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CHANGES IN THE CONTENT OF ZINC AND COBALT IN PLANTS AND SOIL, ABSORPTION OF THESE ELEMENTS BY GOAT'S RUE (*GALEGA ORIENTALIS* LAM.) BIOMASS AND BIOACCUMULATION FACTORS INDUCED BY PHOSPHORUS AND POTASSIUM FERTILIZATION

Barbara Symanowicz, Stanisław Kalembasa, Elżbieta Malinowska, Andrzej Wysokiński

Soil Science and Plant Nutrition Department
Siedlce University of Natural Sciences and Humanities

Abstract

This paper discusses changes in the content of zinc and cobalt in plants and soil under the influence of fertilization with phosphorus and potassium. The absorption of Zn and Co with biomass yield and the factors of their bioaccumulation were calculated. In 2005-2007, goat's rue (*Galega orientalis* Lam.), also known as eastern galega, was cultivated on an experimental field owned by the University of Natural Sciences and Humanities in Siedlce. The study included six fertilized objects (control, P_{50}, $K_{100,}$ $P_{50}K_{150}$, $P_{50}K_{200}$, $P_{50}K_{250}$). In each experimental year, three cuts of the test plant were harvested during the budding phase. The total content of zinc and cobalt in the plants and soil was determined with the ICP-AES method on a plasma-induced emission spectrophotometer. The fertilization with phosphorus and potassium significantly increased the Zn content in goat's rue biomass. The highest concentrations of zinc and cobalt were detected in goat's rue fertilized with the K_{100} dose. The successively higher doses of K with P decreased the content of the two elements in goat's rue biomass. The highest absorption of zinc and cobalt by goat's rue during the vegetation season was induced by the doses of $P_{50}K_{200}$ and $P_{50}K_{150}$, respectively. The highest concentrations of zinc and cobalt were detected in the soil fertilized with $P_{50}K_{250}$ and $P_{50}K_{150}$, respectively. The Zn and Co bioaccumulation factors were on an optimal level.

Keywords: PK fertilization, goat's rue, zinc, cobalt, absorption, bioaccumulation factors.

dr hab. inż. Barbara Symanowicz, prof. nzw. UPH, Soil Science and Plant Nutrition Department, Siedlce University of Natural Sciences and Humanities, Prusa 14, 08-110 Siedlce, Poland, e-mail: bsymanowicz@uph.edu.pl

ZMIANY W ZAWARTOŚCI CYNKU I KOBALTU W ROŚLINIE I GLEBIE, POBRANIU PRZEZ BIOMASĘ RUTWICY WSCHODNIEJ (*GALEGA ORIENTALIS* LAM.) I WSPÓŁCZYNNIKACH BIOAKUMULACJI POD WPŁYWEM NAWOŻENIA FOSFOROWO-POTASOWEGO

Abstrakt

W pracy przedstawiono zmiany w zawartości cynku i kobaltu roślinie i glebie pod wpływem nawożenia fosforowo-potasowego. Obliczono pobranie Zn i Co z plonem biomasy rośliny testowej i współczynniki ich bioakumulacji. Rutwicę wschodnią (*Galega orientalis* Lam.) uprawiano w latach 2005–2007. Doświadczenie polowe prowadzono na polach doświadczalnych Uniwersytetu Przyrodniczo-Humanistycznego w Siedlcach. W badaniach uwzględniono sześć obiektów nawozowych (obiekt kontrolny, P_{50}, K_{100}, $P_{50}K_{150}$, $P_{50}K_{200}$, $P_{50}K_{250}$). W każdym roku badań zbierano trzy pokosy rośliny testowej w fazie pąkowania. Zawartość całkowitą cynku i kobaltu w roślinie i glebie oznaczono metodą ICP-AES na spektrofotometrze emisyjnym z plazmą wzbudzaną indukcyjnie. Nawożenie fosforowo-potasowe wpłynęło istotnie na zwiększenie zawartości cynku w biomasie rutwicy wschodniej. Najwięcej cynku i kobaltu oznaczono w roślinie testowej nawożonej dawką K_{100}. Kolejne dawki nawożenia PK powodowały zmniejszanie zawartości analizowanych pierwiastków w biomasie rutwicy wschodniej. Największe pobranie cynku przez rutwicę wschodnią w ciągu okresu wegetacyjnego uzyskano pod wpływem nawożenia $P_{50}K_{200}$, a kobaltu – $P_{50}K_{150}$. Najwięcej cynku oznaczono w glebie nawożonej dawką $P_{50}K_{250}$, a kobaltu – $P_{50}K_{150}$. Współczynniki bioakumulacji Zn i Co kształtowały się na optymalnym poziomie.

Słowa kluczowe: nawożenie PK, rutwica wschodnia, cynk, kobalt, pobranie, współczynniki bioakumulacji.

INTRODUCTION

Goat's rue (*Galega orientalis* Lam.) is a perennial fabaceae, rich in essential elements and microelements (Raig et al. 2001 Symanowicz et al. 2004, Kalembasa, Symanowicz 2009). It can be used as a fodder ingredient in such feeds as green forage, straw, dry forage, silage and protein concentrate. After perennial cultivation, it may serve as a good preceding crop for winter wheat (Ignaczak, Szczepanek 2005). In studies on ^{15}N isotope carried out in Podlasie, a significant potential for the biological reduction of N_2 by goat's rue was detected during the vegetation season (Kalembasa, Symanowicz 2010), which occurred after the introduction of *Rhizobium galegae* bacteria into soil (Reichel et al. 1984, Peoples et al. 1995, Vanace 1998). The following factors are decisive for the yield and chemical composition of goat's rue biomass: soil, weather conditions, fertilization, growth stage, year of cultivation and strong resistance to fungal and viral diseases (Virkajärvi, Varis 1991, Valkonen 1993).

Zinc and cobalt are trace elements which must be monitored in fodder plants because their excess negatively influences the health of animals as well as the growth and development of plants (Spiak 2000). The literature provides evidence that the presence of cobalt in soil and plants is associated with the biological reduction of N_2 (Ruszkowska et al. 1996). However, there are no data on the qualitative and quantitative changes in the content of these elements in goat's rue biomass and in soil under the influence of different mineral fertilization regimes.

The objective of this study was to determine the impact of fertilization with phosphorus and potassium on changes in zinc and cobalt content in goat's rue (*Galega orientalis* Lam.) biomass and in soil. Another aim was to analyse the absorption of these elements by the biomass of the tested plant and to calculate the factors of Zn and Co bioaccumulation.

MATERIAL AND METHODS

The experiment was carried out in 2005-2007 on a plantation established in 1997 on an experimental field owned by the University of Natural Sciences and Humanities in Siedlce (52°17′N, 22°28′E). The soil on which eastern galega was cultivated was loamy sand (LS) and had neutral reaction. The abundance in absorbable phosphorus and potassium (determined with the Egner-Riehm's method) was medium, whereas the abundance in absorbable magnesium (measured with the Schachtschabel's method) was low. The study included the following fertilization treatments: control, P_{50}, K_{100}, $P_{50}K_{150}$, $P_{50}K_{200}$, $P_{50}K_{250}$. Phosphorus fertilizers as triple superphosphate were applied in autumn while potassium fertilizer in the form of 60% potassium salt was split into two doses (applied in early spring and after the first cut).

The average monthly temperature in the consecutive vegetation seasons was comparable (15.0°C to 15.8°C) and significantly higher compared to the multi-annual records. The average precipitation during the vegetation seasons was lower than the multi-annual sum, except for 2006, when it was slightly higher (by 15.5 mm) because of heavy rainfalls in August, which were three-fold higher than the multi-annual sum (Symanowicz, Kalembasa 2010).

Three cuts of goat's rue at budding were harvested in each year of the study. During the harvesting of subsequent cuts of goat's rue (*Galega orientalis* Lam.), samples of whole plants were collected, dried and ground. Each year in autumn, soil samples were taken, dried and sieved through a 1mm mesh. Analytical solutions were prepared by dry mineralization of the tested plant's biomass and soil in a muffle furnace with a progressive increase in the temperature up to 450°C. Following complete oxygenation of organic compounds in the tested samples, 5 cm^3 HCl (1:1) was poured onto the ash left in a pot in order to degrade carbohydrates, extract silica and produce anions of inorganic acids and chlorides of tested cations. The excess of HCl was evaporated on a sand bath until dry. The content of the pot was again mixed with HCl (10%) and this solution was filtered into a 100 cm^3 measuring flask by a solid filter in order to separate silica. The content left on the filter was washed three times with diluted HCl and the content in the flask was filled up with water to the line representing analytical solution. The total content of Zn and Co in the tested plant and soil was determined with the ICP-AES method on a plasma-induced emission spectrophotometer (SZCZEPANIAK 2005). The absorption of zinc and cobalt by the biomass of eastern galega and the factors of bioaccumulation of the analysed elements were determined.

The results were statistically processed with a three-way analysis of variance (ANOVA) and significant differences were calculated with the Tukey's test at α = 0.05. The Pearson's simple correlation r coefficients were calculated (Statistica 9.1; StatSoft) in order to identify correlations between the content of Zn and Co in the tested plant and soil and the absorption of these elements with the biomass yield. The significance of correlations was determined by comparing empirical values with critical values *r* at α =0.05.

RESULTS AND DISCUSSION

The average content of zinc in the DM of goat's rue harvested at budding was 53.89 mg kg^{-1} (Table 1), being significantly differentiated by the tested factors and their interrelations. The lowest content of zinc was detected in the biomass of eastern galega harvested in the first cut and in the second year of the experiment. The fertilization with phosphorus and potassium did not have any impact on the content of zinc in DM of goat's rue. The successively higher doses of potassium (K_{100}, $P_{50}K_{150}$) resulted in an increase in the Zn content in the biomass of goat's rue. The highest content of zinc was detected in the tested plant fertilized with 100 kg K ha^{-1}. These

Table 1

The content of zinc in biomass of goat's rue (mg kg^{-1} d.m.)

Fertilization	Cuts – means of 3 years			Years – means of 3 cuts			Mean 2005-2007
	I	II	III	2005	2006	2007	
0	43.10	57.88	48.93	69.57	37.75	42.59	49.97
P_{50}	52.09	60.36	56.77	77.33	43.72	48.11	56.39
K_{100}	52.50	62.88	53.89	71.75	45.82	51.70	56.42
$P_{50}K_{150}$	53.31	56.24	55.51	71.15	43.61	50.30	55.02
$P_{50}K_{200}$	55.36	57.89	48.77	77.15	38.42	46.46	54.01
$P_{50}K_{250}$	51.11	57.14	46.33	58.28	43.86	52.44	51.53
Mean	51.24	58.73	51.70	70.87	42.20	48.60	53.89

$LSD_{0.05}$: cuts (C) – 1.33; years (Y) – 1.33; fertilization (F) – 2.29; YxC – 2.30; CxY – 2.30; FxC – 2.80; CxF – 2.30; FxY – 3.97; YxF – 3.25

Table 2

The content of cobalt in biomass of goat's rue (mg kg^{-1} d.m.)

Fertilization	Cuts – means of 3 years			Years – means of 3 cuts			Mean 2005-2007
	I	II	III	2005	2006	2007	
0	0.22	0.22	0.20	0.21	0.22	0.20	0.21
P_{50}	0.24	0.25	0.26	0.26	0.25	0.23	0.25
K_{100}	0.27	0.26	0.23	0.31	0.25	0.21	0.26
$P_{50}K_{150}$	0.28	0.25	0.25	0.27	0.27	0.24	0.26
$P_{50}K_{200}$	0.23	0.21	0.20	0.17	0.26	0.21	0.21
$P_{50}K_{250}$	0.26	0.25	0.20	0.23	0.23	0.24	0.23
Mean	0.25	0.24	0.22	0.24	0.24	0.22	0.24
$LSD_{0.05}$: cuts (C) – 0.01; years (Y) – 0.01; fertilization (F) – 0.01; YxC – 0.01; CxY – 0.01; FxC – 0.02; CxF – 0.01; FxY – 0.02; YxF – 0.02							

results were consistent with the studies carried out by Symanowicz et al. (2004), where changes in the content of heavy metals in goat's rue biomass were tested depending on the type of infection with different microbial strains (*Nostoc* cyanobacteria, *Rhizobium* galegae). The content of zinc in goat's rue biomass harvested in consecutive cuts in the experimental years from the objects fertilized with PK was within the permissible limits set for the content of trace elements in feedstuffs. According to Gorlach (1991), this was an optimal content.

The content of cobalt in the tested plant averaged 0.24 mg kg^{-1} d.m. (Table 2) and was significantly differentiated by the analysed factors and their interrelations. The highest concentration of cobalt was detected in the biomass of the tested plant harvested in the first cut (0.25 mg kg^{-1} d.m.) and also in 2005 and 2006 (0.24 mg kg^{-1} d.m.). The highest content of Co was recorded in goat's rue fertilized with K_{100} and $P_{50}K_{150.}$ These results are within the low range of cobalt concentrations in feedstuffs (Gorlach 1991, Ruszkowska 1996). Presumably, the soil reaction close to neutral explains the lower content of cobalt in the biomass of the tested plant. This conclusion is further supported by the results reported by Kalembasa, Symanowicz (2009) in another study on goat's rue, in which the content of cobalt ranged from 0.24 to 0.32 mg kg^{-1} d.m. with the pH of soil at 6.6.

The average absorption of zinc and cobalt with the yield of goat's rue (*Galega orientalis* Lam.) in 2005-2007 was 454.8 g Zn ha^{-1} and 1.95 g Co ha^{-1} (Tables 3 and 4). The plant absorbed most of the elements in yield after the first cut harvested at budding. The highest total amount of zinc and cobalt in three cuts was detected in the first year (651.3 g Zn ha^{-1} and 2.20 g Co ha^{-1}). Regarding the PK fertilization, the highest amounts of zinc and cobalt were accumulated by goat's rue fertilized with the dose of $P_{50}K_{200}$ and $P_{50}K_{150}$, respectively. These calculations were performed according to the publication by Symanowicz, Kalembasa (2010). The calculated correlation

Table 3

Uptake of zinc in the yield of goat's rue (g Zn ha^{-1})

Fertilization	Cuts – means of 3 years			Years – means of 3 cuts			Mean 2005-2007
	I	II	III	2005	2006	2007	
0	150.8	120.4	58.2	518.3	283.9	227.4	343.2
P_{50}	203.1	176.8	95.4	796.5	369.9	321.4	495.9
K_{100}	195.8	139.0	100.2	625.7	355.6	358.3	446.5
$P_{50}K_{150}$	218.0	176.0	121.6	761.3	396.0	422.0	526.4
$P_{50}K_{200}$	298.9	167.9	115.1	918.1	407.2	441.4	588.9
$P_{50}K_{250}$	174.8	112.0	69.5	353.7	313.2	388.0	351.6
Mean	205.5	148.6	93.1	651.3	354.9	358.2	454.8

Table 4

Uptake of cobalt in the yield of goat's rue (g Co ha^{-1})

Fertilization	Cuts – means of 3 years			Years – means of 3 cuts			Mean 2005-2007
	I	II	III	2005	2006	2007	
0	0.77	0.46	0.24	1.56	1.65	1.07	1.43
P_{50}	0.94	0.73	0.44	2.68	2.11	1.54	2.11
K_{100}	1.01	0.57	0.43	2.70	1.94	1.45	2.03
$P_{50}K_{150}$	1.14	0.78	0.55	2.89	2.45	2.01	2.45
$P_{50}K_{200}$	1.24	0.61	0.47	2.02	2.76	1.99	2.26
$P_{50}K_{250}$	0.89	0.49	0.30	1.40	1.64	1.78	1.61
Mean	1.00	0.61	0.40	2.20	2.02	1.62	1.95

coefficients demonstrated significant correlation (r = 0.92) between the absorption of zinc in goat's rue biomass fertilized with PK and the amount of absorbed cobalt. The content of absorbable forms of compounds in soil substantially affects their absorption by plants (Sienkiewicz et al. 2011).

The average total content of zinc in the humus horizon was 164.1 mg kg^{-1} (Table 5). Statistical calculations demonstrated significant differentiation of the Zn content in the soil. In the third year, the content of zinc in the soil decreased significantly in relation to the first year. In 2007, the concentration of zinc dropped by 12% compared to 2005. The zinc content in the soil decreased steadily in the consecutive years of the study, which was due to the removal of this element together with yields of the test plant. Having analysed the impact of increasing PK doses on the content of Zn in the soil, it was concluded that the concentration of zinc in the soil increased significantly although not steadily versus the control object. The highest content (174.8 mg kg^{-1}) was detected in the soil sampled from the object fertilized with $P_{50}K_{250}$. It is assumed that phosphorus and potassium fertilizers as well as some anthropogenic factors affecting the experimental field could

Table 5

The content of zinc in soil (mg kg^{-1} d.m.)

Fertilization	Years			Mean 2005-2007
	2005	2006	2007	
0	141.4	161.9	143.1	148.8
P_{50}	172.3	169.2	143.2	161.6
K_{100}	173.2	176.1	145.1	164.8
$P_{50}K_{150}$	175.0	175.6	158.7	169.7
$P_{50}K_{200}$	174.8	173.8	146.0	164.8
$P_{50}K_{250}$	181.7	174.4	114.0	174.8
Mean	169.7	171.8	150.7	164.1
$LSD_{0.05}$: years (Y) – 3.2; fertilization (F) – 5.6; FxY – 9.8; YxF – 7.9				

Table 6

The content of cobalt in soil (mg kg^{-1} d.m.)

Fertilization	Years			Mean 2005-2007
	2005	2006	2007	
0	1.27	1.44	1.52	1.41
P_{50}	1.45	2.04	1.55	1.68
K_{100}	1.40	2.04	1.60	1.68
$P_{50}K_{150}$	1.60	2.03	1.77	1.80
$P_{50}K_{200}$	1.49	2.10	1.44	1.68
$P_{50}K_{250}$	1.65	1.93	1.80	1.80
Mean	1.48	1.93	1.61	1.67
$LSD_{0.05}$: years (Y) – 0.10; fertilization (F) – 0.17.; FxY – 0.30.; YxF – 0.24				

have been sources of zinc. In pot experiments carried out by SYMANOWICZ and KALEMBASA (2012) and ŻARCZYŃSKI et al. (2011), the content of zinc was several times lower.

The average content of cobalt in the soil was low: 1.67 mg kg^{-1} (Table 6). The statistical analysis demonstrated the highest amount of this element in the soil sampled after the second year of the study (1.93 mg kg^{-1}). The subsequent doses of phosphorus and potassium fertilizers resulted in an increase in the cobalt concentration, which reached the highest value (1.80 mg kg^{-1}) under the fertilization treatment of $P_{50}K_{150}$. The experiment revealed a significant (r = 0.97) correlation between the content of zinc and cobalt in the soil.

Figures 1 and 2 show factors of Zn and Co bioaccumulation. The mean value of the zinc bioaccumulation factor was 0.33. The lowest value of this factor (0.22) was calculated for the object fertilized with $P_{50}K_{200}$ in the second

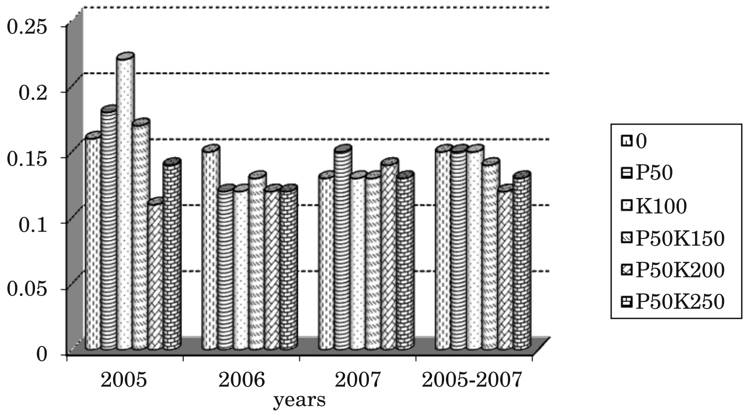

Fig. 1. Bioaccumulation factors of zinc

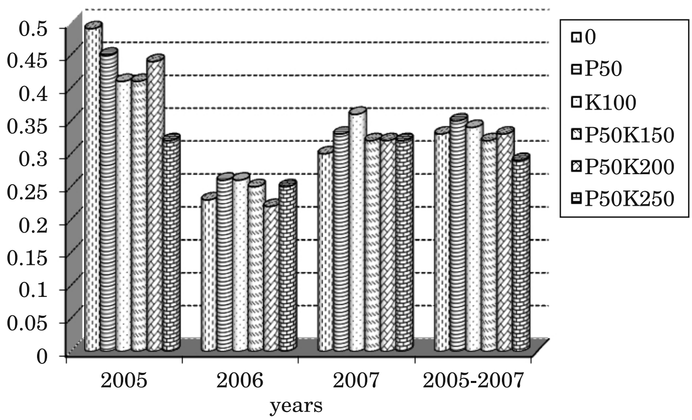

Fig. 2. Bioaccumulation factors of cobalt

year of the experiment. In the following years, the values of the Zn bioaccumulation factor varied (0.42-0.24-0.32). In a study by BARAN and JASIEWICZ (2009), the zinc bioaccumulation factor was approximately ten-fold higher, which most probably was connected with the acid reaction of soil and other test plants.

The calculated factors of cobalt bioaccumulation were on a comparable level of 0.12-0.14 for individual objects and within the range of 0.12-0.16 for the experimental years.

CONCLUSIONS

1. The incremental doses of phosphorus and potassium fertilizers had a significant impact on the zinc content in goat's rue biomass, increasing it versus the control object. The highest content of zinc was detected in the tested plant fertilized with K_{100}. Goat's rue accumulated the highest amounts of cobalt under the fertilization treatments of K_{100} and $P_{50}K_{150}$.

2. The recorded concentrations of zinc and cobalt in goat's rue biomass were within the optimal values in respect of the permissible limits of trace elements in feedstuffs.

3. The highest absorption of zinc and cobalt by goat's rue during the vegetation season was recorded for the fertilization treatments of $P_{50}K_{200}$ and $P_{50}K_{150}$, respectively.

4. The highest content of zinc was detected in the soil fertilized with $P_{50}K_{250}$, and the highest concentration of cobalt was measured in the soil fertilized with $P_{50}K_{150}$ and $P_{50}K_{250}$.

5. The highest values of factors of Zn and Co bioaccumulation were on an optimal level.

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