożenia azot zastosowano w całości przed sadzeniem, natomiast w przypadku potrojonej ilości składników pokarmowych w podłożu – 2/3 dawki przed posadzeniem rozsady, a 1/3 dawki doglebowo po 10 dniach od posadzenia. Skażanie gleby antracenem (ANT) oraz pirenem (PYR) i ich mieszaniną rozpoczęto po 10 dniach od posadzenia sałaty. Wybrane WWA aplikowano doglebowo 5-krotnie w odstępach 5 dni. Zabieg stosowano do końca wegetacji. Oznaczenia makroskładników (N, P, K, Mg, Ca, Na) dokonano standardowymi metodami po mineralizacji (H_2SO_4+H_2O_2) wysuszonego w 60°C materialu roślinnego. Oznaczenia przeprowadzono wobec materiału certyfikowanego (CTA-VTL-2).

Zwiększenie 3-krotnie zasobności składników pokarmowych (N, P, K, Mg, Na, Cl) w podłożu przyczyniło się do wzrostu plonu główek sałaty o 13,7%. Obecne w podłożu związki z grupy WWA zmniejszyły plon sałaty. Wyraźniej ta tendencja występowała w przypadku antracenu niż pirenu. ANT i PYR doprowadziły do spadku zawartości azotu oraz wzrostu koncentracji wapnia, w warunkach niskiej zasobności podłoża w składniki pokarmowe.

Słowa kluczowe: *Lactuca sativa* L., makroskładniki, antracen, piren, gleba, dawki nawozu.

INTRODUCTION

Due to the disturbed balance between their decomposition in soil and the influx from different sources (both natural and anthropogenic ones), polycyclic aromatic hydrocarbons (PAHs) are present in all compartments of the environment (KIPOPOULOU 1999, TSIBULSKY 2001). Most of these compounds eventually accumulate in soil (MALISZEWSKA-KORDYBACH 1992). These substances reach soil from anthropogenic sources, such as man-made air and water contamination, but they also originate from organic and natural fertilisers and are produced as a result of mineralisation of organic matter, either present or introduced to soil (BLUMER 1976). Polycyclic aromatic hydrocarbons can either intensify or inhibit life processes in plants (HUANG et al. 1997, WIECZOREK et al. 2001, 2004).

This paper dicusses the effect of soil contamination with PAHs (anthracene and pyrene or their mixture) on the yield as well as the concentration and uptake of N. P, K, Ca, Mg and Na by cv. Vilmorin butter lettuce (*Lactuca sativa* L.) grown on substrate differently abundant in nutrients.

MATERIAL AND METHODS

A pot experiment was conducted in 2007 and 2008 in a greenhouse of the University of Warmia and Mazury in Olsztyn. It comprised four replicates. Butter lettuce (*Lactuca sativa* L.) of cv. Vilmorin was used for the trials. The lettuce was planted in Kick-Brauckmann pots containing 10 dm^3 mineral substrate. The mineral soil used for the experiment, of pH 6.5 and EC 0.11, contained (in mg dm^{-3}) 4.1. N-NO_3, 5.5 N-NH_4, 44.2 P, 173.3 K, 60.9 Mg, 921.9 Ca, 8.3 Na, 13.4 Cl, 71.7 S-SO_4. Soil in pots was brought to the following level of abundance (in mg dm^{-3}) (N-P-K-Mg-Na-Cl – level I): 60-50-50-40-20-30.8, (N-P-K-Mg-Na-Cl – level II): 180-150-150-120-60-92.4) – factor I. In order to control weeds, the substrate was sprayed with propyzamide, the only active substance allowed to be used for weed control in lettuce cultivation. The amount of propyzamide applied was 0.65 mg dm^{-3} of substrate. The spraying treatment was performed according to the recommendations, i.e. a day before planting lettuce. One gram of anthracene (ANT) or pyrene (PYR) was dissolved in 10 cm^3 of acetonitrile (ACN) and filled up to the volume of 100 cm^3 with deionised water. Working solutions were diluted to the concentration of 100 mg dm^{-3} of ANT, PYR or ANT+PYR. Control trials were watered with the solution of ACN of the same concentration or with deionised water. Soil application of the PAHs (ANT, PYR or ANT+PYR) was performed every 5 days, in the amount of 9 cm^3 of the concentration 100 mg dm^{-3} of anthracene, pyrene or their mixture, starting on day 10 after planting butter lettuce. The contaminants were spread over the surface of soil 5 times, in the volume of 100 cm^3 of deionised water. In total, 4.5 mg of each tested PAH (450 mg kg^{-1} of soil) was introduced to soil – factor II.

Lettuce was harvested after 6 weeks and fresh matter yield of lettuce heads was determined. After wet mineralisation in H_2SO_4, the concentration of macronutrients (N_{og}, P, K, Ca, Na and Mg) was determined with standard methods. In order to verify the correctness of the analytical determinations, certified materal (CTA-VTL-2) was used and the measurements obtained were loaded with the following error: P – 4.5%, K – 2%, Ca – 2.8%, Mg –1.5%, Na – 7%. The results of the determinations underwent statistical analysis using the software application Statistica 7.0.

RESULTS AND DISCUSSION

Butter lettuce responded to the experimental factors, particularly to the increased abundance of substrate in N, P, K, Mg, Na and Cl (Table 1). The three-fold increase in the content of macronutrients in soil caused an increase in the lettuce head fresh matter yield by an average of 23.2 g. The experiment demonstrated a negative effect of the soil contamination with anthracene and pyrene on the amount of produced lettuce biomass. Anthracene had a more adverse influence because its application depressed the mass of lettuce heads by 8.2% versus the control treatment. Similar impact of compounds belonging to the PAH group on lettuce and other plants has been indicated by CHAINEAU et al. (1997), MALISZEWSKA-KORDYBACH and SMERCZAK (1999). The results of our trials suggest that the two polycyclic aromatic hydrocarbons produced different results. Anthracene reduced the mass of lettuce heads irrespective of the substrate abundance whereas the soil application of pyrene stimulated the growth of lettuce heads when the soil abundance in nutrients was low. The mixture of these compounds had an indirect influence on the quantity of fresh mass of lettuce. KUMMEROWA et al. (1995), who examined the influence of another PAH (benzo(a)pyrene), obtained higher growth of roots of lettuce when concentrations of this compound were low (0-50 mg dm^{-3}), while higher rates of this contaminant in the medium retarded the growth.

Table 1

Effect of fertilisation and soil application of PAHs on fresh mass of butter lettuce *(Lactuca sativa* L.) heads

Treatments	Control	ANT	PYR	ANT + PYR	$X \pm SD$
	g head^{-1}				
Fertilisation level I	171.0	164.3	177.2	166.5	169.7 ± 16.27
Fertilisation level II	203.0	182.8	196.6	189.2	192.9 ± 36.63
$X \pm SD$	187.0 ± 21.1	173.5 ± 24.7	186.9 ± 34.5	177.8 ± 38.7	-

Concentrations of nitrogen, potassium and calcium in butter lettuce were modified mainly by the abundance of substrate in nutrients (Figure 1). When it was increased three-fold, the dry matter of lettuce contained more N (by 16.5%), K (by 10.7%) and Ca (by 22.5%) in comparison to the average concentrations of these elements when the abundance of substrate was low. Polycycilc aromatic hydrocarbons also affected the concentrations of N, K and Ca, but these modifications were not confirmed statistically. The influence of PAHs was more evident when the substrate was less abundant in nutrients. Anthracene, pyrene as well as their mixed application caused a higher concentration of calcium in lettuce and a lower one of nitrogen.

Anthracene contributed to depressing the concentration of nitrogen by 2.3 g kg^{-1} d.m., but slightly raised the concentration of potassium in lettuce leaves. The results indicating the concentration of calcium are in accord with the report by Wieczorek et al. (2006), who found out that anthracene introduced to soil increased the concentration of calcium in leaves of yellow lupine.

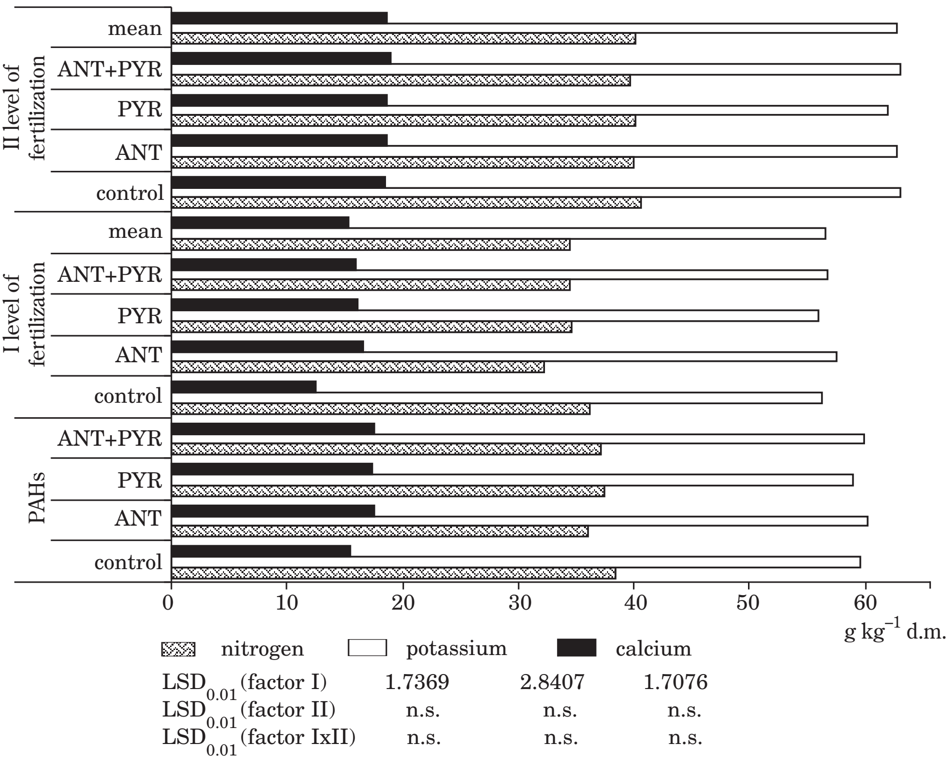
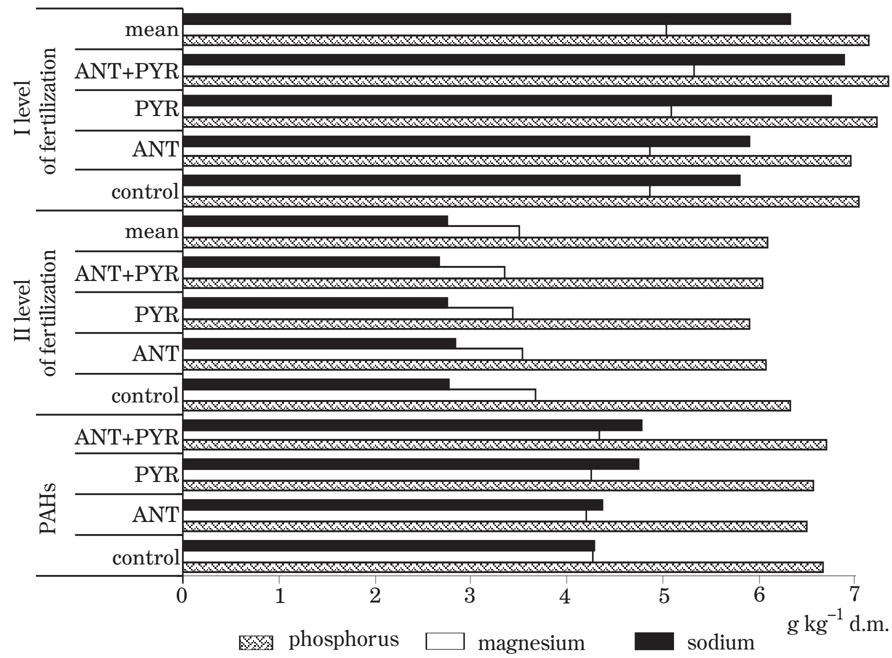


Fig. 1. Effect of fertilisation and soil application of PAHs on the concentration of nitrogen and calcium in butter lettuce

In the experiment presented in this paper, significant influence of the level of fertilisation on the concentration of phosphorus, magnesium and sodium was demonstrated (Figure 2). Lettuce grown on less abundant substrate contained 1.74-fold more phosphorus than magnesium and 2.2-fold more than sodium. Lettuce cultivated on more abundant soil contained just 1.42-fold more phosphorus and 1.12-fold more sodium. Contamination of soil with ANT and PYR did not have any considerable effect on the concentration of the above nutrients. In turn, Wieczorek et al. (2006) demonstrated that anthracene introduced to soil increased the concentration of magnesium in leaves of yellow lupine. In the authors' own experiment, no such effect appeared. Contrary to that, the concentration of magnesium in let-



$LSD_{0.01}$ (factor I)	0.6721	0.1912	0.3479
$LSD_{0.01}$ (factor II)	n.s.	n.s.	n.s.
$LSD_{0.01}$ (factor I×II)	n.s.	n.s.	n.s.

Fig. 2. Effect of fertilisation and soil application of PAHs on the concentration of phosphorus, magnesium and sodium in butter lettuce

tuce grown on less abundant substrate was lower. Contamination of soil more abundant in nutrients with pyrene increased the concentration of P, K and Na in edible parts of butter lettuce.

The tests revealed a significant effect of the applied PAHs on the concentration and competitiveness in the uptake of calcium and potassium ions by butter lettuce (Figure 3). The PAHs modified to a larger extent the ratios between the concentration of Ca to K when soil was less abundant in nutrients. As the concentration of calcium increased, the concentration of potassium decreased, wich was further depressed by the PAHs present in soil. The high correlation coefficients obtained in our tests indicate that these compounds may have a negative effect on the uptake of nutrients, especially when soils are less abundant in nutrients.

The amounts of macronutrients absorbed by butter lettuce depended primarily on the abudnance of substrate in N, P, K, Mg, Na and Cl (Table 2). As a result of the increased concentrations of the nutrients in soil, their uptake by lettuce increased. The uptake of N, P, K and Ca rose by 6% (K) up to 19% (Ca) and in the case of magnesium by 41% while the uptake of

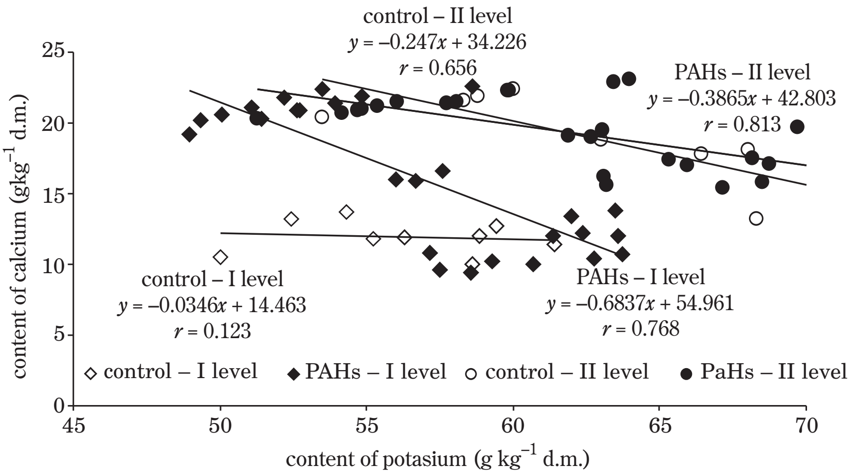


Fig. 3. Correlation between the concentration of calcium and potassium in butter lettuce depending on fertilisation and soil application of PAHs

sodium was two-fold higher. Contamination of soil with anthracene reduced the uptake of nitrogen, phosphorus and magnesium but stimulated the accumulation of potassium, calcium and sodium. Pyrene applied to soil caused an increased uptake of K, Ca, Mg and Na by lettuce. When soil contained larger quantities of PAHs (ANT+PYR), the accumulation of nitrogen, phosphorus and potassium in edible parts of lettuce was negativeluy affected.

CONCLUSIONS

1. Anthracene and pyrene, if present in soil, can cause depressed yields of butter lettuce.

2. The concentration of nutrients and their uptake by butter lettuce depended on the abundance of substrate in N, P, K, Mg, Na and Cl and, to a lesser degree, on the presence of anthracene and pyrene in soil.

3. Anthracene and pyrene caused an increased concentration and uptake of calcium and sodium by lettuce.

4. Low abundance of substrate in conjunction with the soil contamination with ANT and PYR may considerably modify the concentration of nutrients in butter lettuce.

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