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# CONCENTRATION OF SELECTED METALS IN BUTTER LETTUCE (*LACTUCA SATIVA* L. ) CONTAMINATED WITH ANTHRACENE AND PYRENE

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Abstract

PAHs (polycyclic aromatic hydrocarbons) are widespread in the natural environment. They are suspected to have mutagenic, carcinogenic and teratogenic effect on our organisms. Production of wholesome and high quality vegetables does not necessarily involve selection of adequate farmlands or substrates used for vegetable growing. Combustion processes are a source of PAHs in the soil and air. Excessive levels of PAHs are accumulated when the balance between their decomposition in soil and supply from other sources is disrupted. The objective of the present research has been to evaluate the effect of anthracene and pyrene on the concentration of selected trace elements in butter lettuce (*Lactuca sativa* L.), cultivar Vilmorin, grown on substrates with different nutrient abundance. A pot experiment in four replications was carried out twice in the spring of 2007 and 2008. The pots were maintained in a greenhouse at the University of Warmia and Mazury in Olsztyn. Lettuce was grown under the minimum (optimum) and triple (II fertilization level) substrate abundance in nutrients. In the second variant o the experiment, when the nutrient abundance was elevated, nitrogen was split into two doses: 2/3 of the whole rate were added before planting lettuce seedlings, and 1/3 of the rate was introduced 10 days afterwards. In the first variant, when the nutrient abundance was minimal, all of the nitrogen was added in a single dose. Spraying the lettuce plants with anthracene (ANT), pyrene (PYR0 or their mixture started 10 days after planting. Foliar introduction of the tested PAHs continued for 25 days (in the amount of 1.8 $cm^3$ $day^{-1}$ at a concentration of 100 mg $dm^{-3}$) until the vegetative season finished. At the same time, an identical total amount of the PAHs was added to soil as aqueous solution, at 7-day intervals, according to the same pattern. Determinations of the concentrations of heavy metals (Cd, Cu, Ni, Cr, Zn, Mn) was performed with the ASA method, having first mineralised ($HClO_4$+HCl+$HNO_3$) the plant material which had been dried at 60°C. The determinations were compared with certified material (CTA-VTL-2). Concentrations of the trace elements in butter lettuce were

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significantly varied depending on the experimental factors. A more abundant substrate significantly raised the levels of Cd, Zn and Mn, but had no effect on concentrations of Cu, Ni and Cr. The way the PAHs were applied did not have any influence on modifications in the determined levels of the metals. Out of the two analysed PAHs, anthracene, especially when applied to soil, increased the concentrations of Zn nd Cr in lettuce leaves.

Key words: anthracene, pyrene, *Lactuca sativa* L., heavy metals, substrate abundance.

**KONCENTRACJA WYBRANYCH METALI W SAŁACIE (*LACTUCA SATIVA* L. ) SKAŻONEJ ANTRACENEM I PIRENEM**

Abstrakt

WWA (wielopierścieniowe węglowodory aromatyczne) są związkami powszechnie występującymi w środowisku. Przypisuje się im działanie mutagenne, rakotwórcze i teratogenne na nasze organizmy. Produkcja zdrowych i dobrych jakościowo warzyw nie zawsze jest związana z wyborem odpowiednich terenów lub podłoży do ich uprawy. •ródłem WWA zarówno w glebie, jak i w powietrzu są procesy spalania. Do nadmiernego ich nagromadzenia dochodzi na skutek zachwiania równowagi między rozkładem w glebie a przychodem z innych źródeł. Celem badań była ocena wpływu antracenu oraz pirenu na zawartość wybranych pierwiastków śladowych w sałacie masłowej (*Lactuca sativa* L.) odmiany Vilmorin uprawianej w podłożach o zróżnicowanej zasobności. Eksperyment wazonowy w 4 powtórzeniach przeprowadzono dwukrotnie wiosną w latach 2007-2008 w hali wegetacyjnej UWM w Olsztynie. Sałatę uprawiano w warunkach o minimalnej (optymalnej) zasobności podłoża w składniki pokarmowe oraz 3-krotnie zwiększonej (II – poziom nawożenia). Azot wprowadzano w dwóch dawkach przy zwiększonej zasobności podłoża: 2/3 dawki przed posadzeniem rozsady, a 1/3 dawki po 10 dniach od posadzenia oraz w całości w przypadku pierwszego poziomu nawożenia. Oprysk roślin antracenem (ANT) oraz pirenem (PYR) i ich mieszaniną rozpoczęto po 10 dniach od posadzenia. Dolistne wprowadzanie wybranych WWA trwało przez 25 dni (w ilości 1,8 $cm^3$ $doba^{-1}$ o stężeniu 100 mg $dm^{-3}$) do końca okresu wegetacji. Jednocześnie doglebowo dostarczono sumaryczną ilość WWA w takim samym układzie 1 raz na 7 dni w wodnym rozcieńczonym roztworze. Zawartość wybranych metali ciężkich (Cd, Cu, Ni, Cr, Zn, Mn) oznaczano metodą ASA po mineralizacji ($HClO_4$+HCl+$HNO_3$) wysuszonego w temp. 60°C materiału roślinnego. Oznaczenia wykonano wobec materiału certyfikowanego (CTA-VTL-2). Zawartość pierwiastków śladowych w sałacie masłowej była istotnie zróżnicowana w zależności od czynników doświadczalnych. Zasobniejsze podłoże wpływało istotnie na zwiększenie zawartości Cd, Zn i Mn, nie miało zaś wpływu na koncentrację Cu, Ni i Cr. Sposób aplikacji WWA nie miał wpływu na zmiany zawartości oznaczonych metali. Spośród testowanych WWA, szczególnie antracen aplikowany doglebowo zwiększał zawartość Zn i Cr w liściach testowanej rośliny.

Słowa kluczowe: antracen, piren, *Lactuca sativa* L., metale ciężkie, zasobność podłoża.

# INTRODUCTION

Polycyclic aromatic hydrocarbons (PAHs) are among the substances which tend to appear in the natural environment in ever increasing quantities (TSAI et al. 2001). They are generated during incomplete incineration or combustion of various substances, caused by both natural forces (forest wild fires, mineralization of organic matter) or man (tobacco smoking, production and combustion of fuels, transport, etc.) (HENNER et al. 1997, SAMANTA et al. 2002, JOHNSON et al. 2005, RAVINDRA et al. 2008). They are the compounds which re readily bioaccumulated in living organisms and have an adverse effect on our health, producing toxic, carcinogenic, mutagenic and teratogenic effects (PERERA 1997, JIAN et al. 2004). Plants grown on soil which contains PAHs, or otherwise exposed to these compounds, respond to contamination demonstrating certain disorders in physiological processes (photosynthesis, transpiration), biomass production, uptake and concentration of some nutrients (MARWOOD et al. 2001, WIECZOREK et al. 2001, WITTING et al. 2003, WIECZOREK et al. 2004, 2006, KRZEBIETKE, SIENKIEWICZ 2010a,b). In practice, the relevant literature contains no reports on the effect of PAHs on concentrations of heavy metals in plants. The objective of the present study has been to determine the effect of anthracene and pyrene and their mixture, sprayed over leaves or introduced to soil, on concentrations of some metals in butter lettuce (*Lactuca sativa* L.), cv. Vilmorin, grown on substrate with two different levels of nutrient abundance.

## MATERIAL AND METHODS

A three-factor pot experiment was carried out in he spring of 2007 and 2008, in a greenhouse at the University of Warmia and Mazury in Olsztyn. The experiment was run in four replications. The substrate used in the experiment 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 $Cl^-$; 71.7 mg $S\text{-}SO_4$ in $dm^3$ of the pH 6.5 and EC 0.11 mS $cm^{-1}$. The following does of nutrients were added to 1 $dm^3$ of the substrate: 60 or 180 mg N; 50 or 150 mg P; 50 or 150 mg K; 40 or 120 mg Mg; 20 or 60 mg Na and 30.8 or 92.4 mg Cl (1st factor – substrate abundance).

One gram of anthracene and 1 g of pyrene were disolved in 10 $cm^3$ of acetonitrile, filled up with deionised water to 100 $cm^3$ and diluted 100-fold by adding deionised water to the solution. For the fist time, lettuce plants were sprayed 10 days after planting, and the treatment was repeated twice a day for 25 consecutive days, using 1.8 $cm^3$ $day^{-1}$ of the solution at a concentration of 100 mg $dm^{-3}$. The same amounts of the PAHs were introduced to soil, every 7 days, in an aqueous diluted solution. Control plants were sprayed or watered in the same way but without the PAHs ($2^{nd}$ factor – PAH application method).

The tested solutions comprised: anthracene (ANT), pyrene (PYR) or their mixture, and the control plants were sprayed or watered with deionised water mixed with acetonitrile organic solvent (ACN), added in the same amount as need for disolving the PAHs ($3^{rd}$ factor – type of PAHs).

Before the lettuce was planted, the surface of the soil was sprayed with propyzamide in the aound of 0.65 mg $dm^{-3}$ of the substrate. In Kick Brauckmann pots of the capacity of 10 kg soil, six-week old seedlings of cv. Vilmorin butter lettuce (Lactuca sativa L.) were planted, one seedling per pot. The lettuce was harvested after 6 weeks. During the harvest, lettuce heads were weighed. Determinations of the selected metals (Cd, Cu, Ni, Cr, Zn and Mn) were performed with the ASA method, after mineralization ($HClO_4$+HCl+$HNO_3$) of the plant material dried at temp. 60ºC. The determinations were verified with the certified material CTA-VTL-2. The results underwent statistical processing, employing the analysis of variance for a three-factor experiment, in a completely random design – Statistica v. 7.0.

## RESULTS AND DISCUSION

The concentrations of trace metals (Mn, Cd and Zn) in butter lettuce were modified primarily by the abundance of the substrate (Tables 1-3). There are researchers, e.g. Samsře-Petersen et al. 2002, Wennrich et al. 2002, Khan et al. 2008, who demonstrate that soil is the major route of transfer for PAHs to plants. In the present experiment, the way of introducing the PAHs (over leaves or to soil) did not significantly modify the concentrations of the analysed elements in butter lettuce leaves. However, statistically proven differences in the concentrations of chromium or zinc in the tested butter lettuce leaves were found following the application of anthracene or pyrene or their mixtures (Tables 1 and 2). The concentration of chromium rose 2.75-fold in lettuce leaves due to soil contamination with ANT in the substrate with elevated nutrient abundance. When the nutrient levels in the substrate were on the optimum level, the increase in chromium in lettuce leaves was 1.33-fold (Table 1).

In the substrate richer in nutrient, ANT contributed to increased concentrations of Cr in lettuce leaves; a reverse correlation was observed for pyrene. This effect was much stronger when pyrene was supplied to soil and when nutrient availability to plant was higher. A mixture of PAHs (ANT+PYR), regardless of an application method, led to a decrease in the concentration of Cr in butter lettuce plants.

Manganese in butter lettuce leaves occurred in concentrations between 31.16 and 59.17 mg $kg^{-1}$ d.m. (Table 1). The substrate abundance produced a significant effect on this element (a 30.7% increase) only when butter lettuce was grown on the substrate with a three-fold lower abundance in P, K, Mg and Na.

Foliar application of pyrene, under the optimum substrate abundance in nutrients, raised the concentration of nickel in the analysed plant (Table 2). Reverse tendencies were observed in the case of ANT. Under the higher

Table 1

Effect of substrate abundance, soil or foliar application of PAHs, on the concentrations of chromium and manganese in butter lettuce (mg $kg^{-1}$ d.m)

| Treatments | | | | Control | | ANT | | PYR | | ANT+PYR | | Mean | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | Cr | Mn | Cr | Mn | Cr | Mn | Cr | Mn | Cr | Mn |
| Substrate abundance | I level | application PAHs | foliar | 3.27 | 34.91 | 3.25 | 35.61 | 3.49 | 38.90 | 2.45 | 31.16 | 3.12 | 35.15 |
| | | | soli | 3.16 | 47.31 | 4.21 | 49.08 | 3.12 | 45.45 | 3.35 | 44.39 | 3.46 | 46.56 |
| | II level | | foliar | 3.65 | 43.86 | 3.20 | 50.34 | 3.39 | 53.19 | 3.78 | 59.27 | 3.51 | 51.67 |
| | | | soil | 3.75 | 50.71 | 10.30 | 59.18 | 1.02 | 52.77 | 0.93 | 57.98 | 3.99 | 55.16 |
| Mean | | | | 3.45 | 44.20 | 5.23 | 48.55 | 2.76 | 47.58 | 2.63 | 48.20 | – | |
| Substrate abundance | | I level | | 3.21 | 41.11 | 3.73 | 42.34 | 3.30 | 42.18 | 2.90 | 37.78 | 3.29 | 40.85 |
| | | II level | | 3.70 | 47.28 | 6.73 | 54.76 | 2.21 | 52.98 | 2.36 | 58.63 | 3.75 | 53.41 |
| Application PAHs | | foliar | | 3.46 | 39.39 | 3.23 | 42.97 | 3.44 | 46.05 | 3.12 | 45.22 | 3.31 | 43.41 |
| | | soil | | 3.45 | 49.01 | 5.23 | 54.13 | 2.07 | 49.11 | 2.14 | 51.19 | 3.22 | 50.86 |

| $LSD_{0.01}$ | | Cr – chromium | Mn – manganese |
|---|---|---|---|
| Substrate abudance | – factor I | n.s. | 11.5401 |
| Application PAHs | – factor II | n.s. | n.s. |
| PAHs | – factor III | 1.1093 | n.s. |
| Interaction | – factor I×II | n.s. | n.s. |
| | – factor I×III | 1.5688 | n.s. |
| | – factor II×III | 1.5688 | n.s. |
| | – factor I×II×III | 2.2186 | n.s. |

Table 2

Effect of substrate abundance, soil and foliar application of PAHs on the concentrations of zinc and nickel in butter lettuce (mg $kg^{-1}$ d.m)

| Treatments | | | | Control | | ANT | | PYR | | ANT+PYR | | Mean | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | Zn | Ni | Zn | Ni | Zn | Ni | Zn | Ni | Zn | Ni |
| Substrate abundance | I level | application PAHs | foliar | 18.64 | 2.48 | 20.41 | 2.39 | 19.84 | 2.99 | 21.23 | 2.28 | 20.03 | 2.54 |
| | | | soil | 16.90 | 2.34 | 19.26 | 3.06 | 18.00 | 2.79 | 18.26 | 2.55 | 18.10 | 2.69 |
| | II level | | foliar | 25.57 | 2.35 | 24.97 | 2.07 | 25.01 | 2.47 | 26.63 | 2.80 | 25.54 | 2.42 |
| | | | soil | 24.53 | 2.53 | 28.90 | 2.38 | 25.81 | 2.13 | 26.87 | 2.21 | 26.53 | 2.31 |
| Mean | | | | 21.41 | 2.22 | 23.38 | 2.47 | 22.16 | 2.60 | 23.25 | 2.47 | - | |
| Substrate abundance | | I level | | 17.77 | 2.41 | 19.83 | 2.72 | 18.92 | 2.89 | 19.74 | 2.42 | 19.07 | 2.62 |
| | | II level | | 25.05 | 2.44 | 26.94 | 2.22 | 25.41 | 2.30 | 26.75 | 2.48 | 26.04 | 2.37 |
| Application PAHs | | foliar | | 22.10 | 2.42 | 22.69 | 2.23 | 22.42 | 2.73 | 23.95 | 2.54 | 22.79 | 2.48 |
| | | soil | | 20.71 | 2.44 | 24.08 | 2.72 | 21.90 | 2.46 | 22.57 | 2.39 | 22.32 | 2.50 |

| $LSD_{0.01}$ | | Zn – zinc | Ni – nickel |
|---|---|---|---|
| Substrate abudance | – factor I | 0.9446 | n.s. |
| Application PAHs | – factor II | n.s. | n.s. |
| PAHs | – factor III | 1.3358 | n.s. |
| Interaction | – factor I×II | 1.3358 | n.s. |
| | – factor I×III | n.s. | 0.7167 |
| | – factor II×III | 1.8892 | n.s. |
| | – factor I×II×III | n.s. | 1.0135 |

substrate abundance, application of ANT, PYR or ANT+PYR resulted in a 9.5% decrease in the Ni concentration in lettuce leaves.

The content of zinc in lettuce changed depending on the applied polycyclic aromatic hydrocarbons and nutrient abundance of the substrate (Table 2). Its concentration varied from 16.9 to 28.9 mg kg$^{-1}$ d.m. of butter lettuce leaves. Under the higher nutrient abundance of the substrate, the Zn content in lettuce rose by 36.5%. The PAHs caused a higher concentration of zinc in the analysed lettuce. Analogously, positive correlation was found between the concentration of zinc and manganese and the concentration of cadmium in lettuce plants (Figure 1). The more Zn and Mn the plants contained, the more cadmium they accumulated, although the effect of manganese was much stronger. It is highly probable that an increase in the concentration of Mn and Zn in plants can also lead to increased levels of Cd – in the present experiment the determination coefficients were $r$=0.7845 for manganese and $r$=0.4924 for zinc.

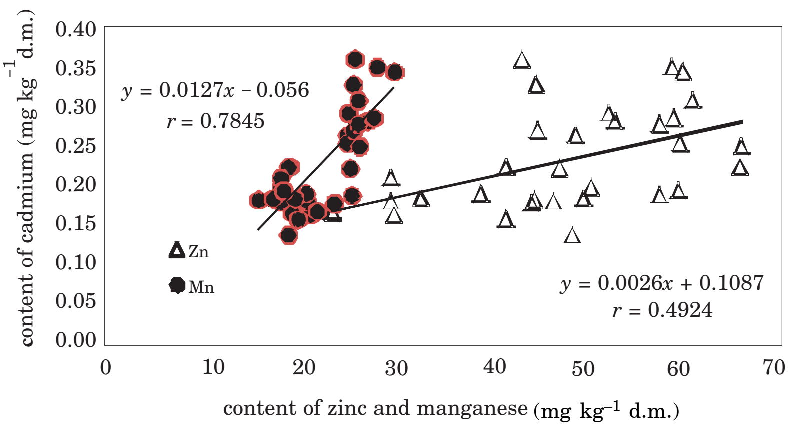


Fig. 1. Correlation between the concentration of cadmium and zinc versus manganese in butter lettuce

The concentration of copper in butter lettuce plants varied from 1.24 to 1.65 mg kg$^{-1}$ d.m. (Table 3). No significant effect of the tested experimental factors was evidenced on the concentration of Cu in lettuce leaves. However, statistically significant differences in the concentration of copper in lettuce leaves were found out for the interaction of the three experimental factors. A mixture of the tested PAHs (ANT+PYR) sprayed over leaves, under the minimal substrate abundance in nutrients, was responsible for a decrease in the Cu content in lettuce leaves.

The concentration of cadmium in lettuce leaves depended mainly on the substrate abundance; the richer substrate raised its concentration by 55.5% (Table 3). The PAHs introduced to the substrate alongside its higher abun-

Table 3

Effect of substrate abundance soil and foliar application of PAHs on the concentrations of copper and cadmium in butter lettuce (mg $kg^{-1}$ d.m.)

| Treatments | | | | Control | | ANT | | PYR | | ANT+PYR | | Mean | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | Cu | Cd | Cu | Cd | Cu | Cd | Cu | Cd | Cu | Cd |
| Substrate abundance | I level | application PAHs | foliar | 1.47 | 0.17 | 1.45 | 0.16 | 1.59 | 0.16 | 1.24 | 0.18 | 1.43 | 0.17 |
| | | | soil | 1.31 | 0.19 | 1.38 | 0.20 | 1.65 | 0.20 | 1.51 | 0.20 | 1.46 | 0.20 |
| | II level | | foliar | 1.41 | 0.34 | 1.41 | 0.28 | 1.61 | 0.24 | 1.63 | 0.27 | 1.52 | 0.28 |
| | | | soil | 1.60 | 0.24 | 1.40 | 0.35 | 1.46 | 0.29 | 1.46 | 0.28 | 1.48 | 0.29 |
| Mean | | | | 1.45 | 0.24 | 1.41 | 0.25 | 1.58 | 0.22 | 1.46 | 0.23 | - | |
| Substrate abundance | | I level | | 1.39 | 0.18 | 1.42 | 0.18 | 1.62 | 0.18 | 1.38 | 0.19 | 1.45 | 0.18 |
| | | II level | | 1.51 | 0.29 | 1.40 | 0.31 | 1.54 | 0.26 | 1.54 | 0.27 | 1.50 | 0.28 |
| Application PAHs | | foliar | | 1.44 | 0.26 | 1.43 | 0.22 | 1.60 | 0.20 | 1.44 | 0.22 | 1.48 | 0.23 |
| | | soil | | 1.46 | 0.22 | 1.39 | 0.28 | 1.56 | 0.25 | 1.48 | 0.24 | 1.47 | 0.25 |

| $LSD_{0.01}$ | | Cu – copper | Cd – cadmium |
|---|---|---|---|
| Substrate abudance | – factor I | n.s. | 0.0190 |
| Application PAHs | – factor II | n.s. | n.s. |
| PAHs | – factor III | n.s. | n.s. |
| Interaction | – factor I×II | n.s. | 0.0270 |
| | – factor I×III | n.s. | 0.0381 |
| | – factor II×III | n.s. | 0.0381 |
| | – factor I×II×III | 0.3893 | 0.0539 |

dance in nutrients caused an increase in Cd in butter lettuce leaves. Anthracene raised the amount of cadmium in lettuce plants grown on the substrate richer in nutrients, whereas pyrene reduced its concentration. Foliar application of the PAHs (ANT, PYR, ANT+PYR) depressed the concentration of cadmium, whereas soil application resulted in higher levels of this metal in lettuce leaves.

## CONCLUSIONS

1. The substrate richer in N, P, K, Mg, Na and Cl significantly increased concentrations of Cd, Zn and Mn in butter lettuce leaves, but had no effect on concentrations of Cu, Ni and Cr.

2. No significant effect of the PAH application path (foliar or to soil) on concentrations of the analysed metals in butter lettuce was demonstrated.

3. Increased content of anthracene in soil may raise the concentration of Zn and Cr in butter lettuce leaves.

4. An increase in the concentration of Mn and Zn in butter lettuce leaves may also entail a higher level of Cd in the tested plant.

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