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Effect of various nitrogen doses on copper and zinc accumulation in yellow lupine biomass

Abstract: Field experiments determined copper and zinc content and accumulation in yellow lupine roots, stems, leaves, flowers, pods and seeds. The test factors included development stages (BBCH 65 and BBCH 90) at which harvest was performed as well as nitrogen doses (0, 30, and 120 kg·ha^{-1}) introduced to the soil prior to sowing. A higher copper content (by an average of 20.9%) and zinc content (by 53.7%) were obtained in the whole mass of lupine harvested at the flowering stage compared to that at the full maturity stage. Yellow lupine fertilised with 120 kg N·ha^{-1} contained and took up more copper and zinc than both lupine cultivated without nitrogen fertilization and fertilised with 30 kg N·ha^{-1}. The application of different nitrogen doses had no significant effect on the contents of the micronutrients in the seeds of the test plant. The amount of copper and zinc accumulated in the seeds was the largest following the application of 120 kg N·ha^{-1}. Lupine accumulated the largest amounts of both elements in the leaves irrespective of the development stage at which the harvest was carried out. The bioaccumulation factor for copper and zinc was higher in the lupine harvested at the flowering stage than in the lupine harvested at full maturity, but it was not significantly determined by the applied nitrogen fertilization. The values of translocation coefficient for the tested heavy metals, usually higher than 1, indicate significant potential for their accumulation in yellow lupine biomass. Under conditions of an increased zinc content in the soil, lupine green matter harvested at the flowering stage contained an above-standard amount of this heavy metal and could not be used for animal feed.

Keywords: copper, zinc, yellow lupine, nitrogen fertilization, growth stage

INTRODUCTION

Copper and zinc are co-factors of many enzymes which are involved in physiological processes occurring in plants (Grzyś 2004). Copper is an essential micronutrient involved, *inter alia*, in the processes of photosynthesis, nitrogen compound conversions and the formation of proteins, DNA, and RNA. Both a deficiency and an excess of copper results in disturbances to these processes (Kabata-Pendias and Pendias 1999). The symptoms of a high content of copper in plants may include growth inhibition and leaf chlorosis (Mourato et al. 2009, Kabata-Pendias and Pendias 1999).

Zinc deficiency disrupts the metabolism of proteins, phosphates and carbohydrates as well as DNA and RNA synthesis, which results in the impaired growth and reproduction of plants (Kabata-Pendias and Mukherjee 2007). Similar to zinc deficiency, an excessive zinc content in the soil and increased uptake by the roots leads to restricted plant development. The symptoms of excess Zn concentrations in plant biomass include chlorotic and necrotic spots on the leaves, which result from photosynthesis limitation (Kabata-Pendias and Pendias 1999).

The mobility and availability of copper and zinc depend on the plant species/genotype as well as chemical and physical properties of the soil, such as the structure, cation exchange capacity, sorption and ion exchange, water retention and movement, the soil air, the pH value of the soil, and the overall metal content (Pinto et al. 2004, Wyszkowska et al. 2013). Nitrogen content in mineral forms in the soil may also affect the absorption of micronutrients by plants. This is mainly due to the effect of nitrogen fertilisers on the change in the pH value of the soil in the root zone of plants (Spiak et al. 2000). Under acidic conditions, the bioavailability of micronutrients which are of importance to agricultural production increases (Murawska et al. 2015). Factors conducive to increasing plant availability, as well as increased content of these elements in the soil, can influence the increased uptake of plants.

The aim of the study was to determine the effect of various doses of nitrogen fertilisation and of the development stage on the content and accumulation of copper and zinc in particular organs of yellow lupine (*Lupinus luteus* L.) cultivated on soil with elevated zinc content, as well as values of bioaccumulation and translocation coefficient of these elements.

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MATERIAL AND METHODS

The field experiment was conducted in Siedlce (N52°10'12.04" E22°17'15.40") in 2008 and 2011. The experiment as conducted on slightly acidic soil with granulometric composition of loamy sand. The content of selected macro- and micronutrients in the soil before the experiment was set up is shown in Table 1. $1m^2$ plots were delineated in a field of yellow lupine of the "Mister" cultivar. Two-factorial experiment was set up in the randomised split-block design, in three replications. Nitrogen fertilisation was the first factor: a) control, with no nitrogen fertilisation; b) with nitrogen applied at a rate equivalent to 30 kg $N \cdot ha^{-1}$; c) with nitrogen applied at a rate equivalent to 120 kg $N \cdot ha^{-1}$. The time of harvest was the second factor (determined as per Bleinholder et al. 2001): a) full flowering stage, 65 BBCH (date I, marked A in table 3–11); b) full maturity stage, 90 BBCH (date II, marked B in table 3–11). Mineral nitrogen was introduced to the soil as ammonium sulphate $(NH_4)_2SO_4$ before yellow lupine was sown. The amounts of phosphorus and potassium were established on the basis of the amounts of the available element forms in soil. Potassium was introduced to the soil in all plots at 100 kg $K \cdot ha^{-1}$ as potassium salt. Because of a very high amount of phosphorus as available forms (Table 1), no phosphorus fertilisation was applied. 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^2. Soil was sprayed with the herbicide Stomp 330 EC at a rate of 4 $dm^3 \cdot ha^{-1}$ on the day following the sowing of lupine. Lupine plants was sprayed with Amistar 250 SC at 1.0 $dm^3 \cdot ha^{-1}$ against anthracnose at the beginning of the budding phase; this procedure was repeated after 10 days. Plants harvested manually during the flowering stage were divided into roots, steams, leaves and flowers, whereas those harvested during the full maturity stage were divided into roots, steams, leaves, pods and seeds.

TABLE 1. Selected properties of soil in humus layer prior used to the field experiments in 2008 and 2011 years

Soils properties	Unit	Years of foundation experiment	
		2008	2011
pH_{KCl}	–	5.90	5.80
C_{tot}	$g \cdot kg^{-1}$	25.7	23.8
N_{tot}		2.04	1.92
P_{tot}		1.10	1.15
K_{tot}		0.85	0.81
Mg_{tot}		0.96	0.93
S_{tot}		0.448	0.56
P_{av}	$mg \cdot kg^{-1}$	369.0	314.0
K_{av}		67.0	59.0
Mo_{tot}		0.12	0.09
Mn_{tot}		155.2	157.9
Cu_{tot}		20.3	18.0
Fe_{tot}		5243	5135
Zn_{tot}		219.1	176.0

P_{av}, K_{av} – available forms for plants; X_{tot} – total content.

The total rainfall in individual months and mean monthly air temperature during the growing season for yellow lupine is 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 growing. 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). In addition to the greater water deficit during the period of intensive growth of lupine (May-June) in 2011, higher temperatures were recorded during the period than in 2008, which probably exacerbated the water deficit and decreased the yield of tested plant, presented in publication of Wysokiński (2013).

TABLE 2. Rainfall and air temperatures during the test crop (according to 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 cooper and zinc in the plant material was determined by the ICP-AES method in the bulk solution obtained by mineralisation of samples at 450°C. The ash obtained by mineralisation was dissolved in HCl 6 $mol \cdot dm^{-3}$ in order to degrade carbonates and evaporated to dryness on a sand bath. A 10% solution of HCl was used to transfer chlorides to volumetric flasks (Krzywy-Gawrońska 2007).

The results were worked out statistically with an analysis of variance. Conclusions regarding the significance of an effect of the factors under study on individual features were based on the Fisher-Snedecor F-test, and the $LSD_{0.05}$ for comparison of the calculated means were calculated by the Tukey test. Moreover, linear correlation coefficients for content, uptake of Cu, Zn and amount and percentage of nitrogen taken up by yellow lupine from the atmosphere were calculated. To these calculations the Statistica 10 PL software package (StatSoft, Tulsa, USA) was used. In addition, the bioaccumulation and translocation coefficient were calculated

Using the bioaccumulation factor (BAF), the ability of plants to take up a heavy metal from the soil was described and information on its translocation from the soil solution to the above-ground parts of the plants and to the roots was obtained (Grzebisz et al. 1998, Jasiewicz and Antonkiewicz 2000). This factor presents the ratio of a metal content in a plant to its amount in the soil.

$$WBx = \frac{CP_{(x)}}{CS_{(x)}}$$

WB_x = bioaccumulation factor
$CP_{(x)}$ = content of element 'x' in the plant
$CS_{(x)}$ = content of element 'x' in the soil

The translocation factor (TF) was used to determine the mobility of copper and zinc in the test plants (Jasiewicz and Antonkiewicz 2000). This parameter was calculated as a ratio of copper and zinc contents in the above-ground parts to the content in the roots.

$$WT_x = \frac{Cpbs_{(x)}}{Cpr_{(x)}}$$

WT_x = translocation factor
$Cpbs_{(x)}$ = content of element 'x' in the above-ground parts of the plant
$Cpr_{(x)}$ = content of element 'x' in the roots

RESULTS AND DISCUSSION

Cooper and zinc contents of whole yellow lupine plants ranged from 5.07 to 9.69 mg·kg^{-1} of Cu and from 87.3 to 161.2 mg·kg^{-1} of Zn (Tables 3 and 5). A higher copper content (by 20.9%) and zinc content (by 53.7%) were found in the lupine harvested at the flowering stage than in the lupine harvested at the full maturity stage (Tables 4 and 6). At the full flowering stage, the highest copper content was noted in the flowers (9.46 mg·kg^{-1}), while the highest zinc content was noted in the leaves (190.1 mg·kg^{-1}). At the full maturity stage, the highest zinc content still remained in the leaves (151.2 mg·kg^{-1}), while the most copper was found in the stems (6.99 mg·kg^{-1}).

Yellow lupine fertilised with 30 kg N·ha^{-1} contained, on average, less copper in the whole biomass than the lupine cultivated without nitrogen fertilization and fertilised with 120 kg N·ha^{-1} (Table 4). The copper content in the roots was higher following the application of 120 kg N·ha^{-1} compared to the copper content in the control object and following the application of 30 kg N·ha^{-1}. Plants harvested from the control object contained a larger amount of this element than following the application of both doses of nitrogen. The leaves contained the largest amount of copper following the application of 120 kg N·ha^{-1}, and the smallest amount of copper following the application of 30 kg N·ha^{-1}. Copper accumulation in the flowers was the highest

TABLE 3. Copper content in yellow lupine, mg Cu·kg^{-1} DM

No. series	Growth stage	Nitrogen dose kg·ha^{-1}	Part of plant					Meanly in plant
			roots	stems	leafs	flowers/ stripped pods[3]	seeds	
I	A	0	9.04	7.99	9.59	10.23	–	8.99
		30	8.75	7.86	9.19	9.46	–	8.67
		120	9.08	8.26	10.95	10.93	–	9.69
	averages		8.96	8.04	9.91	10.21	–	9.12
	B	0	7.26	9.73	7.35	8.79	7.65	8.19
		30	6.81	8.01	7.24	7.13	7.46	7.40
		120	6.84	7.46	7.05	6.18	7.21	6.99
	averages		6.97	8.40	7.21	7.37	7.44	7.53
Averages			7.96	8.22	8.56	–	–	8.32
II	A	0	4.63	5.95	6.88	9.67	–	6.28
		30	6.07	5.71	6.29	8.28	–	6.15
		120	11.07	5.55	6.54	8.21	–	7.11
	averages		7.26	5.73	6.57	8.72	–	6.51
	B	0	4.45	6.12	5.91	4.29	5.16	5.39
		30	4.64	4.95	5.42	4.14	5.63	5.07
		120	5.08	5.67	6.31	5.27	5.90	5.80
	averages		4.72	5.58	5.88	4.57	5.56	5.42
Averages			5.99	5.66	6.23	–	–	5.97
$LSD_{0.05}$ for series			0.47	0.43	0.40	0.60/0.60	0.39	0.17

[3] – in dependence from yellow lupine's growth stage: for blooming stage (A) the tower concerns the flower, but for full maturity stage (B) concerns the stripped pods.

TABLE 4. The averages for investigated factors of copper contents in yellow lupine, mg $Cu \cdot kg^{-1}$ DM

Investigated factor		Part of plant						Meanly in plant
		roots	stems	leafs	flowers	stripped pods	seeds	
N dose $[kg \cdot ha^{-1}]$	0	6.35	7.45	7.43	9.95	6.54	6.41	7.21
	30	6.57	6.63	7.04	8.87	5.64	6.55	6.82
	120	8.02	6.74	7.71	9.57	5.73	6.56	7.40
$LSD_{0.05}$		0.69	0.63	0.60	0.90	0.90	n.i.	0.25
Growth stage	A	8.11	6.89	8.24	9.46	–	–	7.82
	B	5.85	6.99	6.55	–	5.97	6.50	6.47
$LSD_{0.05}$		0.47	n.i.	0.40	–	–	–	0.17
$LSD_{0.05}$ Interaction:								
growth stage/series		n.i.	n.i	0.57	–	–	–	–
N dose/series		0.98	n.i.	n.i.	1.27	1.28	n.i.	0.35
N dose/growth stage		0.98	0.89	n.i.	–	–	–	0.35

TABLE 5. Zinc content in yellow lupine, mg $Zn \cdot kg^{-1}$ DM

No. series	Growth stage	Nitrogen dose $kg \cdot ha^{-1}$	Part of plant					Meanly in plant
			roots	stems	leafs	flowers/ stripped pods[3]	seeds	
I	A	0	114.7	96.2	186.0	97.9	–	139.9
		30	119.5	92.9	196.8	110.1	–	144.1
		120	116.8	92.9	194.8	109.9	–	143.2
	averages		117.0	94.0	192.5	106.0	–	142.4
	B	0	73.1	75.1	136.5	53.6	87.8	94.4
		30	80.3	65.5	132.8	51.2	81.9	87.3
		120	76.3	66.1	140.6	45.5	83.0	88.2
	averages		76.5	68.9	136.6	50.1	84.2	90.0
Averages			96.8	81.5	164.6	–	–	116.2
II	A	0	103.5	100.6	184.4	94.0	–	137.6
		30	130.1	105.7	179.2	100.0	–	142.5
		120	154.1	123.0	199.1	109.4	–	161.2
	averages		129.2	109.8	187.6	101.1	–	147.1
	B	0	58.0	69.8	153.9	51.8	83.0	92.6
		30	74.4	63.6	167.6	53.2	85.2	96.8
		120	69.1	82.0	175.5	58.4	91.7	106.0
	averages		67.2	71.8	165.7	54.5	86.6	98.5
Averages			98.2	90.8	176.6	–	–	122.8
$LSD_{0.05}$ for series			n.i.	3.3	5.5	n.i./n.i.	n.i.	2.8

in the control object, while the lowest was noted following the application of 30 kg $N \cdot ha^{-1}$. An increase in nitrogen dose from 30 to 120 $kg \cdot ha^{-1}$ had no significant effect on the copper content in the stems, flowers or pods. Copper content in the seeds was not significantly different depending on the applied nitrogen fertilisation.

Yellow lupine fertilised with 120 kg $N \cdot ha^{-1}$ contained more zinc than the lupine cultivated on the control object and fertilised with 30 kg $N \cdot ha^{-1}$ (Table 6). Zinc content in the roots of lupine fertilised with both nitrogen doses was higher than that in the control object. A higher zinc content in the stems and leaves was noted following the application of 120 kg $N \cdot ha^{-1}$ than that following the fertilisation with 30 kg $N \cdot ha^{-1}$ and without nitrogen fertilisation. Zinc content in the flowers was the highest in the lupine fertilised with 120 kg $N \cdot ha^{-1}$ and was the lowest in the control object. Fertilisation with various nitrogen doses did not significantly differentiate the content of this micronutrient in generative organs, i.e. the pods and seeds.

The copper content in all organs and, on average, in the whole biomass of yellow lupine was higher in 2008, which was characterised by both a lower

TABLE 6. The averages for investigated factors of zinc contents in yellow lupine, mg $Zn \cdot kg^{-1}$ DM

Investigated factor		Part of plant						Meanly in plant
		roots	stems	leafs	flowers	stripped pods	seeds	
N dose [$kg \cdot ha^{-1}$]	0	87.3	85.4	165.2	96.0	52.7	85.4	116.1
	30	101.1	81.9	169.1	105.1	52.2	83.6	117.7
	120	104.1	91.0	177.5	109.7	52.0	87.4	124.7
$LSD_{0.05}$		9.4	4.8	7.4	11.5	n.i.	n.i	4.1
Growth stage	A	123.1	101.9	190.1	103.6	–	–	144.8
	B	71.9	70.4	151.2	–	52.3	85.4	94.2
$LSD_{0.05}$		6.3	3.3	5.0	–	–	–	2.8
$LSD_{0.05}$ Interaction:								
growth stage/series		9.0	4.6	7.1	–	–	–	n.i.
N dose/series		13.3	6.8	n.i.	n.i.	10.2	8.9	5.8
N dose/growth stage		13.3	n.i.	n.i.	–	–	–	5.8

temperature during the growing season and more favourable temperature distribution as compared to 2011 (Table 3). For zinc, significant differences in terms of the year were noted for the stems and leaves as well as the average content in the biomass and the obtained relationships were reverse to those noted for copper (Table 5). In both years of the study, no significant differences were obtained for zinc content in the roots, flowers, pods or seeds.

Yellow lupine cultivated in 2008 took up more copper and zinc than in 2011 (Tables 7 and 9). The contents of copper and zinc accumulated in the whole lupine biomass was higher following the application of 120 kg $N \cdot ha^{-1}$ than following the application of 30 kg $N \cdot ha^{-1}$ and on the control object (Tables 8 and 10). The amounts of copper and zinc taken up by lupine harvested at the full maturity stage were higher (by 64.6% and 29.1%, respectively) than those in the flowering stage. The test plant accumulated the largest amounts of both elements in the leaves, irrespective of the development stage at which it was harvested.

The application of physiologically acidic fertilisers such as ammonium sulphate (which lower the pH value of the soil) increased the content of phyto-available forms of heavy metals in the soil. The consequence

TABLE 7. The amount of copper taken up by yellow lupine, g $Cu \cdot ha^{-1}$

No. series	Growth stage	Nitrogen dose $kg \cdot ha^{-1}$	Part of plant					Meanly in plant
			roots	stems	leafs	flowers/ stripped pods[3]	seeds	
I	A	0	4.92	7.21	12.04	1.09	–	25.27
		30	5.17	8.90	13.44	1.23	–	28.73
		120	5.73	10.64	18.06	1.22	–	35.66
	averages		5.27	8.92	14.51	1.18	–	29.89
	B	0	2.25	12.00	14.46	9.66	8.67	47.04
		30	2.55	10.67	13.96	9.47	11.23	47.89
		120	2.63	11.70	15.50	9.42	14.95	54.20
	averages		2.48	11.46	14.64	9.52	11.62	49.71
Averages			3.88	10.19	14.58	–	–	39.80
II	A	0	2.38	5.47	8.35	1.18	–	17.38
		30	3.23	5.40	8.05	1.07	–	17.74
		120	6.11	5.59	8.46	1.07	–	21.24
	averages		3.90	5.49	8.29	1.11	–	18.78
	B	0	1.91	7.74	9.13	4.02	6.93	29.73
		30	1.86	6.24	8.48	4.25	7.38	28.21
		120	1.81	7.38	10.21	5.23	8.66	33.30
	averages		1.86	7.12	9.27	4.50	7.66	30.41
Averages			2.88	6.30	8.78	–	–	24.60
$LSD_{0.05}$ for series			0.58	1.06	1.38	n.i./1.53	1.65	3.48

TABLE 8. The averages of amount for investigated factors of copper taken up by yellow lupine, g $Cu \cdot ha^{-1}$

Investigated factor		Part of plant						Meanly in plant
		roots	stems	leafs	flowers	stripped pods	seeds	
N dose [$kg \cdot ha^{-1}$]	0	2.87	8.11	11.00	1.14	6.84	7.80	29.86
	30	3.20	7.80	10.98	1.15	6.86	9.31	30.64
	120	4.07	8.83	13.06	1.15	7.33	11.81	36.10
$LSD_{0.05}$		0.86	n.i.	2.04	n.i.	n.i.	2.48	5.16
Growth stage	A	4.59	7.20	11.40	1.14	–	–	24.34
	B	2.17	9.29	11.96	–	7.01	9.64	40.06
$LSD_{0.05}$		0.58	1.06	n.i.	–	–	–	3.48
$LSD_{0.05}$ Interaction:								
growth stage/series		n.i.	n.i.	n.i.	–	–	–	4.93
N dose/series		n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.
N dose/growth stage		1.21	n.i.	n.i.	–	–	–	n.i.

TABLE 9. The amount of zinc taken up by yellow lupine, g $Zn \cdot ha^{-1}$

No. series	Growth stage	Nitrogen dose $kg \cdot ha^{-1}$	Part of plant					Meanly in plant
			roots	stems	leafs	flowers/ stripped pods3	seeds	
I	A	0	62.4	86.9	233.4	10.5	–	393.1
		30	70.6	105.1	287.7	14.3	–	477.8
		120	73.7	119.6	321.2	12.3	–	526.9
	averages		68.9	103.9	280.8	12.4	–	465.9
	B	0	22.7	92.6	268.6	59.0	99.5	524.4
		30	30.1	87.3	256.0	67.9	123.3	564.6
		120	29.4	103.7	309.4	69.4	172.1	683.9
	averages		27.4	94.5	278.0	65.4	131.6	597.0
Averages			48.2	99.2	279.4	–	–	531.5
II	A	0	53.2	92.6	223.7	11.5	–	380.9
		30	69.1	100.0	229.2	12.9	–	411.2
		120	85.1	124.0	257.9	14.3	–	481.3
	averages		69.1	105.5	236.9	12.9	–	424.5
	B	0	24.9	88.4	237.5	48.6	111.4	510.8
		30	29.8	80.2	262.5	54.5	111.8	538.8
		120	24.7	106.8	284.2	58.0	134.6	608.3
	averages		26.4	91.8	261.4	53.7	119.3	552.6
Averages			47.8	98.7	249.2	–	–	488.6
$LSD_{0.05}$ for series			n.i.	n.i.	23.3	n.i./10.5	n.i.	40.6

TABLE 10. The averages of amount for investigated factors of zinc taken up by yellow lupine, g $Zn \cdot ha^{-1}$

Investigated factor		Part of plant						Meanly in plant
		roots	stems	leafs	flowers	stripped pods	seeds	
N dose [$kg \cdot ha^{-1}$]	0	40.8	90.1	240.8	11.0	53.8	105.5	456.8
	30	49.9	93.2	258.9	13.6	61.2	117.6	498.1
	120	53.2	113.5	293.2	13.3	63.7	153.4	575.1
$LSD_{0.05}$		7.2	14.4	34.5	n.i.	n.i.	30.8	60.2
Growth stage	A	69.0	104.7	258.9	12.6	–	–	445.2
	B	26.9	93.2	269.7	–	59.6	125.5	574.8
$LSD_{0.05}$		4.9	9.7	n.i.	–	–	–	40.6
$LSD_{0.05}$ Interaction:								
growth stage/series		n.i.	n.i.	n.i.	–	–	–	n.i
N dose/series		n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.
N dose/growth stage		10.2	n.i.	n.i.	–	–	–	n.i.

of this process was an increase in the accumulation of these elements in plants (Sady and Smoleń 2004). In addition, increasing nitrogen doses result in more intense uptake of copper from the soil (Rabikowska and Piszcz 2004). Moreover, positive interactions between an increasing nitrogen content and zinc content in the plant were noted (Sady and Smoleń 2004). A slightly different view is presented by Spiak (2000) who states that nitrogen salts introduced to the soil may either alleviate or aggravate the symptoms of zinc deficiency in plants. However, acidification of the soil results in an increase in the solubility of various chemical forms of zinc and increases its bioavailability and activity (Curyło and Jasiewicz 1998, Mercik et al. 2003). In the study, higher contents and larger amounts of copper and zinc were taken up by yellow lupine fertilised with 120 kg N·ha^{-1} than following the application of 30 kg N·ha^{-1} or under cultivation without ammonium sulphate fertilisation.

According to Kabata-Pendias and Pendias (1999), the roots accumulate large amounts of copper, both in deficiency and in excess. Due to the low mobility of this element in plants, both young parts of plants and generative organs are affected by its absence (Rosada and Przewocka 2016). As demonstrated by a study by Sękara et al. (2005), the copper content in the roots of lucerne was three times higher than in the stems. The obtained results of the author's own study did not confirm the above-mentioned relationships, and the copper contents in particular organs were often similar.

The experiment demonstrated that irrespective of nitrogen dose and the stage at which the harvest is performed, the leaves of lupine contained (and took up) more zinc than other organs. Sękara et al. (2005) obtained 20% more zinc in the stems than in the roots. Shure and Macfie (2006) presented different study results which indicated the highest zinc content in the roots of soybean, while leaves contained 33% less zinc.

In order to assess the degree and direction of the translocation of copper and zinc in the test plant, their translocation and bioaccumulation factors were calculated. The bioaccumulation factor value reflected the plant's potential to take up a metal from the soil (Baran and Jasiewicz 2009). This factor for copper was higher in the first year of the experiment, while for zinc it was higher in the second year, and it was higher for both elements in the lupine harvested at the flowering stage than in the lupine harvested at the full maturity stage (Table 11). The bioaccumulation factor values for copper ranged from 0.28 to 0.48, while for zinc they ranged from 0.42 to 0.92; no significant contribution of nitrogen fertilisation to an increase in the value of this factor was noted. A study performed on eastern galega also did not confirm the effect of various doses of nitrogen fertilisation on the a ailability of heavy metals (Symanowicz et al. 2015).

TABLE 11. The value of bioaccumulation and translocation coefficient

No. series	Growth stage	Nitrogen dose kg·ha^{-1}	Bioacumulation coefficient		Translocation coefficient	
			Cu	Zn	Cu	Zn
I	A	0	0.44	0.64	0.99	1.27
		30	0.43	0.66	0.99	1.25
		120	0.48	0.65	1.08	1.27
	averages		0.45	0.65	1.02	1.26
	B	0	0.40	0.43	1.14	0.97
		30	0.36	0.40	1.09	0.91
		120	0.34	0.40	1.02	0.88
	averages		0.37	0.41	1.08	0.92
Averages			0.41	0.53	1.05	1.09
II	A	0	0.35	0.78	1.44	1.40
		30	0.34	0.81	1.02	1.12
		120	0.40	0.92	0.56	1.06
	averages		0.36	0.84	1.00	1.19
	B	0	0.30	0.53	1.23	1.65
		30	0.28	0.55	1.10	1.32
		120	0.32	0.60	1.15	1.57
	averages		0.30	0.56	1.16	1.51
Averages			0.33	0.70	1.08	1.35

The translocation factor provides information on the possibility for translocation of metals from the roots to the above-ground parts (Ociepa et al. 2014). Of the two tested metals, zinc was translocated more easily than copper, which is indicated by slightly higher values of the translocation factors obtained in the two years of the study. According to the literature data, a translocation factor value > 1 indicates hyperaccumulation potential for a particular species in relation to a metal. Both own study and that carried out by Ehsan et al. (2015) confirmed such potential for lupine.

The assessment of metal contents in lupine was performed in order to determine the possibilities for the use of the produced biomass for animal feed. For copper and zinc, the following max limit values were adopted: 30 mg Cu kg^{-1} d.m. and 100 mg Zn·kg^{-1} d.m. (Kabata-Pendias et al. 1993). Generally, copper content in lupine fell within the desired range, while zinc concentration in the lupine harvested at the flowering stage significantly exceeded the adopted standard values. Taking the obtained values into account, the lupine green fodder harvested during the study was not suitable for animal feed. The accumulation of significant amounts of zinc in lupine biomass could be an effect of the elevated content of this element in the soil.

CONCLUSIONS

1. Copper and zinc contents in the seeds of yellow lupine were not significantly determined by the applied nitrogen fertilisation. On average, the content of both elements in the whole lupine biomass was the highest following the application of 120 kg $N \cdot ha^{-1}$.
2. Yellow lupine harvested at the full maturity stage contained less copper and zinc but took up more of these elements than at the full flowering stage.
3. The largest amounts of copper and zinc were accumulated by yellow lupine in the leaves.
4. The application of various doses of nitrogen fertilisation had no significant effect on the values of accumulation and translocation factors for copper and zinc. A higher value of the bioaccumulation factor for copper and zinc was obtained at the flowering stage than at the full maturity stage. The translocation factor values at both development stages were similar.
5. Green fodder of yellow lupine cultivated under conditions of elevated zinc content in the soil was excessively (above the standard) contaminated with this heavy metal.

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Wpływ zróżnicowanych dawek azotu na zawartość i akumulację miedzi oraz cynku w łubinie żółtym

Streszczenie: W doświadczeniach polowych określono zawartość oraz ilość zakumulowanej miedzi i cynku w: korzeniach, łodygach, liściach, kwiatach, strączynach i nasionach łubinu żółtego. Badanymi czynnikami były faza rozwojowa (65 BBCH i 90 BBCH), w której nastąpił zbiór oraz ilość azotu (0, 30 i 120 kg $N \cdot ha^{-1}$) wprowadzona do gleby przed wysiewem. Większą zawartość miedzi średnio o 20,9% oraz cynku o 53,7% uzyskano w całej masie łubinu zbieranego w fazie kwitnienia niż w fazie pełnej dojrzałości. Łubin żółty nawożony 120 kg $N \cdot ha^{-1}$ zawierał i pobrał więcej miedzi i cynku w porównaniu z jego uprawą bez nawożenia azotem i po zastosowaniu 30 kg $N \cdot ha^{-1}$. Zróżnicowane nawożenie azotem nie miało istotnego wpływu na zawartość obydwu badanych mikroelementów w nasionach badanej rośliny. Ilość miedzi i cynku zakumulowanych w nasionach była największa po zastosowaniu 120 kg $N \cdot ha^{-1}$. Niezależnie od fazy rozwojowej, w której nastąpił zbiór, największe ilości obu pierwiastków łubin zakumulował w liściach. Współczynnik bioakumulacji miedzi i cynku był większy w łubinie zbieranym w fazie kwitnienia niż w pełnej dojrzałości, ale nie był istotnie uzależniony od zastosowanego nawożenia azotem. Wartości współczynnika translokacji badanych metali ciężkich najczęściej wynoszące ponad 1, wskazują na znaczny potencjał do ich akumulacji w biomasie łubinu żółtego. W warunkach podwyższonej zawartości cynku w glebie zielona masa łubinu zbierana w fazie kwitnienia zawierała ponadnormatywną ilość tego metalu ciężkiego i nie mogła być przeznaczona na paszę dla zwierząt.

Słowa kluczowe: miedź, cynk, łubin żółty, nawożenie azotem, faza rozwojowa