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EFFECT OF FOLIAR APPLICATION OF ANTHRACENE AND PYRENE (PAH) ON YIELDS AND CHEMICAL COMPOSITION OF BUTTERHEAD LETTUCE (*LACTUCA SATIVA* L.) GROWN UNDER VARIED ABUNDANCE OF SUBSTRATE IN NUTRIENTS

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Abstract

The purpose of this study has been to evaluate the effect of foliar application of PAH organic chemical compounds (anthracene, pyrene) on yield, chemical composition and uptake of nutrients by cv. Vilmorin butterhead lettuce (*Lactuca sativa* L.). Lettuce was grown under minimum and three-fold higher abundance of substrate in nutrients, as determined according to threshold amounts. A pot trial was established in four replicates and performed twice, in the spring of 2008 and 2009, in a greenhouse at the University of Warmia and Mazury in Olsztyn. Lettuce seedlings were planted in pots containing 10 dm^3 of mineral substrate. Fertilization (N, P, K, Mg, Na and Cl) was carried out prior to the planting of lettuce. Under the minimum nutrient abundance of the substrate, all the dose of nitrogen was supplied as a pre-sowing treatment, whereas when the abundance in nutrients was raised three-fold, the nitrogen dose was divided (2/3 pre-sowing and 1/3 10 days after planting). Contamination of lettuce plants with anthracene (ANT) or pyrene (PYR) and their mixture started 10 days after planting lettuce. Foliar application of either of the PAHs continued for 25 days until the vegetative growth of lettuce terminated. Determination of the concentration of macronutrients (N, P, K, Mg, Ca and Na) was performed using standard methods on the mineralised (H_2SO_4+H_2O_2), previously dried at 60şC lettuce plant material. The determinations were completed by referring to certified material (CTA-VTL-2). The amount of fresh mass of butterhead lettuce depended primarily on the abundance of the substrate in nutrients. The three-fold increase in the substrate's abundance in N, P, K,

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Mg, Na and Cl caused an increment in the yield of lettuce head mass by 13.3%. Foliar application of ANT and PYR caused an increase in the yield of lettuce head mass. The concentration of N, K, Na, Mg, Ca and Mg in lettuce was modified first of all by the abundance of the substrate and, to a lesser degree, by the applied PAHs.

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

WPŁYW DOLISTNEJ APLIKACJI ANTRACENU I PIRENU (WWA) NA PLONOWANIE I SKŁAD CHEMICZNY SAŁATY MASŁOWEJ (*LACTUCA SATIVA* L.) UPRAWIANEJ W WARUNKACH ZRÓŻNICOWANEJ ZASOBNOŚCI PODŁOŻA W SKŁADNIKI POKARMOWE

Abstrakt

Celem badań była ocena oddziaływania wpływu dolistnej aplikacji organicznych związków chemicznych z grupy WWA (antracen oraz piren) na plon, skład chemiczny oraz pobranie składników pokarmowych przez sałatę masłową (*Lactuca sativa* L.*)* odmiany Vilmorin. Sałatę uprawiano w warunkach minimalnej i 3-krotnie zwiększonej zasobności podłoża w składniki pokarmowe, którą ustalono wg liczb granicznych. Doświadczenie wazonowe w 4 powtórzeniach prowadzono 2-krotnie wiosną w latach 2007-2008, w hali wegetacyjnej UWM w Olsztynie. Rozsadę sałaty wysadzono do wazonów o pojem. 10 dm^3 podłoża mineralnego. Nawożenie (N, P, K, Mg, Na i Cl) zastosowano przed sadzeniem sałaty. W warunkach minimalnej zasobności podłoża azot również podano w całości przedsiewnie a w warunkach 3-krotnie zwiększonej zasobności podzielono na 2 części (2/3 przedsiewnie i 1/3 po 10 dniach od posadzenia). Skażanie roślin antracenem (ANT) oraz pirenem (PYR) i ich mieszaniną rozpoczęto po 10 dniach od posadzenia sałaty. Aplikacja dolistna wybranych WWA trwała przez 25 dni do końca okresu wegetacji. Oznaczenia zawartości makroskładników (N, P, K, Mg, Ca, Na) dokonano standardowymi metodami po mineralizacji (H_2SO_4+H_2O_2) wysuszonego w 60ºC materialu roślinnego. Oznaczenia wykonano wobec materiału certyfikowanego (CTA-VTL-2). Ilość świeżej masy sałaty masłowej zależała przede wszystkim od zasobności podłoża w składniki pokarmowe. Trzykrotne zwiększenie zasobności podłoża w N, P, K, Mg, Na i Cl spowodowało przyrost plonu masy główek o 13,3%. Dolistnie aplikowane związki ANT i PYR powodowały przyrost masy główek sałaty masłowej. Zawartość N, K, Na, Mg, Ca i Mg w sałacie była modyfikowana przede wszystkim przez zasobność podłoża i w mniejszym zakresie przez aplikowane wybrane WWA.

Słowa kluczowe: *Lactuca sativa* L., makroelementy, antracen, piren, dawki nawozów.

INTRODUCTION

Intensive growth of particular branches of both industrial and agrcultural production, coupled by an increase in the human population and its expansiveness, means that growing amounts of carcinogenic and mutagenic substances, including polycyclic aromatic hydrocarbon compounds (PAHs), are emitted into the environment. Eventually, about 90% of such compounds end in soil (by sedimenting on plants) and are practically present in all elements of the natural environment in which we live (Wcisło 1998, Oleszczuk 2002, Kluska 2003). PAHs are mainly created during processes of in-

complete combustion of organic matter, but also as a product of its mineralisation. The fact that there are few reports dealing with the effect of PAHs on the chemical composition of crops (WIECZOREK et al. 2006), especially when nutrients are deficient or excessive in substrate, stimulated our study.

The aim has been to assess the effect of foliar sedimentation of the PAH organic chemical compounds (anthracene and pyrene) on the yield, chemical composition and uptake of nutrients by cv. Vilmorin butterhead lettuce (*Lactuca sativa* L.), which was grown under the minimum and threefold enhanced abundance of substrate in nutrients.

MATERIAL AND METHODS

A two-factor pot experiment, in four replicates, was set up on cv. Vilmorin butterhead lettuce (*Lactuca sativa* L.) grown in a greenhouse at the University of Warmia and Mazury in Olsztyn. Six-week seedlings of lettuce were purchased and planted on 25 April 2007 and 7 April 2008 in pots containing 10 dm^3 of substrate. The substrate used for the trials had the following chemical properties: 4.1 mg $N\text{-}NO_3$, 5.5 mg $N\text{-}NH_4$, 44.2 mg P, 173.3 mg K, 60.9 mg Mg, 921.9 mg Ca, 8.3 mg Na, 13.4 mg Cl^-, 71. mg $S\text{-}SO_4$ in dm^3, pH – 6.5, EC – 0.11 mS cm^{-1}. The following rates of nutrients were added per 1 dm^3 of the substrate: 60, 180 mg N; 50, 150 mg P; 50, 150 mg K; 40, 120 mg Mg; 20, 60 mg Na and 30.8 oraz 92.4 mg Cl^- (1^{st} factor). Before planting the lettuce seedlings, the soil surface was sprayed with propyzamide in the amount of 0.65 mg dm^{-3} of substrate. One gram of anthracene (ANT) or pyrene (PYR) was dissolved in 10 cm^3 of acetonitrile (ACN), filled up to 100 cm^3 with deionised water. Afterwards, 10 cm^3 of this solution was trasferred to 1000 cm^3 flasks and filled up to 1 dm^3 with deionised water. Control plants were sprayed with a solution of ACN of the same concentration or with deionised water. The first spraying treatment was performed 10 days after the lettuce had been planted and conducted twice daily for the next 25 days, supplying 1.8 cm^3 day^{-1} of ACN, ANT, PYR or ANT+PYR solutions of the concentration of 100 mg dm^{-3} (2^{nd} factor). The lettuce plants were harvested six weeks after planting the seedlings. During the harvest, lettuce heads were weighed. The chemical analysis involved only aerial parts of each lettuce plant (the treatments were not aggregated into combinations). The determinations of the concentration of N_{org} (Kjeldahl method), P (the vanadium-molybdene method), K, Ca, Na (the flame atomic emission spectrometric method, ESA), and Mg (the atomic absorption spectrometric method, AAS), having first wet mineralised the material in H_2SO_4. The determinations were completed with reference to cetrified material (CTA VTL-2), at the following error: P – 4.5%, K – 2%, Ca – 2.8%, Mg – 1.5%, Na – 7%.

The results were processed statistically using analysis of variance for a two-factor experiment in a completely random design, with an aid of the software package Statistica v. 7.0. The results are given as means from 8 replications for two years.

RESULTS AND DISCUSSION

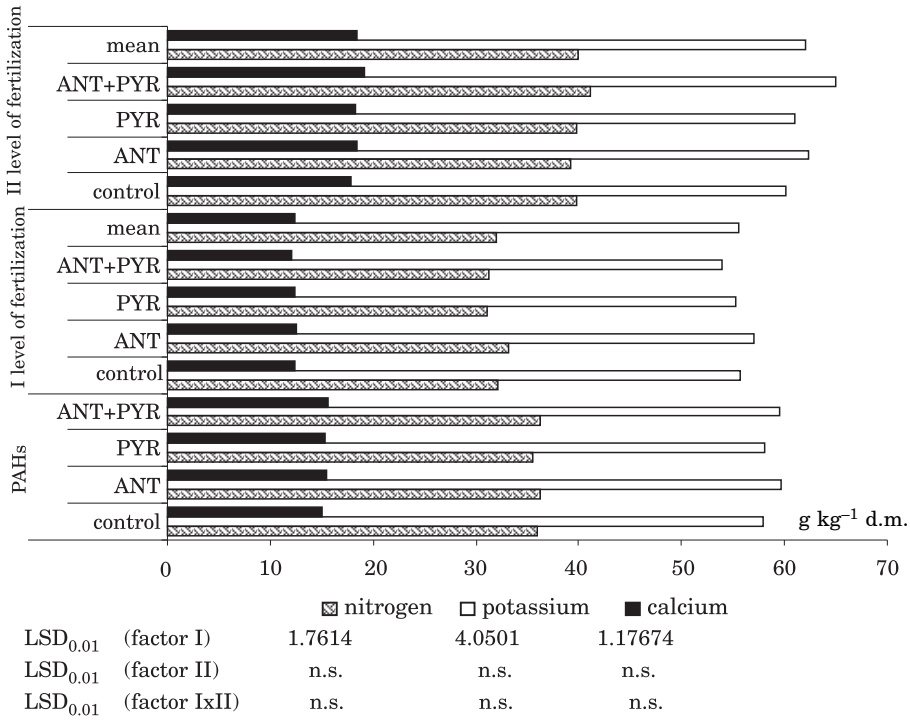
The increase in fresh matter of aerial parts of butterhead lettuce was significantly dependent mainly on the concentration of nutrients in the substrate (Table 1). The three-fold increase in the abundance of soil in nutrients (N, P, K, Mg, Na, Cl) caused an over 1.13-fold increase in the weight of lettuce heads. Foliar application of PAHs (ANT, PYR) raised an increase in the lettuce biomass. ANT demonstrated a stronger effect on the produced fresh mass of butterhead lettuce grown on soil less abundant in nutrients, while PYR – on substrate three-fold richer in nutrients. In a study completed by WIECZOREK et al. (2003), the increase in the aerial part of lettuce obtained after foliar application of anthracene was smaller. Such a result was observed in the present experiment only when substrate was more abundant in nutrients (on average, a 2.3 g per pot increase in weight).

Table 1

Effect of fertilization and foliar application of PAHs on the average mass of butterhead lettuce (*Lactuca sativa* L.) heads (g)

Fertilization level	Control	ANT	PYR	ANT + PYR	X ± SD
I	174.7	182.7	180.0	174.9	178.1 ± 22.47
II	196.2	193.9	202.8	214.0	201.7 ± 18.68
X ± SD	185.5 ± 21.2	188.3 ± 17.9	191.4 ± 23.6	194.5 ± 31.4	-
$LSD_{0.01}$ (factor I) – 13.84		$LSD_{0.01}$ (factor II) – n.s.		$LSD_{0.01}$ (factor I´II) – n.s.	

The concentration of nitrogen, potassium and calcium in leaves of butterhead lettuce was dependent mainly on the availability of nutrients in substrate (Figure 1). The substrate three-fold richer in nutrients resulted in an average increase in the concentration of nitrogen by 28.9% and potassium by 11.9% in leaves of the tested lettuce. Although calcium was not supplied with the fertilizers, its concentration in plants changed largely depending on the abundance of substrate in nutrients. The three-fold increase in quantities of available nutrients in substrate caused a 49.2% increased Ca concentration in plant tissues. In an experiment conducted by other researchers, such as MICHAŁOJĆ (2000), JAROSZ, DZIDA (2006), the concentration of nitrogen in leaves also increased following nitrogen fertilization, while the

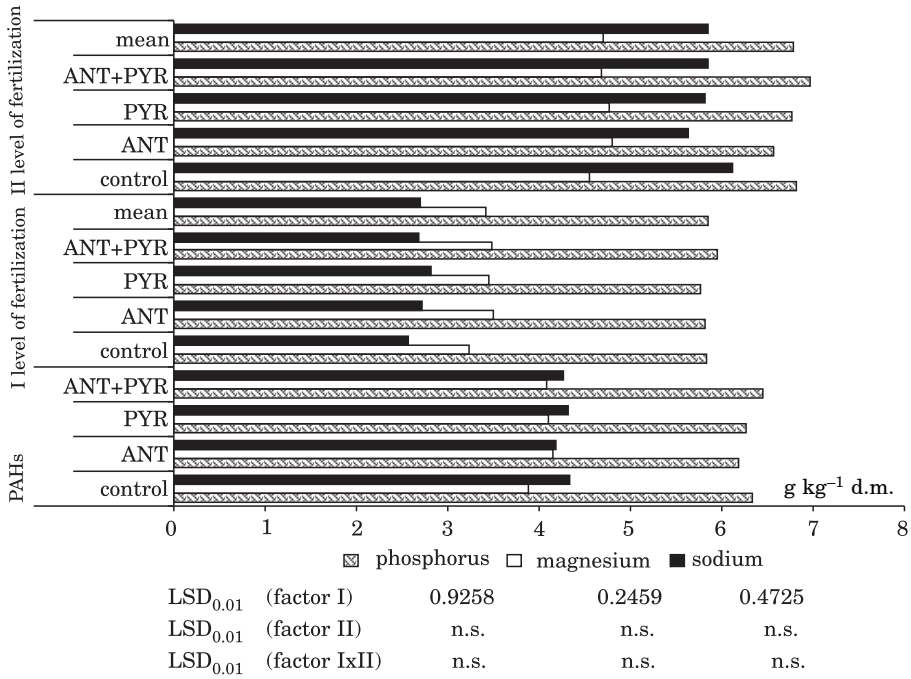


$LSD_{0.01}$	(factor I)	1.7614	4.0501	1.17674
$LSD_{0.01}$	(factor II)	n.s.	n.s.	n.s.
$LSD_{0.01}$	(factor IxII)	n.s.	n.s.	n.s.

Fig. 1. Effect of fertilization and foliar application of PAHs on the concentration of nitrogen, potassium and calcium in dry matter of butterhead lettuce heads (g kg^{-1} d.m.)

concentration of Ca did not change. This, however, did not lead to its increased content in substrate. PAHs reach plants predominantly via atmospheric precipitations or airborne dust.

Plants can also be contaminated with soil dust from substrate on which vegetables are grown (Maliszewska-Kordybach 1986, Bell, Failey 1991, Lodovici et al. 1994). There are very few reports on the influence of these compounds on the chemical composition of plants. In our study, the concentration of nitrogen in lettuce leaves affacted by the PAHs was less changed when lettuce grew on a substrate richer in nutrients than on a poorer one. The examined hydrocarbons raised the concentration of potassium in lettuce plants growing on a substrate three-fold richer in nutrients. When PAHs were sprayed over leaves of lettuce growing on a substrate poor in nutrients, the concentration of potassium in plant tissues was nearly unchanged or even decreased when ANT+PYR was applied. A reverse tendency was observed when lettuce was cultivated under improved availability of nutrients. Based on this observation, it can be concluded that the influence of PAHs on the concentration of Ca in lettuce was predominantly conditioned by the abundance of substrate in nutrients. In a study completed by



$LSD_{0.01}$	(factor I)	0.9258	0.2459	0.4725
$LSD_{0.01}$	(factor II)	n.s.	n.s.	n.s.
$LSD_{0.01}$	(factor IxII)	n.s.	n.s.	n.s.

Fig. 2. Effect of fertilization and foliar application of PAHs on the concentration of calcium and phosphorus in dry matter of butterhead lettuce heads (g kg^{-1} d.m.)

WIECZOREK et al. (2006), foliar application of anthracene raised the concentration of magnesium and calcium in leaves, potassium and sodium in stems and caclium and sodium in seeds of lupine.

The concentration of phosphorus, magnesium and sodium in lettuce was also significantly dependent on fertilization (Figure 2). Increasing amounts of P, Mg and Na in substrate led to an increase in their concentration in butterhead lettuce leaves by 16.1% for phosphorus, 37.4% for magnesium and 116% for sodium. No significant effect of ANT and PYR on the concentration of the analysed nutrients has been demonstrated. However, an increasing concentration of magnesium after foliar application of anthracene, pyrene as well as their mixture was observable, irrespective of the abundance of substrate. In turn, anthracene when sprayed over lettuce plants caused a decline in the concentration of phosphorus and sodium in leaves of butterhead leaves, especially when the substrate was highly abundant in nutrients.

The accumulation of macronutrients (N, P, K, Ca, Mg and Na) in aerial parts of burtterhead lettuce was significantly dependent on the availability of nutrients in soil (Table 2). The three-fold increase in the amounts of available nutrients in soil led to their enhanced uptake. The uptake of sodi-

Table 2

Accumulation of macronutrients in aerial parts of butterhead lettuce depending on fertilization and foliar application of PAHs

Treatments			N	P	K	Ca	Mg	Na
			mg pot^{-1}					
Factor I	fertilization 1st level	control	383.3	68.93	658.4	147.8	38.52	31,10
		ANT	382.0	68.30	663.2	144.4	41.00	31,90
		PYR	372.2	68.23	660.4	148.0	41.10	33,52
		ANT+PYR	366.5	69.70	640.0	142.7	41.50	31,57
		mean	376.0	68.8	655.5	145.7	40.50	32.03
	fertilization 2nd level	control	483.1	80.80	730.7	222.9	56.48	74.90
		ANT	474.7	76.20	761.9	230.2	58.23	66.35
		PYR	485.4	81.90	751.4	227.6	57.38	68.80
		ANT+PYR	511.9	82.70	809.3	243.7	59.02	72.70
		mean	488.8	80.40	763.3	231.1	57.8	70.69
Factor II PAHs		control	433.2	74.87	694.6	185.3	47.50	53.00
		ANT	428.4	72.23	712.6	187.3	49.62	49.13
		PYR	428.8	75.07	705.9	187.8	49.24	51.15
		ANT+PYR	439.2	76.18	724.7	193.2	50.16	52.13
$LSD_{0.01}$ (factor I)			20.74	6.823	25.72	23.72	3.292	4.978
$LSD_{0.01}$ (factor II)			n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
$LSD_{0.01}$ (factor I II)			n.s.	n.s.	n.s.	n.s.	n.s.	n.s.

um was raised the most evidently, followed by that of calcium (even though the substrate was not fertilised with this element), magnesium, nitrogen and – finally – phosphorus and potassium, whose uptake increased to a comparable degree. The effect of the other factor was not significant, although it could be noticed that under the effect of anthracene, the uptake of nitrogen, phosphorus and sodium declined while the accumulation of potassium, calcium and magnesium increased. The same PAH applied in conjunction with pyrene caused an increased uptake of all the macronutrients except for sodium.

CONCLUSIONS

1. The yield of butterhead lettuce heads was mainly affected by the fertilization and, to a lesser extent, by the contamination of the plants with anthracene, pyrene or their mixture.

2. The concentration of macronutrients in lettuce was predominantly modified by the amounts of nutrients available to lettuce plants from substrate.

3. Anthracene and pyrene as well as their mixture, when applied as foliar sprays, increased significantly the mass of butterhead lettuce heads.

4. Butterhead lettuce accumulated significantly more nutrients in aerial parts when growing on a substrate three-fold more abundant in N, P, K, Mg, Na and Cl.

5. No significant effect of foliar application of anthracene and pyrene on the accumulation of N, P, K, Ca, Mg and Na in aerial parts of butterhead lettuce has been observed.

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