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EFFECT OF IRON, MOLYBDENUM AND COBALT ON THE AMOUNT OF NITROGEN BIOLOGICALLY REDUCED BY *Rhizobium galegae*

WPŁYW ŻELAZA, MOLIBDENU I KOBALTU NA ILOŚĆ AZOTU BIOLOGICZNIE ZREDUKOWANEGO PRZEZ *Rhizobium galegae*

Abstract: Iron and molybdenum are microelements which have a decisive effect on the amount of nitrogen fixed by legume plants. They are mainly necessary in the synthesis of nitrogenase. Cobalt also takes part in the process of biological reduction of N_2.

In order to determine the effect of Fe, Mo and Co on the amount of nitrogen fixed by fodder galega (*Galega orientalis* Lam.), a field experiment was conducted in the years 2005–2007 at the experimental station of the University of Natural Sciences and Humanities in Siedlce. Nitrogen ^{15}N enriched at 10.3 at % was applied in early spring as $(^{15}NH_4)_2SO_4$ at 1.66g per $1m^2$. Simultaneously with fodder galega (*Galega orientalis* Lam.), a plant unable to reduce nitrogen N_2 was cultivated (spring barley – *Hordeum sativum*), which was also fertilised with ^{15}N as $(^{15}NH_4)_2SO_4$ with 10.3 at % enrichment. Upon harvesting the test plant in the budding phase (three cuts), samples were taken, dried and ground. Soil samples were also taken before each cut. Fe, Mo and Co content in soil and the plant was determined by the ICP-AES, following its dry mineralisation. Statistical calculations revealed significant differences in the content of iron, molybdenum and cobalt in the soil and in the plant. The soil contained the largest amount of iron, less cobalt and the smallest amount of molybdenum. The iron amount found in biomass of fodder galega lay within the optimum limits specifying the acceptable content of trace elements in fodder, whereas the molybdenum content was too high and that of cobalt was too low. The correlation coefficients indicate a significant relationship between the content of iron, molybdenum and cobalt in soil and the amount of nitrogen reduced biologically by *Rhizobium galegae* bacteria which live in symbiosis with fodder galega.

Keywords: fodder galega, nitrogen, iron, molybdenum, cobalt, soil, plant

Iron, molybdenum and cobalt are among the elements which are essential to plants and animals [1, 2]. The optimum content of the microelements in plants is an important quality feature in assessment of their usability as fodder. Restricting organic fertilisation

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and using exclusively mineral fertilisers leads very frequently to a negative balance of microelements in soil, which may affect a plant's ability to take them up [3]. Moreover, insufficient amounts of the elements available to plants may lead to disturb generative development of plants [4, 5]. Iron and molybdenum also have a decisive effect on the amount of nitrogen fixed by legume plants. Iron and molybdenum are essential in the synthesis of nitrogenase, whereas cobalt is incorporated in the molecules of porphyrins which are parts of the cobalamine, which is a coenzyme directly participating in the process of fixing N_2.

The process of biological reduction of nitrogen involves incorporation of molecular nitrogen in the biological system [6–10]. This ability is used by *Rhizobium* bacteria which live in symbiosis with legume plants, as well by free-living bacteria (*Azotobacter, Clostridium*) as well as fungi (*Rhizopus*) and actinobacteria (*Streptomyces*) [11, 12]. Their common feature is the presence of nitrogenase – the main enzyme which is responsible for fixing elemental nitrogen. Nitrogenase consists of two protein complexes: Protein comprising Mo-Fe is an enzyme which reduces N_2, whereas the protein that contains only Fe provides electrons necessary for reduction.

Recently, much interest has been attracted by the comprehensive research into fodder galega, conducted by Andrzejewska and Ignaczak [13], Ignaczak [14], Sienkiewicz et al [15], Symanowicz and Kalembasa [16]. It is well-known that the presence of Fe, Mo and Co in soil is associated with the process of biological reduction of N_2. However, there are no data on the effect of those elements on the process of fixing nitrogen by symbiotic and free-living bacteria.

The aim of the study was to determine the content of iron, molybdenum and cobalt in soil and plants as well as to determine the effect of those elements on the amount of nitrogen fixed by fodder galega (*Galega orientalis* Lam.) in three consecutive cuts and vegetation periods.

Materials and methods

A field experiment was conducted in the years 2005–2007 in a multi-year plantation of fodder galega (9th, 10th and 11th year of cultivation). Field experiments were conducted at the experimental station of the University of Natural Sciences and Humanities in Siedlce, on soil formed from loamy sand, with pH measured in 1mol KCl $\cdot$ dm^{-3} – 6.9. The soil on which the experiment was conducted contained 31.2 g $\cdot$ kg^{-1} of total carbon and 3.6 g $\cdot$ kg^{-1} of total nitrogen. The content of available phosphorus and potassium was determined to be of a medium level and that of magnesium to be low. The total content of iron in the soil on which galega was cultivated was equal to 4738.1 mg $\cdot$ kg^{-1}, that of molybdenum was 0.31 mg $\cdot$ kg^{-1} and cobalt was 1.98 mg $\cdot$ kg^{-1}. Nitrogen ^{15}N with 10.3 % enrichment was applied in early spring as $(^{15}NH_4)_2SO_4$ at 1.66 g per 1m^2. Simultaneously with fodder galega (*Galega orientalis* Lam.), a plant unable to reduce nitrogen N_2 was cultivated (spring barley – *Hordeum sativum*) which was also fertilised with ^{15}N as $(^{15}NH_4)_2SO_4$ at 10.3 % enrichment.

Three cuts of the test plant during the budding phase were harvested in each year of the study. Samples of soil from the humus horizon were taken three times (before the first cut, before the second cut and before the third cut) and were subsequently dried and sifted through a 1 mm mesh sieve. During consecutive harvests of fodder galega (*Galega orientalis* Lam.), samples of entire plants were taken, which were subsequently dried and ground. The content of iron, molybdenum and cobalt in soil and in the plant was determined following dry mineralisation, by the ICP-AES method [17].

The results were worked out statistically and the analysis of variance was applied. Significant differences were calculated with Tukey's test at the level of significance of $\alpha = 0.05$. The effect of the content of iron, molybdenum and cobalt on the amount of nitrogen biologically reduced by fodder galega (*Galega orientalis* Lam.) was determined with correlation coefficients.

Results and discussion

Rainfall and temperature data for the 2005–2007 vegetation seasons are shown in Table 1.

Table 1

Air temperatures and rainfall in the vegetation in the years 2005–2007. Reported by the measurement centre in Siedlce

Means air temperature [°C]								
Months	Years	IV	V	VI	VII	VIII	IX	Mean
Mean monthly	2005	8.6	13.0	15.9	20.2	17.5	15.0	15.0
	2006	8.4	13.6	17.2	22.3	18.0	15.4	15.8
	2007	8.3	14.5	18.2	18.5	18.6	13.1	15.2
Multiyear mean		7.7	10.0	16.1	19.3	18.0	13.0	11.4
Total monthly rainfall [mm]								Sum
Sum monthly	2005	12.3	64.7	44.1	86.5	45.4	15.8	268.8
	2006	29.8	39.6	24.0	16.2	227.6	22.0	359.2
	2007	21.2	59.1	59.9	70.2	31.1	67.6	309.1
Multiyear sum		52.3	50.0	68.2	45.7	66.8	60.7	343.7

The average monthly temperature in consecutive vegetation periods was similar (15.0 °C to 15.8 °C) and it was much higher than the multi-year average. The temperatures recorded during the vegetation period favoured the process of biological reduction of N_2 [18]. The average rainfall during the vegetation period was lower than the multi-year total. Only in 2006 it was slightly higher (by 15.5 mm). This was a result of high rainfall in August, which was three times higher than the multi-year average.

The average iron content in the humus level of soil was equal to 4065.3 mg $\cdot$ kg^{-1} (Table 2) and it was significantly varied by the analysed factors and their combinations. The significantly highest iron content was determined in the soil sample taken before the first cut of fodder galega (4254.4 mg $\cdot$ kg^{-1}). The content of iron in 2005 and 2007 was similar (4285.8 and 4282.9 mg · kg^{-1}). Significant differences were found between iron content in soil in 2005 and 2006 and between 2006 and 2007. The results were confirmed by a study conducted by Kalembasa and Symanowicz [19].

Table 2

The content of total iron in the soil [mg · kg^{-1}]

Cuts (A)	Research years (B)			Mean
	2005	2006	2007	
I	4581.8	3732.1	4449.3	4254.4
II	4328.7	3670.1	4292.7	4097.2
III	3947.0	3479.1	4106.8	3844.3
Mean	4285.8	3627.1	4282.9	4065.3

$LSD_{0.05}$ for: cuts (A) – 31.8; research years (B) – 31.8; interaction (AxB) – 55.0.

The correlation coefficients indicate a significant positive relationship (r = 0.96*) between iron and molybdenum content in soil. A significant negative correlation was also shown to exist between iron content in soil and: cobalt content in soil (r = –0.99*); iron content in the plant (r = –0.95*); molybdenum content in the plant (r = –0.98*); cobalt content in the plant (r = –0.97*).

The average molybdenum content, determined at the humus horizon, was equal to 0.28 mg · kg^{-1} and was regarded as low (Table 3). The content was significantly differentiated by the analysed factors and their combinations. A statistical analysis showed that the largest amounts of the element were determined before harvesting the first cut of fodder galega (0.35 mg · kg^{-1}) and in the first year of the study (2005 – 0.29 mg · kg^{-1}). An analysis of variance showed a steady decrease in molybdenum content in soil in the consecutive years of the study. The correlation coefficients between the content of molybdenum in soil and the content of cobalt in soil, iron in the plant, molybdenum in the plant and cobalt in the plant all revealed a significant negative relationship.

Table 3

The content of total molybdenum in the soil [mg · kg^{-1}]

Cuts (A)	Research years (B)			Mean
	2005	2006	2007	
I	0.28	0.25	0.32	0.28
II	0.39	0.34	0.27	0.33
III	0.21	0.27	0.22	0.23
Mean	0.29	0.28	0.27	0.28

$LSD_{0.05}$ for: cuts (A) – 0.01; research years (B) – 0.01; interaction (A×B) – 0.02.

The cobalt content in soil was significantly varied for different cuts and years of the study and for combinations of the analysed factors (Table 4). The average cobalt content in soil was equal to 1.89 mg · kg^{-1}. Analyses of the soil from the humus horizon performed before re-growth of the consecutive cuts of fodder galega showed a steady increase in cobalt content in soil; the value decreased significantly in the third year of the study compared with the first year. The cobalt content in soil was significantly positively correlated with the content of iron, molybdenum and cobalt in the plant.

Table 4

The content of total cobalt in the soil [mg · kg^{-1}]

Cuts (A)	Research years (B)			Mean
	2005	2006	2007	
I	1.72	1.72	1.60	1.68
II	1.92	2.06	1.73	1.90
III	2.05	2.11	2.12	2.09
Mean	1.90	1.96	1.82	1.89

$LSD_{0.05}$ for: cuts (A) – 0.08; research years (B) – 0.08; interaction (A×B) – 0.14.

The average iron content in dry matter of fodder galega harvested during the budding phase was equal to 163.74 mg · kg^{-1} (Table 5) and it was significantly varied between the first and third cut and between the second and the third cut. A statistical analysis revealed significant differences for combinations of the analysed factors. The iron content in consecutive years of the study was similar (162.17–165.39 mg · kg^{-1} of d.m.). The results were confirmed by a study conducted by Kalembasa and Symanowicz [19], which examined changes in the content of iron in biomass of fodder galega depending on its development phase and years of cultivation. In addition, Kabata-Pendias and Pendias [2] report that iron content changes during the vegetation period and such changes are different for different plant organs. The content for legume plants may range from 75 to 400 mg · kg^{-1} of d.m. The iron content in biomass of fodder galega harvested in consecutive cuts lay within the limit values of the acceptable trace elements content in fodder [20]; according to Gorlach [21] and Jamroz et al [22], the content was optimal.

Table 5

The content of iron [mg · kg^{-1} d.m.] in the dry mass
of goat's rue (*Galega orientalis* Lam.) of fertilization ^{15}N

Cuts (A)	Research years (B)			Mean
	2005	2006	2007	
I	136.76	114.36	126.82	125.98
II	112.68	154.16	107.20	124.68
III	241.54	227.65	252.51	240.56
Mean	163.66	165.39	162.17	163.74

$LSD_{0.05}$ for: cuts (A) – 14.62; research years (B) – n.s.; interaction (A×B) – 25.3.

The average molybdenum content in the test plant was equal to 4.13 mg · kg^{-1} of d.m. (Table 6) and it was significantly varied by the analysed factors and their combinations. The highest significant molybdenum content was determined in the biomass of the test plant harvested in the third cut (4.78 mg · kg^{-1} of d.m.), as well as in 2007 (4.46 mg · kg^{-1} of d.m.). A statistical analysis found significant differences in molybdenum content between cuts of fodder galega. The results exceed the optimum and acceptable levels of molybdenum content in fodder [20–25]. It is supposed that the elevated content of molybdenum in the test plant biomass was caused by the neutral pH value of the soil. This causes the element to be more soluble and phyto-absorbable [2, 23, 26]. Such reasoning has been corroborated by the findings of a study conducted by Symanowicz and Kalembasa [27], in which molybdenum content in corn ranged from 0.89 to 0.96 mg · kg^{-1} of d.m. at a soil pH equal to 5.3. Moreover, Wysokinski et al [28] conducted some research on soil at pH 4.0 and determined the molybdenum content in dry matter of corn to be 0.57–0.68 mg · kg^{-1} and in that of silage sunflower to range from 0.71 to 1.12 mg · kg^{-1}.

Table 6

The content of molybdenum [mg · kg^{-1} d.m.] in the dry mass of goat's rue (*Galega orientalis* Lam.) of fertilization ^{15}N

Cuts (A)	Research years (B)			Mean
	2005	2006	2007	
I	3.78	3.47	5.56	4.27
II	4.56	4.01	4.74	4.44
III	3.71	4.22	3.09	3.67
Mean	4.02	3.90	4.46	4.13

$LSD_{0.05}$ for: cuts (A) – 0.42; research years (B) – 0.42; interaction (A×B) – 0.72.

The analysed factors significantly differentiated the total content of cobalt in biomass of fodder galega (*Galega orientalis* Lam.) (Table 7).

Table 7

The content of cobalt [mg · kg^{-1} d.m.] in the dry mass of goat's rue (*Galega orientalis* Lam.) of fertilization ^{15}N

Cuts (A)	Research years (B)			Mean
	2005	2006	2007	
I	0.21	0.19	0.10	0.17
II	0.23	0.13	0.21	0.19
III	0.15	0.48	0.49	0.37
Mean	0.20	0.27	0.27	0.24

$LSD_{0.05}$ for: cuts (A) – 0.06; research years (B) – 0.06; interaction (A×B) – 0.11.

The average cobalt content was low and equal to 0.24 mg · kg^{-1} of d.m. These findings confirm the results of earlier studies conducted in similar soil conditions by

Kalembasa and Symanowicz [29]. The highest significant content of the element was determined in dry matter of the test plant harvested in the third cut in 2006 and 2007 (0.48 and 0.49 mg · kg^{-1}) and the values lay within the limits of the optimum range [20, 21]. According to Kabata-Pendias and Pendias [23], cobalt content in dry matter of plants to be used as fodder, especially for ruminants, should not be lower than 0.08–0.1 mg · kg^{-1}, whereas Gorlach [21] determined animals' demand for trace elements and found the value of 0.3 mg Co · kg^{-1} of d.m. as a deficit. Cobalt is used by ruminants in the synthesis of vitamin B_{12}.

Fodder galega absorbed an average of 288.63 kgN · ha^{-1} by bio-reduction of N^2 from the air during a vegetation season (Table 8). The analysis of variance has shown that the amount of nitrogen from the air was significantly differentiated by the analysed factors and their combinations. The largest amounts of bio-reduced nitrogen were found in the biomass of the first cut of fodder galega harvested during the budding phase. The largest total amounts of nitrogen in three cuts were found in the first year of the study (2005) – 376.03 kgN · ha^{-1}. The results were similar to the findings of a study conducted by Symanowicz et al [30], in which the amount of nitrogen bio-reduced by fodder galega harvested during the blossoming phase was equal to 379.7 kgN · ha^{-1}. The correlation coefficient ($r = 0.99^*$) indicates a significant relationship between the amount of bioreduced nitrogen in consecutive cuts and years of the study. The calculations were based on a paper by Kalembasa and Symanowicz [31].

Table 8

Amount of nitrogen biologically reduced for *Rhizobium galegae* cultures living together by goat's rue (*Galega orientalis* Lam.) [kgN · ha^{-1}]

Cuts (A)	Research years (B)			Mean
	2005	2006	2007	
I	280.34	183.75	91.65	185.25
II	81.33	37.07	81.18	66.53
III	14.36	39.98	56.23	36.86
Sum	376.03	260.80	229.06	288.63

$LSD_{0.05}$ for: cuts (A) – 0.72; research years (B) – 0.72; interaction (A×B) – 1.25.

The coefficients of simple correlation were calculated in order to determine the effect of the content of iron, molybdenum and cobalt in soil on the amount of nitrogen bio-reduced by *Rhizobium galegae* bacteria which live in symbiosis with fodder galega. The findings of the study have shown that there is a significant relationship between the content of iron ($r = 0.91^*$), molybdenum ($r = 0.99^*$) and cobalt (-0.96^*) in soil and the amount of nitrogen bioreduced by *Rhizobium galegae* bacteria which live in symbiosis with fodder galega (Table 9). The correlation coefficients have also shown a significant negative correlation (-0.97^*) between the content of molybdenum in the test plant and the amount of nitrogen bio-reduced by *Rhizobium galegae*.

Table 9

Values of the correlation coefficients between the average content of Fe, Mo, Co in the soil and content of Fe, Mo, Co in the dry mass goat's rye and N_2 biologically reduced from air

	N_2	Fe_s	Mo_s	Co_s	Fe_p	Mo_p	Co_p
N_2	1.00						
Fe_s	0.91*	1.00					
Mo_s	0.99*	0.96*	1.00				
Co_s	–0.96*	–0.99*	–0.99*	1.00			
Fe_p	–0.72	–0.95*	–0.81*	0.88*	1.00		
Mo_p	–0.97*	–0.98*	–0.99*	0.99*	0.87	1.00	
Co_p	–0.78	–0.97*	–0.86	0.92*	0.99*	0.91*	1.00

* – significant at $\alpha = 0.05$; N_2 – N_2 biologically reduced from air; Fe_s – iron in the soil; Mo_s – molybdenum in the soil; Co_s – cobalt in the soil; Fe_p – iron in the plant; Mo_p – molybdenum in the plant; Co_p – cobalt in the plant.

Conclusions

1. The soil contained the largest amount of iron, less cobalt and the smallest amount of molybdenum.
2. The iron amount found in biomass of fodder galega lay within the optimum limits specifying the acceptable content of trace elements in fodder, whereas the molybdenum content was too high and that of cobalt was too low.
3. The average amount of nitrogen bio-reduced during the vegetation period by fodder galega (*Galega orientalis* Lam.) was equal to 288.63 kg N $\cdot$ ha^{-1}, and it decreased in consecutive cuts.
4. The simple correlation coefficients indicate a significant relationship between the amount of N_2 bioreduced by *Rhizobium galegae* and the content of Fe, Mo and Co in the soil and the plant.

References

[1] Gorlach E, Gambuś F. Potencjalnie toksyczne pierwiastki śladowe w glebach (nadmiar, szkodliwość i przeciwdziałanie). Zesz Probl Post Nauk Roln. 2000;472:275-296.
[2] Kabata-Pendias A, Pendias H. Biogeochemia pierwiastków. Warszawa: PWN; 1999.
[3] Szulc W, Rutkowska B, Łabętowicz J. Bilans mikroelementów zmianowaniu w trwałym doświadczeniu nawozowym. Zesz Probl Post Nauk Roln. 2004;502:363-369.
[4] Grzyś E. Rola i znaczenie mikroelementów w żywieniu roślin. Zesz Probl Post Nauk Roln. 2004;502:89-99.
[5] Sowiński J, Szyszkowska A. The effect of harvesting methods on the quantity and quality of fodder galega (Galega orientalis Lam) forage. Reu Technical. 2002;66:110-112.
[6] Borowiecki J. Nowe aspekty symbiotycznego wiązania azotu. Post Nauk Roln. 2004;2:9-18.
[7] Broos K, Beyens H, Smolders E. Survival of rhizobia in soil is sensitive to elevated zinc in the absence of the host plant. Soil Biol & Biochem. 2005;37:573-579.
[8] Hiechel G, Barnes DK, Vance CP, Henjum KJ. N_2 Fixation and N and dry matter partitioning during a 4-year alfaalfa stand. Crop Sci. 1984;24:811-815.
[9] Peoples MB, Herridge DF, Ladha JK. Biological nitrogen fixatin: An efficient source of nitrogen for sustainable agricultural production. Plant and Soil. 1995;174:3-28.

[10] Rennie RJ. Quatifying dinitrogen (N_2) fixation in saybeans by ^{15}N isotope dilution: the quuestion of the non-fixing control plant. Cand J Bot. 1982;60:856-861.
[11] Kalembasa S. Zastosowanie izotopów ^{15}N i ^{13}N w badaniach gleboznawczych i chemiczno-rolniczych. Warszawa: WNT; 1995.
[12] Trabelsi D, Pini F, Aouani ME, Bazzicalupo M, Mengoni A. Development of real-time PCR assay for detection and quantification of Sinorhizobium meliloti in soil and plant tissue. Lett Appl Microbiol. 2009;48:355-361.
[13] Andrzejewska J, Ignaczak S. Effectiveness of symbiosis between fodder galega (Galega orientlis Lam) and Rhizobium galegae on follow land. EJPAU, Agronomy. 2001;4(2), www.ejpau.media.pl
[14] Ignaczak S. Wartość zielonki z rutwicy wschodniej (Galega orientalis Lam) jako surowca dla różnych form paszy. Zesz Probl Post Nauk Roln. 1999;468:145-157.
[15] Sienkiewicz S, Wojnowska T, Pilejczyk D. Plonowanie rutwicy wschodniej (Galega orientalis Lam) oraz zawartość związków organicznych w zależności od zróżnicowanego nawożenia fosforowo-potasowego. Zesz Probl Post Nauk Roln. 1999;468:223-232.
[16] Symanowicz B, Appel Th, Kalembasa S. "Goat's rue" (Galega orientalis Lam) a plant with multi-directional possibilities of use for agriculture. Part III: The influence of the infection of Galega orientalis seeds on the content of trace elements. Polish J Soil Sci. 2004;XXXVII(1):11-20.
[17] Szczepaniak W. Metody instrumentalne w analizie chemicznej. Warszawa: PWN; 2005:165-168.
[18] Vanace CP. Legume symbiotic nitrogen fixation. Agronomic aspects. In: The rhizobiaceae. Spaink HP, Kondorosi A, Hooykaas PJ, editors. Dordrecht, Boston, London: Kluwer Acad Pub; 1998.
[19] Kalembasa S, Symanowicz B. Wpływ procesu biologicznej redukcji N_2 na zmiany zawartości żelaza i manganu w biomasie rutwicy wschodniej (Galega orientalis Lam) w kolejnych latach uprawy. Zesz Probl Post Nauk Roln. 2009;541:181-188.
[20] Anke M. Kolloquien des Instituts für Pflanzenernährung. Jena. 1987;2:110-111.
[21] Gorlach E. Zawartość pierwiastków śladowych w roślinach pastewnych jako miernik ich wartości. Zesz Nauk AR w Krakowie. 1991;34(262):13-22.
[22] Jamroz D, Buraczewski S, Kamiński J. Żywienie zwierząt i paszoznawstwo. Cz 1: Fizjologiczne i biochemiczne podstawy żywienia zwierząt. Warszawa: Wyd Nauk PWN; 2001.
[23] Kabata-Pendias A. Trace elements in soils and plants. 4rd ed. Boca Raton: CRC Press; 2010.
[24] Ruszkowska M, Wojcieska-Wyskupajtys U. Fizjologiczne i biochemiczne funkcje miedzi i molibdenu w roślinach. Zesz Nauk Komitetu "Człowiek i Środowisko". 1996;14:104-110.
[25] Schüfer U, Seifert M. Trace Elements Electrolytes. 2006;23:150-161.
[26] Stanisławska-Glubiak E, Sienkiewicz U. Reakcja odmian jęczmienia jarego na kwaśny odczyn gleby oraz wapnowanie i nawożenie molibdenem. Zesz Probl Post Nauk Roln. 2004;502:349-356.
[27] Symanowicz B, Kalembasa S. Zawartość Mn, B, Mo i Co w biomasie kukurydzy nawożonej odpadowymi materiałami organicznymi, popiołem i NPKMg. Zesz Probl Post Nauk Roln. 2010;547:347-357.
[28] Wysokiński A, Kalembasa S, Symanowicz B. Wpływ alkalizacji oraz kompostowania osadów ściekowych na zawartość boru i molibdenu w roślinach. Zesz Probl Post Nauk Roln. 2008;526:487-495.
[29] Kalembasa S, Symanowicz B. The changes of molybdenum and cobalt contents in biomass of Goat's rue (Galega orientalis Lam). Fresenius Environ Bull. 2009;18(6):1150-1153.
[30] Symanowicz B, Pala J, Kalembasa S. Wpływ procesu biologicznej redukji N_2 na pobranie azotu przez rutwicę wschodnią (Galega orientalis Lam). Acta Sci Polon Agricult. 2005;4(2):93-99.
[31] Kalembasa S, Symanowicz B. Quantitative ablities of biological nitrogen reduction for Rhizobium galegae cultures by goat's rue. Ecol Chem Eng A. 2010;17(7):757-764.

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Abstrakt: Żelazo i molibden to mikroelementy, które mają decydujący wpływ na ilość azotu biologicznie związanego przez rośliny bobowate. Są one niezbędne do budowy nitrogenazy i innych enzymów. Również kobalt pośrednio uczestniczy w procesie biologicznej redukcji N_2. W celu określenia wpływu Fe, Mo i Co na ilość azotu związanego przez rutwicę wschodnią (*Galega orientalis* Lam.) przeprowadzono doświadczenie polowe w latach 2005–2007 na terenie należącym do stacji doświadczalnej UP-H w Siedlcach. Azot ^{15}N o wzbogaceniu 10,3 at % stosowano w formie $(^{15}NH_4)_2SO_4$ w ilości 1,66 g na 1 m^2 wczesną wiosną. Równolegle z uprawą rutwicy wschodniej (*Galega orientalis* Lam.) uprawiano roślinę nie mającą zdolności biologicznej redukcji N_2 (jęczmień jary – *Hordeum sativum*), którą również nawożono ^{15}N w formie $(^{15}NH_4)_2SO_4$ o wzbogaceniu 10,3 at %. Podczas zbioru rośliny testowej w fazie pąkowania (trzy pokosy) pobierano próbki, które następnie wysuszono i rozdrobniono. Przed każdym pokosem pobierano także próbki gleby. Zawartość Fe, Mo i Co w glebie i roślinie oznaczono metodą ICP-AES po mineralizacji „na sucho". Obliczenia statystyczne wykazały istotne zróżnicowanie w zawartości żelaza, molibdenu i kobaltu w glebie i roślinie. W glebie najwięcej oznaczono żelaza, mniej kobaltu, a najmniej molibdenu. Oznaczona zawartość żelaza w biomasie rutwicy wschodniej mieściła się w optymalnym zakresie liczb granicznych określających dopuszczalne ilości pierwiastków śladowych w paszy, natomiast molibden występował w nadmiarze, a kobalt w niedoborze. Obliczone współczynniki korelacji wskazują na istotną zależność między zawartością żelaza, molibdenu i kobaltu w glebie a ilością biologicznie zredukowanego azotu przez bakterie *Rhizobium galegae* żyjące w symbiozie z rutwicą wschodnią.

Słowa kluczowe: rutwica wschodnia, azot, żelazo, molibden, kobalt, gleba, roślina