

## THE EFFECT OF DIFFERENT NITROGEN FERTILIZATION DOSES ON LEAD AND CADMIUM ACCUMULATION AND TRANSLOCATION IN YELLOW LUPINE

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### ABSTRACT

**Background.** Heavy metals in the soil are often found in quantities exceeding their natural level. Given the high degree of harmfulness of some of them (e.g. Pb and Cd) to living organisms, their ingress into the food chain should be monitored.

**Material and methods.** The aim of the study was to determine if different nitrogen doses had an effect on lead and cadmium accumulation and translocation at different growth stages in yellow lupine. The test factors were nitrogen doses (0, 30, 120 kg·ha<sup>-1</sup>) introduced to the soil prior to sowing and two development stages (full blooming and full maturity) of the tested species.

**Results.** Different levels of nitrogen fertilization had no significant effect on the mean content of lead in yellow lupine. Only the highest level of nitrogen application (120 kg N·ha<sup>-1</sup>) increased the cadmium content in this species. Each applied dose of nitrogen increased the amount of Cd uptake by yellow lupine. Lead uptake was also increased by the application of 120 kg N·ha<sup>-1</sup>. Yellow lupine harvested at the full maturity stage usually contained less Cd and Pb, but had taken up more of these heavy metals than in the blooming stage. The bioaccumulation factor values indicate a high potential for lupine to absorb Cd and a low potential for Pb absorption. The values of the translocation factor indicate the possibility of Cd hyperaccumulation by this plant, whereas in the case of Pb that capacity was recorded only on soil that was lightly contaminated with this heavy metal.

**Conclusion.** Regardless of the applied nitrogen dose, green feed and lupine seeds grown on unpolluted soil and on soil lightly contaminated with lead (II<sup>o</sup>) were not excessively contaminated with these heavy metals and can be used as animal feed.

**Key words:** development stage, fertilization, forage, heavy metals, *Lupinus luteus* L.

### INTRODUCTION

Lead and cadmium content in the environment that exceeds natural amounts - the so-called geochemical background, is most often caused by anthropogenic action. These elements belong to the group of heavy metals having a high potential environmental risk (Ociepa-Kubicka and Ociepa, 2012; Lou *et al.*, 2013).

The harmfulness of cadmium and lead results from their high toxicity to humans and other living organisms, and it is well-documented (Makokha *et al.*, 2008; Dokmeci *et al.*, 2009; Khan and Ghouri, 2011; Wyszowska *et al.*, 2013). Plants are the first link in a trophic chain in which cadmium and lead accumulate. They probably do not have any physiological functions in plants as they demonstrate

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negative effects on growth and development and generally they can be described as purely toxic (Smolders, 2001; Ociepa-Kubicka and Ociepa, 2012; Tran and Popova, 2013). High concentrations of these metals in tissue can result in metabolic disorders, inhibition of plant growth, and can even lead to death (Tripathi *et al.*, 2007; Wyszowska *et al.*, 2013). Cadmium and lead are absorbed mainly by plant roots and there is usually thought to be the highest accumulation of cadmium and lead in these organs. In plants with the possibility of adsorption of these elements through their leaves (e.g. growing near sources of the metals emission into the atmosphere), high concentrations of heavy metals are also recorded in aboveground parts (Khan and Ghouri, 2011; Shahid *et al.*, 2017). In general, in the case of heavy metals, an increasing accumulation has been noted in subsequent food chain links (Singh and Kalamdhad, 2011; Butt *et al.*, 2018). In the case of lead, food products of animal origin generally contain more of this element than plant products (Devkota and Schmidt, 2000). According to many researchers, human activity has led to environmental pollution by heavy metals, therefore their mobility should be monitored and their negative impacts on the environment and human beings should be reduced (Smolders, 2001; Siebielec *et al.*, 2012; Chang *et al.*, 2014). Nitrogen fertilizers, which by changing the soil pH value in the root zone of plants may affect the plant uptake of certain elements, are among many factors affecting heavy metal bioavailability (Spiak, 2000).

The aim of the study was to determine the effect of various doses of nitrogen fertilization and of the growth stage of yellow lupine (*Lupinus luteus* L.), cultivated on soil contaminated (II°) and uncontaminated with lead, on the bioaccumulation and translocation of lead and cadmium. The study also assessed the possibility of using the obtained yields as animal feed.

## MATERIAL AND METHODS

The field experiment was conducted in Siedlce (52°10' N; 22°17' E) in 2008 and 2011. The experiment was conducted on slightly acidic soil with the granulometric composition of loamy sand. The content of selected elements in the soil before the

experiment was set up is shown in Table 1. According to the IUNG-PIB guidelines in Puławy (Siebielec *et al.*, 2012), the soil on which the study was carried out in the first year showed weak lead contamination (II°) and a natural cadmium content. In the second year of the study, the soil was characterized by a natural content of both of these heavy metals. 1m<sup>2</sup> plots were delineated in a field of yellow lupine of the 'Mister' cultivar. A two-factorial experiment was set up in three replications. Nitrogen fertilization was the first factor: a) control object, with no nitrogen fertilization; b) with nitrogen applied at a rate equivalent to 30 kg N·ha<sup>-1</sup> – recommended dose; c) with nitrogen applied at a rate equivalent to 120 kg N·ha<sup>-1</sup> – a dose aimed at enabling the lupine to take up nitrogen in a form that was easily available and less energy-consuming for the plant. The time of harvest was the second factor: a) full flowering stage, 65 BBCH; b) full maturity stage, 90 BBCH. Mineral nitrogen was introduced into the soil as (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> before the yellow lupine was sown. Potassium was introduced into the soil in all plots at the rate of 100 kg K·ha<sup>-1</sup> as KCl. Because of a very high amount of phosphorus in available forms (Table 1), no phosphorus fertilization was applied.

**Table 1.** Selected properties of soil (0.25 m) used in the field experiments

Soils properties	Unit	Year of study	
		2008 (1 <sup>st</sup> )	2011 (2 <sup>nd</sup> )
pH <sub>KCl</sub>	–	5.90	5.80
C <sub>tot</sub>		25.7	23.8
N <sub>tot</sub>		2.04	1.92
P <sub>tot</sub>	g·kg <sup>-1</sup>	1.10	1.15
K <sub>tot</sub>		0.845	0.810
Mg <sub>tot</sub>		0.961	0.927
S <sub>tot</sub>		0.448	0.561
P <sub>av</sub>		369.0	314.0
K <sub>av</sub>	mg·kg <sup>-1</sup>	67.0	59.0
Cd <sub>tot</sub>		0.437	0.460
Pb <sub>tot</sub>		186.6	43.0

P<sub>av</sub>, K<sub>av</sub> – available forms for plants

X<sub>tot</sub> – total content

Before sowing, seeds of yellow lupine were inoculated with a vaccine containing *Rhizobium lupini*. Sowing was performed in early April at 100 germinating seeds per 1 m<sup>2</sup>. Soil was sprayed with the herbicide Stomp 330 EC at a rate of 4 dm<sup>3</sup>·ha<sup>-1</sup> on the day following the sowing of the lupine. The plants were harvested manually by digging them up with a spade inserted to a depth of 0.25m. Lupine plants harvested during the flowering stage were divided into roots, stems, leaves and flowers, whereas those harvested during the full maturity stage were divided into roots, stems, leaves, stripped pods and seeds.

Precipitation in individual months and mean monthly air temperature during the growing seasons for yellow lupine are shown in Table 2. It shows that both growing seasons were rather favourable for the growth, development and yielding of yellow lupine. The total rainfall during the 2008 and 2011 growing seasons satisfied the plants needs in full. However, it was not properly distributed over the months of growth. The amount of rainfall in June 2008 and in May and June 2011 was lower than required for yellow lupine, as reported by Dzieżyc *et al.* (1987).

**Table 2.** Rainfall and air temperatures during the test crops, data from IMGW-PIB Warszawa

Weather parameter	Month	Study period		Multiyear [1981-2007]
		2008	2011	
Monthly rainfall (mm)	IV	43.5	38.1	32.9
	V	72.7	55.6	54.2
	VI	56.7	44.3	68.8
	VII	108.8	204.2	64.9
	VIII	85.1	55.4	61.8
Averages monthly temperatures (°C)	IV	8.7	9.8	7.9
	V	12.5	13.5	13.7
	VI	17.0	18.1	16.1
	VII	18.1	18.1	18.3
	VIII	18.3	18.1	17.6

The content of Pb and Cd in the plant material was determined by the ICP-AES method after dry mineralisation of samples at 450°C.

The results were worked out statistically with an analysis of variance. Conclusions regarding the significance of the effect of the factors under study on individual features were based on the Fisher-Snedecor F-test, and the LSD<sub>0.05</sub> for a comparison of the calculated means we used the Tukey's test. For these calculations the Statistica 13.1 PL software package and MS Excel were used. In addition, the bioaccumulation and translocation coefficients of Pb and Cd were calculated.

The bioaccumulation factor (BAF) presents the ratio of the elements content in a plant to its amount in the soil (Rezvani and Zaefarian, 2011).

$$BAF = \frac{C_p}{C_s}$$

BAF<sub>x</sub> = bioaccumulation factor  
 C<sub>p</sub> = content of Pb and Cd in the plant  
 C<sub>s</sub> = content of Pb and Cd in the soil

The translocation factor (TF) was calculated as a ratio of the elements content in the above-ground parts to the content in the roots (Rezvani and Zaefarian, 2011).

$$TF = \frac{C_{pbs}}{C_{pr}}$$

TF = translocation factor  
 C<sub>pbs</sub> = content of Pb and Cd in the above-ground parts of the plant  
 C<sub>pr</sub> = content of Pb and Cd in the roots.

## RESULTS AND DISCUSSION

No significant effect of the differentiated nitrogen doses on the cadmium content in roots, stems and flowers of yellow lupine was obtained in the experiment (Table 3). However, the content of this heavy metal in the leaves, seeds and on average in the whole lupine mass was higher after the application of 120 kg N·ha<sup>-1</sup> than in the control object. The application of 30 kg N·ha<sup>-1</sup> did not significantly affect the cadmium content in leaves and seeds of the test plant and on average in its whole mass in comparison to the control object without nitrogen

fertilization and after the application of 120 kg N·ha<sup>-1</sup> dose. The highest content of this heavy metal in the stripped pods was obtained after the application of 30 kg N·ha<sup>-1</sup>, and the lowest after the application of 120 kg N·ha<sup>-1</sup>. Lead content in roots, stems, leaves, stripped pods, seeds and on average in the whole

yellow lupine mass was not significantly dependent on the applied nitrogen dose (Table 4). Only in the flowers of lupine fertilized with 120 kg N·ha<sup>-1</sup> was the content of this heavy metal higher than in both of the other objects (0 and 30 kg N·ha<sup>-1</sup>).

**Table 3.** Cadmium content in yellow lupine, mg Cd·kg<sup>-1</sup> dry mass (D.M.)

Investigated factor		Part of plant						Mean in plants (weighted average)
		roots	stems	leaves	flowers	stripped pods	seeds	
N dose (kg·ha <sup>-1</sup> )	0	0.399 <sup>a</sup>	0.407 <sup>a</sup>	0.513 <sup>a</sup>	0.254 <sup>a</sup>	0.390 <sup>ab</sup>	0.379 <sup>a</sup>	0.458 <sup>a</sup>
	30	0.354 <sup>a</sup>	0.380 <sup>a</sup>	0.621 <sup>ab</sup>	0.303 <sup>a</sup>	0.471 <sup>b</sup>	0.473 <sup>ab</sup>	0.499 <sup>ab</sup>
	120	0.376 <sup>a</sup>	0.438 <sup>a</sup>	0.691 <sup>b</sup>	0.306 <sup>a</sup>	0.316 <sup>a</sup>	0.549 <sup>b</sup>	0.538 <sup>b</sup>
Growth stage (BBCH)	65	0.501 <sup>b</sup>	0.491 <sup>b</sup>	0.698 <sup>b</sup>	0.288	–	–	0.577 <sup>b</sup>
	90	0.251 <sup>a</sup>	0.325 <sup>a</sup>	0.519 <sup>a</sup>	–	0.392	0.467	0.420 <sup>a</sup>
Year of study	1 <sup>st</sup>	0.242 <sup>a</sup>	0.293 <sup>a</sup>	0.419 <sup>a</sup>	0.142 <sup>a</sup>	0.104 <sup>a</sup>	0.128 <sup>a</sup>	0.313 <sup>a</sup>
	2 <sup>nd</sup>	0.510 <sup>b</sup>	0.523 <sup>b</sup>	0.798 <sup>b</sup>	0.433 <sup>b</sup>	0.680 <sup>b</sup>	0.805 <sup>b</sup>	0.684 <sup>b</sup>

*a, b, ...* – means with different letters in the columns are significantly different,  $P < 0.05$

When harvested at the blooming stage the cadmium content in roots, stems and leaves, as well as on average for the whole yellow lupine plant was higher than it was when the lupine was harvested at full maturity (Table 3). The lead concentration in the roots of lupine harvested at the blooming stage was higher than at the full maturity stage, while in the case of leaves the relationships was reversed (Table 4). The content of this heavy metal in the stems, and in the whole mass of the test plants, on average, was not significantly differentiated in either of the development stages.

The cadmium content in all organs and, on average, in the whole mass of yellow lupine was lower in the first year as compared to the second year of experiment (Table 3). In the case of lead, significant differences in terms of the year were noted for all separate parts, except for the roots, and for the average content in the biomass (Table 4), however, the obtained relationships were the reverse to those

noted for cadmium. No significant differences were obtained for lead content in the roots when comparing the two study years.

The amount of heavy metals taken up by yellow lupine in both years of the study were subject to similar relationships as to their content in the separated parts, as well as to the whole mass of this plant. Yellow lupine cultivated in 2008 absorbed less cadmium and more lead than it did in 2011 (Tables 5 and 6).

The amount of lead taken up by the whole yellow lupine plant was higher following the application of 120 kg N·ha<sup>-1</sup> than it was after both the application of 30 kg N·ha<sup>-1</sup> and on the control object without nitrogen fertilization (Table 6). The amount of this heavy metal accumulated in flowers was the highest after the 120 kg N·ha<sup>-1</sup> application, in seeds it was higher after both levels of nitrogen application than it was in the control object, whereas in the roots, stems, leaves and stripped pods it was not significantly differentiated by the level of nitrogen fertilization.

The amount of cadmium accumulated in the entire lupine plant was the highest after the 120 kg N·ha<sup>-1</sup> application and lowest in the control object (Table 5). The amount of this heavy metal accumulated in stems, leaves and seeds was the highest after the 120 kg N·ha<sup>-1</sup> application, whereas in the roots, flowers and stripped pods it was not significantly differentiated by the level of nitrogen fertilization.

The amount of cadmium and lead accumulated in the roots of lupine was higher at the blooming stage

than after full maturity. The amount of lead accumulated in stems and leaves was higher at the stage of full maturity than during blooming, whereas the amount of cadmium accumulated in these organs was not significantly different at these developmental stages. A higher accumulation of cadmium and lead in yellow lupine mass harvested at the full maturity stage (by 41.4% and 85.1%, respectively) than in that found at the blooming stage were noted.

**Table 4.** Lead content in yellow lupine, mg Pb·kg<sup>-1</sup> D.M.

Investigated factor		Part of plant						Mean in plants (weighted average)
		roots	stems	leaves	flowers	stripped pods	seeds	
N dose (kg·ha <sup>-1</sup> )	0	4.673 <sup>a</sup>	3.189 <sup>a</sup>	6.626 <sup>a</sup>	2.724 <sup>a</sup>	3.941 <sup>a</sup>	1.751 <sup>a</sup>	4.505 <sup>a</sup>
	30	5.060 <sup>a</sup>	2.926 <sup>a</sup>	6.169 <sup>a</sup>	2.857 <sup>a</sup>	3.025 <sup>a</sup>	1.949 <sup>a</sup>	4.189 <sup>a</sup>
	120	5.270 <sup>a</sup>	2.661 <sup>a</sup>	6.118 <sup>a</sup>	4.223 <sup>b</sup>	3.107 <sup>a</sup>	1.839 <sup>a</sup>	4.197 <sup>a</sup>
Growth stage (BBCH)	65	5.674 <sup>b</sup>	2.818 <sup>a</sup>	5.407 <sup>a</sup>	3.268	–	–	4.483 <sup>a</sup>
	90	4.327 <sup>a</sup>	3.033 <sup>a</sup>	7.202 <sup>b</sup>	–	3.358	1.846	4.120 <sup>a</sup>
Year of study	1 <sup>st</sup>	4.909 <sup>a</sup>	4.097 <sup>b</sup>	7.328 <sup>b</sup>	5.788 <sup>b</sup>	4.917 <sup>b</sup>	3.259 <sup>b</sup>	5.433 <sup>b</sup>
	2 <sup>nd</sup>	5.092 <sup>a</sup>	1.754 <sup>a</sup>	5.281 <sup>a</sup>	0.748 <sup>a</sup>	1.798 <sup>a</sup>	0.433 <sup>a</sup>	3.170 <sup>a</sup>

**Table 5.** Cadmium uptake by yellow lupine, g Cd·ha<sup>-1</sup>

Investigated factor		Part of plant						Total by plants
		roots	stems	leaves	flowers	stripped pods	seeds	
N dose (kg·ha <sup>-1</sup> )	0	0.196 <sup>a</sup>	0.425 <sup>a</sup>	0.718 <sup>a</sup>	0.031 <sup>a</sup>	0.377 <sup>a</sup>	0.496 <sup>a</sup>	1.790 <sup>a</sup>
	30	0.175 <sup>a</sup>	0.422 <sup>a</sup>	0.925 <sup>ab</sup>	0.038 <sup>a</sup>	0.506 <sup>a</sup>	0.626 <sup>a</sup>	2.108 <sup>b</sup>
	120	0.187 <sup>a</sup>	0.545 <sup>b</sup>	1.103 <sup>b</sup>	0.038 <sup>a</sup>	0.346 <sup>a</sup>	0.852 <sup>b</sup>	2.453 <sup>c</sup>
Growth stage (BBCH)	65	0.278 <sup>b</sup>	0.496 <sup>a</sup>	0.944 <sup>a</sup>	0.036	–	–	1.754 <sup>a</sup>
	90	0.094 <sup>a</sup>	0.432 <sup>a</sup>	0.886 <sup>a</sup>	–	0.410	0.658	2.480 <sup>b</sup>
Year of study	1 <sup>st</sup>	0.119 <sup>a</sup>	0.361 <sup>a</sup>	0.685 <sup>a</sup>	0.016 <sup>a</sup>	0.139 <sup>a</sup>	0.207 <sup>a</sup>	1.346 <sup>a</sup>
	2 <sup>nd</sup>	0.253 <sup>b</sup>	0.567 <sup>b</sup>	1.146 <sup>b</sup>	0.055 <sup>b</sup>	0.680 <sup>b</sup>	1.108 <sup>b</sup>	2.887 <sup>b</sup>

**Table 6.** Lead uptake by yellow lupine, g Pb·ha<sup>-1</sup>

Investigated factor		Part of plant						Total by plants
		roots	stems	leaves	flowers	stripped pods	seeds	
N dose (kg·ha <sup>-1</sup> )	0	2.143 <sup>a</sup>	3.372 <sup>a</sup>	10.118 <sup>a</sup>	0.296 <sup>a</sup>	4.136 <sup>a</sup>	2.007 <sup>a</sup>	18.853 <sup>a</sup>
	30	2.448 <sup>a</sup>	3.463 <sup>a</sup>	9.991 <sup>a</sup>	0.366 <sup>a</sup>	3.780 <sup>a</sup>	3.071 <sup>b</sup>	19.520 <sup>a</sup>
	120	2.590 <sup>a</sup>	3.635 <sup>a</sup>	10.861 <sup>a</sup>	0.476 <sup>b</sup>	4.463 <sup>a</sup>	3.573 <sup>b</sup>	21.342 <sup>b</sup>
Growth stage (BBCH)	65	3.169 <sup>b</sup>	2.921 <sup>a</sup>	7.491 <sup>a</sup>	0.379	–	–	13.961 <sup>a</sup>
	90	1.618 <sup>a</sup>	4.058 <sup>b</sup>	13.156 <sup>b</sup>	–	4.133	2.884	25.842 <sup>b</sup>
Year of study	1 <sup>st</sup>	2.354 <sup>a</sup>	4.979 <sup>b</sup>	12.982 <sup>b</sup>	0.665 <sup>b</sup>	6.437 <sup>b</sup>	5.173 <sup>b</sup>	26.492 <sup>b</sup>
	2 <sup>nd</sup>	2.393 <sup>a</sup>	2.001 <sup>a</sup>	7.665 <sup>a</sup>	0.094 <sup>a</sup>	1.829 <sup>a</sup>	0.595 <sup>a</sup>	13.318 <sup>a</sup>

The amount of heavy metals absorbed by the root system of plants depends on the metals content and availability in soil (Kibria *et al.*, 2006; Ociepa-Kubicka and Ociepa, 2012). The metals uptake is particularly easy when they are present in the form of free ions in the soil. The application of fertilizers that lower the pH value of the soil, such as ammonium sulphate, can increase the content of phyto-available heavy metal forms in the soil. According to (Sady and Smoleń, 2004) the consequence of this process is an increase in the accumulation of these elements in plants. In this study, the small nitrogen dose of 30 kg N·ha<sup>-1</sup>, applied in the form of ammonium sulphate, did not significantly increase the content of either of the heavy metals in yellow lupine. However, after applying 120 kg N·ha<sup>-1</sup> the cadmium content increased in the test plants, whereas the concentration of lead did not change significantly in comparison to the control object. Each of the applied doses of nitrogen significantly increased the amount of cadmium absorbed by the yellow lupine. In the case of lead, only after applying 120 kg N·ha<sup>-1</sup> was there a significantly higher amount of lead accumulated in the whole lupine mass as compared to the control object. This dependence was not the result of a higher lead content in the lupine biomass after the 120 kg N·ha<sup>-1</sup> application, but was the result of the higher volume of harvested biomass with this fertilizer variant than was the case in the control object. The calculated correlation coefficients between the amount of lupine mass reported by Wysokiński

(2013) and the amount of cadmium and lead absorbed by this plant were +0.087 and +0.836, respectively.

Heavy metals have a negative effect on the nitrogen metabolism in plants by inhibiting nitrate reductase. They can also increase the synthesis of active oxygen forms, which has a destructive effect on all macromolecules of a cell thus causing oxidative stress in it (Kopcewicz and Lewak, 2002). The calculated correlation coefficients indicate that the content of cadmium and lead in roots and in the whole mass of lupine, on average, did not significantly have an affect on the amount of nitrogen derived in this plant from the atmosphere presented by Wysokiński (2013).

In order to assess the mobility of cadmium and lead between soil and plant and within the test plant itself their BAF and TF coefficients were calculated (Table 7). The BAF value reflects the potential of a plant to absorb metal from soil (Baran and Jasiewicz, 2009). The lead uptake from soil by plant roots is low, whereas in the case of cadmium, it is relatively easy to absorb (Ociepa-Kubicka and Ociepa, 2012; Chang *et al.*, 2014). This thesis is in line with this current research where the value of the lead BAF for yellow lupine cultivated on soil with low lead contamination (II<sup>o</sup>) was 0.08 on average (in the 1<sup>st</sup> year of the experiment), whereas on soil with a natural content of this element (in the 2<sup>nd</sup> year of experiment) it was 0.03 on average. The BAF values for cadmium were much higher than for lead. In the



first year of the study the average value of this coefficient was 0.72, while in the second year it was 1.49. The values of lead BAF were similar at the stages of blooming and full maturity of lupine. In the case of cadmium, the values calculated for this coefficient were higher at the blooming stage than at the full maturity stage of the test plants. No effect of nitrogen fertilization on an increase in the value of lead BAF was observed in the conducted studies. In the case of cadmium, a slight increase in the value of this coefficient was observed in line with an increase in the nitrogen doses. In other studies carried out on *Galega orientalis* Lam. the effect of varied nitrogen fertilization on the availability of heavy metals has been shown (Symanowicz *et al.*, 2015).

**Table 7.** The values of BAF and TF coefficients

Investigated factor		BAF		TF	
		Cd	Pb	Cd	Pb
N dose (kg·ha <sup>-1</sup> )	0	1.01	0.06	1.47	1.02
	30	1.10	0.05	1.75	0.84
	120	1.19	0.05	2.18	0.80
Growth stage (BBCH)	65	1.28	0.06	1.47	0.77
	90	0.92	0.05	2.13	1.00
Year of study	1 <sup>st</sup>	0.72	0.08	1.80	1.15
	2 <sup>nd</sup>	1.49	0.03	1.80	0.62

The mobility of lead in plants is very limited, and the literature data indicate that in general the majority of this element (sometimes over 90%) is accumulated in the roots (Asadi Kapourchal *et al.*, 2009; Ociepa-Kubicka and Ociepa, 2012; Stefanowicz *et al.*, 2016). However, the author's own research did not confirm this thesis. During the blooming stage 22.7% of the total amount of lead absorbed by yellow lupine was found in roots while 77.3% was found in the aboveground parts. At the stage of full maturity for this plant only 6.3% of the total amount of lead collected was accumulated in the roots while 93.7% was transported to the aboveground organs. In the aboveground organs, those constituting harvest residues (stems, leaves and stripped pods)

accumulated 82.5% of the lead collected by the whole plant, whereas seeds accumulated only 11.2%. In contrast to lead, cadmium is easily transported through the root system to all plant organs (Ociepa-Kubicka and Ociepa, 2012; Page and Feller, 2015; Song *et al.*, 2017). In this current experiment the percentage of cadmium accumulated in roots was lower than it was in the case of lead and amounted to 15.8% at the blooming stage and 3.8% at full maturity. In the aboveground part harvested at the blooming stage, which is a potential roughage feed, lupine accumulated 84.2% of the cadmium, and in the seed yield obtained at the full maturity stage – 26.5% of the total absorbed amount of cadmium. The study shows that large amounts of these heavy metals can be fed further up the food chain when the green mass of yellow lupine harvested during the blooming stage is used as an animal feed. When cultivating this plant for seeds, only a small part of the cadmium and lead is found in these organs (yield) while the vast majority, 73.5% of Cd and 88.8% of Pb, respectively, goes back to the soil in the form of post-harvest residues. The TF factor provides information about the susceptibility of heavy metals to be displaced from the plant roots to aboveground parts (Ociepa *et al.*, 2014). Among the studied metals cadmium was more easily displaced than lead, as evidenced by the higher values of TF obtained in both years of the study. A value of TF above 1 indicates the hyperaccumulation potential for the specific species in relation to the metal. Our own studies confirmed such a potential for yellow lupine in relation to cadmium. Ehsan *et al.* (2015) did not find any hyperaccumulation of cadmium by *Lupinus uncinatus*, but only that this species can tolerate high concentrations of that heavy metal in the substrate. In the case of lead, lupines cultivated on soil showing weak contamination with this metal (II° in the 1<sup>st</sup> year of the experiment) also demonstrated the potential for its hyperaccumulation. However, under conditions of cultivation of this plant on soil with a natural lead content no tendency to hyperaccumulation of this heavy metal was observed. Tozser *et al.* (2017), when carrying out research on willow, found that all parts of this plant species accumulated significantly more Cd and Pb from contaminated soils than from uncontaminated soils and willow demonstrated a tendency to have a large accumulation of metal in twigs and leaves.

The cadmium and lead content in yellow lupine was assessed in terms of the potential for fodder use, of the green mass at the blooming stage and the seeds at the full maturity stage, by using the limit values provided in the Regulation of the Minister of Agriculture and Rural Development of 6<sup>th</sup> February 2012, on the content of undesirable substances in feeding stuffs (MRiRW, 2012). In the case of the content of the investigated heavy metals in feed materials of plant origin the following limit values have been adopted: 1 mg Cd·kg<sup>-1</sup> and 10 mg Pb·kg<sup>-1</sup>. The content of both of these heavy metals, in the green mass at the blooming stage and in lupine seeds, did not exceed the given standards (Table 3, 4).

## CONCLUSIONS

The application of 30 kg N·ha<sup>-1</sup> did not significantly affect the mean content of cadmium and lead in yellow lupine. Application of 120 kg N·ha<sup>-1</sup> increased the cadmium content without significantly affecting the concentration of lead in the test plants. Fertilization with each of the doses of nitrogen increased the amount of cadmium absorbed by lupine. Only after the higher nitrogen dose was applied did the test plant absorb more lead than the control plant. Yellow lupine harvested at the full maturity stage contained less cadmium and a similar concentration of lead than at full blooming, but it had absorbed more of these elements. Yellow lupine showed the ability to hyperaccumulate cadmium, whereas in the case of lead this ability was visible only on the soil demonstrating weak contamination with this heavy metal. In terms of the amount of cadmium and lead that can reach subsequent stages of the food chain it was found to be more advantageous to harvest yellow lupine at the full maturity stage and to use the seeds for fodder rather than to harvest it at the blooming stage and use the green forage for fodder. In the conditions of cultivating yellow lupine on soils with a natural cadmium and lead content, as well as on soil lightly contaminated with lead (II°), the green fodder and seeds were not excessively contaminated with these heavy metals and could be used for animal feed.

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## WPLYW ZRÓŻNICOWANEGO NAWOŻENIA AZOTEM NA AKUMULACJĘ I TRANSLOKACJĘ OŁOWIU I KADMU W ŁUBINIE ŻÓŁTYM

### Streszczenie

Metale ciężkie w środowisku występują często w ilościach przekraczających ich naturalną zawartość. Ze względu na dużą szkodliwość niektórych z nich (np. kadmu i ołowiu) dla organizmów żywych należy monitorować ich włączanie do łańcucha pokarmowego. Celem przeprowadzonych badań było określenie wpływu zróżnicowanych dawek azotu oraz fazy rozwojowej na akumulację i translokację ołowiu i kadmu w łubinie żółtym. Dawki azotu wprowadzonego do gleby przed siewem nasion wynosiły odpowiednio 0, 30

i  $120 \text{ kg} \cdot \text{ha}^{-1}$ , natomiast zbiór roślin przeprowadzono w fazach pełni kwitnienia i pełnej dojrzałości. Zróżnicowane nawożenie azotem nie miało istotnego wpływu na zawartość ołowiu w łubinie żółtym, natomiast zastosowanie dawki  $120 \text{ kg N} \cdot \text{ha}^{-1}$  zwiększyło zawartość kadmu w tej roślinie. Każda zastosowana dawka azotu zwiększyła ilość kadmu pobranego przez łubin. W przypadku ołowiu zależność tę uzyskano po zastosowaniu  $120 \text{ kg N} \cdot \text{ha}^{-1}$ . Łubin żółty zbierany w fazie pełnej dojrzałości najczęściej zawierał mniej kadmu i ołowiu, ale pobrał więcej tych metali ciężkich niż w fazie kwitnienia. Wartości współczynnika bioakumulacji wskazują na duży potencjał łubinu do pobierania kadmu oraz niewielki do pobierania ołowiu. Wartości współczynnika translokacji wskazują na możliwość hiperakumulacji kadmu przez tę roślinę, natomiast w przypadku ołowiu zdolność tę odnotowano tylko na glebie słabo zanieczyszczonej tym metalem ciężkim. Plony zielonej masy oraz nasion łubinu żółtego uprawianego na glebie lekko zanieczyszczonej ( $\text{II}^{\circ}$ ) i niezanieczyszczonej ołowiem nie były nadmiernie zanieczyszczone tym metalem ciężkim i mogły stanowić paszę dla zwierząt.

**Słowa kluczowe:** faza rozwojowa, *Lupinus luteus* L., metale ciężkie, nawożenie, pasza