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POSSIBILITIES OF REDUCING THE PHYTOTOXIC EFFECT OF NICKEL*

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A b s t r a c t. A pot experiment was conducted in the years 2009-2010. Its aim was to determine the limit of toxicity of nickel to orchard grass (*Dactylis glomerata* L.) and methods of its reduction by liming and by the addition of straw or brown coal to soil. The addition of nickel to soil, regardless of its dose, increased its content in the test plant and in soil, especially in fraction F_1. The content of nickel in biomass of orchard grass exceeded the limit values in pots where it was added to soil. Soil liming reduced the content of nickel in orchard grass and in fractions isolated from soil – soluble fraction F_1, reducible fraction F_2 and oxidisable fraction F_3. The addition of rye straw and brown coal to the soil reduced the content of nickel in the test grass and the content in bioavailable fraction F_1. Liming and the addition of rye straw and brown coal to soil reduced the phytoavailability of nickel.

K e y w o r d s: nickel, liming, straw, brown coal, fractions of Ni in soil

INTRODUCTION

The amount of substances which are present in the natural environment and cause its degradation increases. This is a consequence of such factors as agricultural activities aimed at increasing crop yields and using large doses of mineral fertilisers and pesticides (Gorlach and Gambuś 2000, Węglarzyk 2014, Kacálková *et al*. 2014). A particular threat is posed by increasing amounts of heavy metals which are not biodegraded and which can remain in ecosystems for many years (Kabata-Pendias 2011). Many heavy metals play important functions in plant metabolism (Zn, Cu, Mo, Ni, Fe). For life processes to run properly, their amounts in the cells of organisms must be kept at safe levels. Increased amounts of heavy metals can be toxic. The physiological boundary between the states of deficiency and toxicity is thin (Olko 2009). It is important for plant nutrition and, consequently, for food safety, to determine the content of the metals in those compound-fractions of soil

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which can be directly absorbed by plants (bioabsorbable, bioavailable) (Jaremko and Kalembasa 2011, Kuziemska and Kalembasa 2013, Hülya *et al*. 2013). The amounts of metals in the different fractions do not depend only on their total content, but also on the environmental conditions – natural amounts in the bedrock, particle size distribution, pH of the soil solution, organic matter content, redox potential, system of cultivation (Weng *et al*. 2004, Campel *et al*. 2006, Nieminen *et al*. 2007). Bioavailability of heavy metals for plants can be reduced by liming or by introducing organic matter into the soil (Wyszkowski *et al.* 2016).

Nickel is one of those metals whose content in the natural environment is increasing steadily. It is a micronutrient necessary for plant growth and development (Eisler *et al*. 2000, Kuziemska 2009, Molas 2000, Koszelnik and Bielecki 2013), but in larger amounts it can be toxic to organisms, including plants and microorganisms in soil (Kabata-Pendias and Pendias 1999).

The metal non-specifically activates some of the enzymes of the citric acid cycle and it stimulates many stages of nitrate metabolism (Dickson *at al*. 1975). It takes part in nitrogen transport from roots to the aboveground plant parts and it affects the process of reduction of molecular nitrogen from the air (Spiak 1995, Jasiewicz *et al*. 2010) It binds with porphyrin compounds and chlorophyll, in which it replaces iron and magnesium (Zasadowski and Spodniewska 1995). One of the methods to reduce the amount of nickel in the soil is phytoremediation (Antonkiewicz *et al*. 2016, Korzeniowska and Stanislawska-Glubiak 2018).

The aim of the study was to determine the limit of toxicity of nickel to orchard grass and methods of its reduction by liming or the addition of straw or brown coal to soil. In order to determine the content of nickel in the bioavailable fraction, it was isolated from soil by the BCR procedure.

MATERIALS AND METHODS

A two-year pot experiment was conducted in the years 2009-2010. The following factors were taken into account in the design of the experiment conducted in 4 replicates: I – liming (calcium as $CaCO_3$): 0 Ca (pots with no liming), pots with Ca dose according to 1 Hh (hydrolytic acidity) (20g of $CaCO_3$ was added to the pot); II – addition of organic waste: control (0) – no such materials; pots with an addition of rye straw (at the dose of 4 t ha^{-1}, that is 1.33 g kg^{-1} of soil); pots with an addition of brown coal (at the dose of 40 t ha^{-1}, that is 13.3 g kg^{-1} of soil); III – the addition of nickel to soil (as aqueous solution of $NiSO_4$ $7H_2O$): control (0) – no nickel, pots with the addition of 75, 150 and 225 mg Ni kg^{-1} of soil.

Pots (with the capacity of 10 dm^3) were filled with 15 kg of soil (from the humus horizon, Albic Luvisol – Systematics of Soils of Poland 2011) with the particle size distribution of loamy sand, with acidic pH (pH_{KCl} 5,5), containing: N 0.98 g kg^{-1},

C_{org} 7.9 g kg^{-1}, available phosphorus 69 mg kg^{-1}, available potassium 75 mg kg^{-1}, nickel 5.67 mg kg^{-1}. Liming, the addition of organic material (rye straw cut into chaff and brown coal dust from the Turów Brown Coal Mine) and the addition of nickel were applied in 2008. The rye straw and brown coal contained 3.84 and 5.10 mg Ni kg^{-1} DM, respectively. A detailed experimental scheme and chemical composition of used organic materials was given in previous studies (Kalembasa *et al*. 2014, Kuziemska *et al*. 2014). The content of nickel was determined in orchard grass biomass (in its aboveground parts) of every crop in the first and second year of the experiment, by the ICP-AES method.

After the cultivation was completed in each year of the experiment, the total nickel content was determined in the soil by the ICP – OES method, as well as its content in the fractions isolated by the 3-step method of sequential fractionation, as proposed by the Community Bureau of Reference (BCR) (Raulet *et al.* 1999) (Tab.1). The experiment results were processed statistically by analysis of variance using the Fisher-Snedecor F distribution, in accordance with the F.R.Anal.var 4.4 program. $LSD_{(0,05)}$ was calculated by Tukey's test. The analysis of variance was not performed for the content of nickel in the grass biomass in either of the experiment years due to an excessively large number of pots where no crop was harvested (the toxic effect of a large amount of nickel in soil).

Table 1. Scheme of sequential fractionation method proposed by Community Bureau o Reference (BCR) (Rauret *et al*. 1999)

No.	Fraction names	Exctraction reagents	pH
F_1	Exchangeable and acid soluble	0.1 M CH_3COOH	3.0
F_2	Reducible	0.5 M $NH_2OH \cdot HCl$	1.5
F_3	Oxidisable	8.8 M H_2O_2 + 1 M CH_3COONH_4	2.0
F_4	Residual	Calculated as difference between total content and sum of three previously separated fractions	–

RESULTS AND DISCUSSION

The mechanism of plant tolerance to the presence of heavy metals in the environment has not been elucidated sufficiently, which is why researchers have been studying the varied resistance of plants to their excessive concentration in soil and plant tissues (Kuziemska 2009). The content of nickel in Polish grasses ranges from 0.01 to 19 mg kg^{-1}, with the average content of 0.84 mg kg^{-1} DM (Kabata-Pendias and Pendias 1999). The content of nickel in the orchard grass biomass determined in this experiment was as follows: in the first year 5.61-519.7 mg kg^{-1} DM and in the second year it was 9.37-339.2 mg kg^{-1} DM. It depended on the liming applied, the addition of organic waste and the content of nickel in soil, and on the year of cultivation (Table 2 and 3). The results of the first year of the experiment (Table 2) are difficult to interpret because of the large number of pots in which nickel content proved toxic and no crop was harvested. The chemical analyses of the plant material showed that liming as well as the addition of straw and brown coal to soil reduced nickel content in orchard grass biomass. A tendency of nickel content in plants to decrease as a result of liming was observed by Domańska (Domańska 2009). A decrease in the bioavailability of nickel and other heavy metals following the application of organic materials was observed by Gibczyńska and Stankowski (Gibczyńska and Stankowski 2011). It was found in this experiment that the effect of straw and brown coal on nickel content in the grass under study was particularly noticeable in the pots where liming was applied. The content of nickel in biomass of the test grass increased with its total content in soil and content in the soluble and exchangeable fraction F1 (Table 2). An increase in the concentration of nickel was significant after its lowest dose (75 mg Ni kg^{-1} of soil) was applied, and its highest concentration was found in the plants grown in pots with the highest dose of nickel (225 mg kg^{-1} soil).

Table 2. Nickel content (mg kg^{-1} d.m.) in cocksfoot grass biomass in 1st year of the pot experiment

Fertilisation treatment	Cuts	Treatment without liming				Treatment with liming			
		Doses of nickel (mg kg^{-1} soil)							
		0	75	150	225	0	75	150	225
Without organic fertilisation	I	10.72	–	–	–	9.30	8.80	–	–
	II	12.27	–	–	–	11.07	95.72	107.3	–
	III	10.34	–	–	–	9.54	78.30	115.5	–
	IV	10.10	–	–	–	8.18	85.06	119.0	–
mean		0.86	–	–	–	9.52	79.47	–	–
Rye straw	I	11.64	–	–	–	14.13	61.65	170.1	–
	II	19.26	173.6	–	–	13.59	120.6	140.8	203.5
	III	6.99	121.9	–	–	6.64	27.31	84.81	129.2
	IV	10.02	113.7	–	–	8.48	55.25	95.10	121.4
mean		11.98	–	–	–	10.71	66.20	122.7	–
Lignite	I	16.41	187.0	–	–	11.91	88.60	341.5	519.7
	II	13.34	206.6	352.5	–	10.51	56.27	228.0	358.5
	III	5.61	83.46	191.4	–	3.88	31.93	173.0	214.0
	IV	11.60	118.5	206.1	–	6.90	41.19	100.8	149.4
mean		11.74	148.7	–	–	8.30	54.49	225.8	310.4
Mean for treatments		11.52	–	–	–	9.51	66.72	–	–

„–” no yield obtained

A higher content of nickel in the second year of the experiment was found in biomass of the grass harvested in the pots without lime added than with lime added (Table 3). The addition of straw and brown coal reduced the content of nickel in orchard grass. Its lowest content was found in the test grass harvested in the pots where brown coal was added. The addition of growing amounts of nickel to the soil significantly increased its content in plants. Moreover, even in pots with the smallest dose of nickel, its content in the grass was higher than in the control pots. The largest amounts of nickel were found in plants in the pots with its largest dose added (225 mg kg^{-1} of soil). Grass grown in pots where the soil was not limed and where

75 mg Ni kg^{-1} soil was added contained an average of 4 times as much nickel, and with the dose of 150 mg Ni kg^{-1} of soil, over 10 times as much nickel as the grass grown in control pots. The amount of nickel in plants in pots with limed soil and an addition of nickel of 75 mg Ni kg^{-1} was 4 times higher, and with the dose of nickel of 150 mg Ni kg^{-1} – 7.5 times higher, and with the dose of nickel of 225 mg Ni kg^{-1} – 10.3 times higher, compared to the plants grown in pots where no nickel was added.

Table 3. Nickel content (mg kg^{-1} d.m.) in cocksfoot grass biomass in 2nd year of the pot experiment

Fertilisation treatment	Cuts	Treatment without liming				Treatment with liming			
		Doses of nickel (mg kg^{-1} soil)							
		0	75	150	225	0	75	150	225
Without organic fertilisation	I	17.81	129.4	339.2	–	17.10	80.90	175.6	248.6
	II	20.10	105.5	263.5	–	17.10	81.50	146.6	200.0
	III	20.14	91.10	204.1	–	13.65	69.30	134.9	186.9
	IV	33.00	73.20	214.2	–	27.10	65.00	129.4	169.5
mean		22.76	99.80	255.2	–	18.74	74.17	146.6	201.2
Rye straw	I	21.03	120.5	330.1	–	17.82	109.0	140.3	204.1
	II	21.10	92.20	200.6	–	19.94	79.10	130.1	197.1
	III	23.65	82.05	161.1	–	20.30	72.72	128.8	176.8
	IV	26.50	31.30	196.2	–	24.50	55.56	120.3	159.9
mean		23.07	89.01	222.0	–	20.64	79.10	129.9	184.5
Lignite	I	11.80	111.3	254.5	–	9.37	84.45	165.5	211.7
	II	20.65	84.70	196.3	–	19.60	65.50	130.4	188.0
	III	25.54	68.92	191.5	–	17.65	65.20	152.9	173.9
	IV	28.30	70.35	187.0	–	16.82	58.92	97.15	150.5
mean		21.58	83.82	207.3	–	15.86	68.51	136.5	181.0
Mean for treatments		22.47	90.88	228.2	–	18.41	73.92	137.7	188.9

„–" no yield obtained

An analysis of the findings from two years of the experiment showed that the addition of nickel to soil had a greater effect on its content in biomass of orchard grass in the first year of the experiment. Liming and the addition of straw and brown coal proved to be a good way of reducing the toxicity of nickel, which was also confirmed in the study of Badora (Badora 2002).

The largest amount of nickel in soil after the first and second year of the experiment was found in pots where its largest amount was added (225 mg Ni kg^{-1}), and the smallest in the soil of the control pots, where no nickel was added (Tables 4 and 6).

The statistical analysis did not reveal any clear effect of liming or the application of organic waste on the total content of nickel (sum of its content in fractions) in the soil of different pots in the experiment.

The proportion of nickel of different fractions in its total content was changed by liming and the addition of straw and brown coal (Tables 5 and 7). The analyses of soil samples taken after each of the years of the experiment showed that liming reduced the amount of nickel in the soluble and exchangeable fraction F_1, in the reducible fraction F_2 and oxidisable fraction F_3, and increased the amount of nickel in the residual fraction F_4. This confirms the well-known fact that liming is one of the factors which reduces metal mobility (Smolińska and Król 2011). The addition of organic waste to soil reduced the amount of nickel in the soluble fraction F_1 and increased the amount of nickel in the oxidisable fraction F_3 bound with organic matter and sulphides.

Table 4. Nickel content (mg kg^{-1} of soil) in fractions determined by the sequential fractionation method proposed by Community Bureau o Reference (BCR) in the analysed soil (1st year)

Fertilisation treatment	Fraction	Treatment without liming				Treatment with liming			
		Doses of nickel (mg kg^{-1} soil)							
		0	75	150	225	0	75	150	225
Without organic fertilisation	F_1	0.46	60.22	112.8	163.2	0.34	48.42	84.91	131.6
	F_2	0.72	11.29	20.08	27.05	0.58	7.91	14.84	19.99
	F_3	1.18	5.43	14.40	19.59	1.05	5.73	11.17	19.04
	F_4	3.31	3.54	9.12	21.73	3.27	18.36	45.98	60.97
Sum of fractions		5.67	80.48	156.40	231.6	5.72	80.42	156.9	231.6
Rye straw	F_1	0.41	42.36	79.53	119.0	0.37	33.96	63.47	90.54
	F_2	0.78	10.09	17.34	25.87	0.60	8.22	13.89	21.14
	F_3	1.67	20.60	37.65	53.96	1.15	18.67	31.55	49.28
	F_4	2.84	7.66	21.98	32.92	3.60	18.89	47.89	70.64
Sum of fractions		5.70	80.71	156.5	231.8	5.72	80.74	156.8	231.8
Lignite	F_1	0.42	42.47	75.50	123.2	0.41	32.96	65.96	106.8
	F_2	0.77	11.10	18.30	25.20	0.59	9.74	15.81	22.91
	F_3	1.78	20.28	34.61	57.03	1.04	13.38	30.11	46.75
	F_4	2.77	7.07	28.49	26.53	3.66	24.64	44.94	55.47
Sum of fractions		5.74	80.92	156.9	232.0	5.70	80.72	156.8	231.9

$LSD_{0.05}$ for:	Fraction				
	F_1	F_2	F_3	F_4	Σ
liming	7.12	1.39	2.09	9.26	n.s.
organic fertilisation	12.50	n.s.	9.76	n.s.	n.s.
doses of nickel	17.88	2.10	11.98	15.40	1.06

Table 5. Share (%) of individual nickel fractions in the total Ni content in the analysed soil (1st year)

Fertilisation treatment	Fraction	Treatment without liming				Treatment with liming			
		Doses of nickel (mg kg^{-1} soil)							
		0	75	150	225	0	75	150	225
Without organic fertilisation	F_1	8.12	74.83	72.12	70.48	6.47	60.21	54.12	56.82
	F_2	12.70	14.03	12.84	11.68	10.14	9.84	9.46	8.63
	F_3	20.81	6.75	9.21	8.46	18.36	7.12	9.00	8.22
	F_4	58.37	4.39	5.83	9.38	65.03	22.83	27.42	26.33
Sum of fractions		100	100	100	100	100	100	100	100
Rye straw	F_1	7.19	52.48	50.82	51.36	6.47	42.06	40.48	39.06
	F_2	13.68	12.50	11.08	11.16	10.49	10.18	8.86	9.12
	F_3	29.35	25.56	24.06	23.28	20.10	23.12	20.12	21.26
	F_4	49.78	9.47	14.04	14.20	62.94	24.64	30.34	30.56
Sum of fractions		100	100	100	100	100	100	100	100
Lignite	F_1	7.32	52.48	48.12	53.12	7.19	40.83	42.06	46.04
	F_2	13.41	13.60	11.66	10.86	10.35	12.07	10.08	9.88
	F_3	31.01	25.06	22.06	24.58	18.25	16.58	19.20	20.16
	F_4	48.26	8.86	18.46	11.44	64.21	30.52	27.94	23.92
Sum of fractions		100	100	100	100	100	100	100	100

Straw and brown coal immobilised nickel in soil by incorporating it in permanent mineral-organic complexes. According to Molas (Molas 2000), such a reduction of phytoavailability of nickel consists in its incorporation in complexes with low-molecular organic compounds, formed by decomposition of straw or brown coal. No clear effect of the addition of organic waste to soil on the content of nickel in compounds of fraction F_2 (bound with iron and manganese oxides) was demonstrated. An increase in the amount of nickel added to soil was followed by a multiple increase in its content in the bioavailable soluble and exchangeable fraction F_1 and a decrease in fractions F_2, F_3 and F_4.

Table 6. Nickel content (mg. kg^{-1} of soil) in fractions determined by the BCR method in the analysed soil (2nd year)

Fertilisation treatment	Fraction	Treatment without liming				Treatment with liming			
		Doses of nickel (mg kg^{-1} soil)							
		0	75	150	225	0	75	150	225
Without organic fertilisation	F_1	0.44	56.76	107.8	155.5	0.34	37.14	60.26	139.4
	F_2	0.65	12.60	21.20	40.30	0.60	7.80	14.60	28.50
	F_3	1.25	6.46	12.70	30.50	1.21	7.10	13.55	28.30
	F_4	3.27	4.48	13.90	4.35	3.55	28.30	67.29	34.30
Sum of fractions		5.61	80.30	155.6	230.6	5.70	80.34	155.7	230.5
Rye straw	F_1	0.33	39.40	72.30	112.6	0.39	30.30	54.70	105.5
	F_2	0.72	13.50	22.35	34.6	0.40	9.27	16.00	26.40
	F_3	2.76	20.70	40.90	61.6	1.37	19.60	29.80	41.20
	F_4	1.83	7.00	20.10	21.70	3.51	21.44	55.20	57.61
Sum of fractions		5.64	80.60	155.6	230.5	5.67	80.61	155.7	230.7
Lignite	F_1	0.33	35.69	66.00	115.5	0.34	30.53	59.40	101.2
	F_2	0.76	12.80	21.20	29.30	0.42	9.91	17.40	20.60
	F_3	2.45	25.20	37.50	60.60	1.12	18.50	30.70	40.00
	F_4	2.13	6.90	31.15	25.15	3.76	21.74	48.00	68.77
Sum of fractions		5.67	80.59	155.8	230.5	5.64	80.68	155.5	230.6

$LSD_{0.05}$ for:	Fraction				
	F_1	F_2	F_3	F_4	Σ
liming	8.47	1.07	1.91	0.83	n.s.
organic fertilisation	12.55	1.58	2.83	1.23	n.s.
doses of nickel	16.00	2.02	3.61	1.44	0.36

The average percentage of total nickel in fractions isolated from the soil to which no additional nickel was added formed the following series of decreasing values: $F_4>F_3>F_2>F_1$.

Significant correlations were found to exist between the total content of nickel in soil and its content in fraction F1, in both years of the experiment, for which the correlation coefficients were r = 0.950** (after year I) and r = 0.951** (after year II).

The pot experiment and chemical analyses of the plant material and soil showed that all the factors under study, i.e. liming, organic material – straw and brown coal, as well as variable amount of nickel added to the soil, had a significant effect on nickel content in biomass of orchard grass and in fractions isolated from soil. The addition of nickel to soil increased its amount in orchard grass and in the bioavailable fraction F_1

in soil. Only in pots with no nickel added did the content of nickel in grass not deviate from the average levels for grassy plants in Poland. The content of nickel in the test grass, harvested in pots to which nickel had been added, was even several dozen times higher than the levels considered to be safe (Koszelnik-Leszek and Bielecki 2013).

Table 7. Share (%) of individual nickel fractions in total Ni content in the analysed soil (2nd year)

Fertilisation treatment	Fraction	Treatment without liming				Treatment with liming			
		Doses of nickel (mg kg^{-1} soil)							
		0	75	150	225	0	75	150	225
Without organic fertilisation	F_1	7.85	70.68	69.28	67.41	5.96	46.23	38.70	60.48
	F_2	11.59	15.69	13.62	17.47	10.53	9.71	9.38	12.36
	F_3	22.28	8.05	8.16	13.24	21.23	8.84	8.70	12.28
	F_4	58.28	5.58	8.94	1.88	62.28	35.22	43.22	14.88
Sum of fractions		100	100	100	100	100	100	100	100
Rye straw	F_1	5.85	48.88	46.45	48.84	6.88	37.59	35.13	45.73
	F_2	12.76	16.76	14.36	15.01	7.05	11.49	10.28	11.44
	F_3	48.94	25.68	26.27	26.74	24.17	24.33	19.14	17.86
	F_4	32.44	8.68	12.92	9.41	61.90	26.59	35.45	24.97
Sum of fractions		100	100	100	100	100	100	100	100
Lignite	F_1	5.82	44.28	42.36	50.09	6.03	37.84	38.19	43.89
	F_2	13.40	15.88	13.60	12.72	7.45	12.28	11.18	8.93
	F_3	43.21	31.27	24.06	26.28	19.85	22.93	19.76	17.35
	F_4	37.57	8.57	19.98	10.91	66.67	26.95	30.87	29.83
Sum of fractions		100	100	100	100	100	100	100	100

CONCLUSIONS

1. Soil liming reduced the content of nickel in orchard grass and in fractions isolated from soil – bioavailable (soluble and exchangeable) fraction F_1, oxidisable fraction F_2 and reducible fraction F_3.

2. The addition of rye straw and brown coal to the soil reduced the content of nickel in the test grass and content in fraction F_1.

3. The addition of nickel to soil, regardless of its dose, increased its content in the test plant and in soil, especially in fraction F_1.

4. The content of nickel in biomass of orchard grass exceeded the limit values in pots where it was added at 75, 150 and 225 mg Ni kg^{-1} of soil. Application of nickel to the soil at the dose 75 mg kg^{-1} was toxic to the cocksfoot grass.

5. Liming and addition of rye straw and brown coal to soil reduced the phytoavailability of nickel.

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MOŻLIWOŚCI ZMNIEJSZENIA FITOTOKSYCZNEGO DZIAŁANIA NIKLU

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Streszczenie: W latach 2009-2010 przeprowadzono doświadczenie wazonowe, którego celem było ustalenie granicy toksyczności niklu dla kupkówki pospolitej (*Dactylis glomerata* L.) i sposobów jego zmniejszenia, przez zastosowanie wapnowania, dodatku słomy i węgla brunatnego do gleby. Dodatek do gleby niklu, niezależnie od jego dawki, powodował zwiększenie jego zawartości w roślinie testowej oraz w glebie, zwłaszcza we frakcji F_1. Na obiektach z dodatkiem niklu do gleby jego zawartość w biomasie kupkówki pospolitej przekraczała zawartości graniczne. Wapnowanie gleby wpłynęło na zmniejszenie zawartości niklu w kupkówce pospolitej oraz w wydzielonych frakcjach z gleby – rozpuszczalnej F_1, redukowalnej F_2 i utlenialnej F_3. Wprowadzenie do gleby słomy żytniej i węgla brunatnego spowodowało zmniejszenie zawartości niklu w testowej trawie oraz jego ilości we frakcji biodostępnej F_1. Wapnowanie, jak też zastosowane do gleby słoma żytnia i węgiel brunatny ograniczyły fitoprzyswajalność niklu.

Słowa kluczowe: nikiel, wapnowanie, słoma, węgiel brunatny, frakcje Ni w glebie