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CONTENTS OF CADMIUM, LEAD AND NICKEL AT DIFFERENT DEVELOPMENT STAGES OF SELECTED *Miscanthus* GENOTYPES

ZAWARTOŚĆ KADMU, OŁOWIU I NIKLU W RÓŻNYCH OKRESACH ROZWOJU WYBRANYCH GENOTYPÓW TRAWY *Miscanthus*

Abstract: Bioaccumulation of three heavy metals (Cd, Pb, and Ni) at the second cultivation year of *Miscanthus sinenis* (2 diploid genotypes) and *Miscanthus sinensis x gigantheus* (3 triploid genotypes) was examined on objects with no fertilization and with NPK nutrition in five dates. Higher mean contents of analyzed heavy metals in triploid than diploid genotypes biomass was found. Mineral nutrition significantly affected the cadmium level in diploid genotypes biomass as well as nickel in all studied genotypes. The highest cadmium concentration was recorded in Miscanthus biomass at the beginning of June, lead – at the beginning of September and October, whereas nickel – at the beginning of July.

Keywords: *Miscanthus*, diploid genotypes, triploid genotypes, cadmium, lead, nickel, mineral nutrition

Miscanthus grass is an interesting alternative plant species, the biomass of which may be utilized for many ways. Species that may be every time adjusted to specific cultivation and utilization conditions, are required as renewable plant-origin material [1]. Among 20 different species including *Miscanthus* genus, *Miscanthus sinensis x gigantheus* finds its greatest interests in Europe due to its high efficiency [2, 3]. Mineral fertilization level does not directly influence on heavy metals accumulation within the plant, while indirectly through the soil acidity changes or precipitation of hardly soluble phosphates, mineral fertilizers can intensify or reduce the availability of these elements by plants [4].

Present research aimed at evaluating the dynamics of cadmium, lead, and nickel intake and accumulation under differentiated development stages, by five *Miscanthus* genotypes depending on mineral nutrition.

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Material and methods

The experiments were carried out in the field on the soil of loamy sand granulometric composition (according to PN-R-04033, pH_{KCl} = 6.73). Organic carbon content was 37.4 g · kg^{-1}; total contents of selected heavy metals [mg · kg^{-1} soil] amounted to: Cd – 0.960; Pb – 63.99; Ni – 5.53, that were determined after combustion by means of ICP-EAS technique.

The experimental plots with *Miscanthus* grass cultivation were set in autumn 2000. Studies included five genotypes of the grass: two diploid (2×) representing *Miscanthus sinensis* species, including one *Hybriden* (clone No. 1) and one German form named Goliath – MGo (clone No. 19), while other three genotypes belonged to triploid (3×) *Miscanthus sinensis x gigantheus* hybrid differing with their origin: clone No. 53 came from Germany, clone No. 63 from Denmark, and clone POL from Poland. Seedlings were achieved from the rhizome reproduction. Particular clones were set on plots of 1.5 m^2 area in three replications in completely randomized pattern. The biomass yields of studied genotypes were presented in earlier publication [3].

The plant samples (10 leaved stems) were collected at the beginning of June, July, August, September and October 2002. Mineral nutrition at the levels of: N – 60 kg · ha^{-1} (ammonium nitrate), P – 50 kg · ha^{-1} (triple superphosphate), K – 100 kg · ha^{-1} (potassium sulfate) was applied every year early spring before vegetation period. The experiment included control object (with no nutrition) and objects fertilized with NPK.

The plant samples were ground till 0.25 mm particle diameter and aliquots of 1 g were weighed into the crucibles. The organic matter was then combusted in muffle furnace at 450 ^{o}C for 15 hours. After that, 10 cm^3 of diluted HCl (1:1) was added to the crucible and evaporated on sand bath to decompose carbonates and separate silicates. The contents of the crucibles, after adding 5 cm^3 10 % HCl, were then transferred into 100 cm^3 capacity measure flask and adjusted volume using distilled water. Such prepared basic solution was subjected to determinations for cadmium, lead, and nickel concentrations by means of ICP-EAS technique on Optima 3200 RL device (Perkin Elmer). Differences between mean values for fertilization, genotypes, and sampling dates were evaluated by means of variance analysis; in the case of their significance, $LSD_{0.05}$ values were calculated applying Tukey's test. All calculations were made with a help of FR Analvar 3.2 software.

Results and discussion

Varied cadmium, lead, and nickel contents were found in the biomass of five *Miscanthus* genotypes depending on genotype, fertilization, and study date, in the second growing year. Less cadmium was recorded on control, and more cadmium on objects treated with NPK in the biomass of diploid than triploid genotypes (Table 1). More cadmium accumulation was found than in diploid, mineral fertilized genotypes than with no fertilization; diploid No. 1 was characterized by much higher cadmium content than No. 19. The element concentration (mean for fertilization) decreased along with the increase of biomass of cultivated grass (date): at the beginning of June, it

Table 1

Total content of cadmium [mg · kg^{-1} d.m.] in the biomass of *Miscanthus* grass at different development stages

Genotypes	Month																
	June			July			August			September			October			Mean	
	0	NPK	mean	0	NPK	mean	0	NPK	mean	0	NPK	mean	0	NPK	mean	0	NPK
1	0.102	0.123	0.113	0.110	0.180	0.145	0.078	0.138	0.108	0.105	0115	0.110	0.089	0.103	0.096	0.097	0.132
19	0.098	0.083	0.091	0.046	0.039	0.043	0.068	0.097	0.083	0.095	0.039	0.067	0.072	0.098	0.085	0.076	0.071
Mean for diploid genotypes	0.100	0.103	0.102	0.078	0.110	0.094	0.073	0.116	0.095	0.100	0.077	0.089	0.081	0.101	0.091	0.086	0.101
$LSD_{0,05}$ for: A – fertilization B – genotypes C – month A/B; B/A; C/A; A/C; C/B; B/C – interaction	A = 0.007 B = 0.007 C = 0.017			B/A = 0.011 C/A = 0.015 A/B = 0.011			A/C = 0.011 C/B = 0.024 B/C = 0.017										
53	0.199	0.202	0.201	0.139	0.106	0.123	0.051	0.141	0.096	0.081	0.054	0.068	0.099	0.078	0.089	0.114	0.116
63	0.128	0.088	0.108	0.153	0.061	0.107	0.056	0.129	0.093	0.126	0.085	0.106	0.104	0.095	0.099	0.113	0.092
POL	0.116	0.098	0.107	0.098	0.063	0.081	0.092	0.111	0.102	0.122	0.046	0.084	0.094	0.086	0.090	0.104	0.081
Mean for triploid genotypes	0.148	0.129	0.139	0.130	0.077	0.104	0.083	0.127	0.105	0.109	0.062	0.086	0.099	0.086	0.093	0.114	0.096
Mean	0.124	0.116	0.120	0.104	0.094	0.099	0.078	0.122	0.100	0.105	0.069	0.087	0.090	0.094	0.092	0.100	0.099
$LSD_{0,05}$ for: A – fertilization B – genotypes C – month A/B; B/A; C/A; A/C; C/B; B/C – interaction	A = n.s. B = n.s. C = 0.048			B/A = n.s. C/A = 0.053 A/B = n.s.			A/C = 0.037 C/B = n.s. B/C = n.s.										

n.s. – not significant difference

amounted to 0.102 mg · kg^{-1}, while at the beginning of October 0.091 mg · kg^{-1}. Similar dependence was recorded for triploid genotypes, for which cadmium content were 0.139 and 0.093 mg · kg^{-1}, respectively. The sampling date had significant influence on cadmium content in triploid clones, while genotype and mineral nutrition did not considerably differentiate the metal level.

Total cadmium contents [mg · kg^{-1}] in biomass of studied *Miscanthus* genotypes can be lined up in the following sequences (mean values for study dates and nutrition):

– June: No. 53 (0.201) > No. 1 (0.113) > No. 63 (0.108) > POL (0.107) > No. 19 (0.091);

– July: No. 1 (0.145) > No. 53 (0.123) > No. 63 (0.107) > POL (0.081) > No. 19 (0.043);

– August: No. 1 (0.108) > POL (0.102) > No. 53 (0.096) > No. 63 (0.093) > No. 19 (0.083);

– September: No. 1 (0.110) > No. 63 (0.106) > POL (0.084) > No. 53 (0.068) > No. 19 (0.067);

– October: No. 63 (0.099) > No. 1 (0.096) > POL (0.090) > No. 53 (0.089) > No. 19 (0.085).

Studied experimental factors had not significant influence on lead content in *Miscanthus* grass, except from some interactions (Table 2). The metal bioaccumulation varied in diploid and triploid genotypes. Triploid genotypes contained more lead than diploid ones. Lead level decreased in subsequent study dates for triploid biomass. The lowest lead content was recorded in June for all studied genotypes, while the highest in September and October. A systematic increase of lead quantities in osier in following harvest dates was also observed by Kaniuczak et al [5].

Total lead contents [mg · kg^{-1}] in *Miscanthus* biomass can be lined up in the following sequence (mean values for study dates and fertilization):

– June: No. 63 (1.05) > No. 19 (0.882) > POL (0.822) = No. 1 (0.822) > No. 53 (0.698);

– July: No. 63 (1.98) > No. 1 (1.74) > POL (1.68) > No. 19 (1.42) > No. 53 (1.41);

– August: No. 63 (2.16) > POL (1.90) > No. 53 (1.68) > No. 1 (1.49) > No. 19 (1.48);

– September: No. 53 (2.83) > POL (2.04) > No. 19 (1.99) > No. 1 (1.63) > No. 63 (1.63);

– October: No. 53 (2.70) > POL (2.27) > No. 63 (1.96) > No. 1 (1.39) > No. 19 (1.30).

Nickel content in tested grass biomass depended on the genotype, mineral nutrition, and study date (Table 3). Chemical analyses revealed less nickel in diploid than triploid genotypes. The highest bioaccumulation of that element was recorded in July, whereas the lowest in October (for diploid) and August (for triploid genotypes). The largest amounts of nickel on objects with no fertilization were found in the biomass of clone POL, while the lowest – clone No. 1; on objects treated with NPK – clone No. 53 and clone No. 19, respectively.

Table 2

Total content of lead [mg · kg^{-1} d.m.] in the biomass of *Miscanthus* grass at different development stages

Genotypes	Month																
	June			July			August			September			October			Mean	
	0	NPK	mean	0	NPK	mean	0	NPK	mean	0	NPK	mean	0	NPK	mean	0	NPK
1	0.826	0.817	0.822	2.39	1.090	1.74	0.932	2.05	1.49	1.75	1.51	1.63	1.42	1.36	1.39	1.46	1.37
19	0.882	0.881	0.882	2.15	0.684	1.42	0.923	2.03	1.48	2.23	1.74	1.99	1.38	1.21	1.30	1.51	1.31
Mean for diploid genotypes	0.854	0.849	0.852	2.27	0.887	1.58	0.928	2.04	1.48	1.99	1.63	1.81	1.40	1.33	1.37	1.49	1.34
$LSD_{0,05}$ for: A – fertilization B – genotypes C – month A/B; B/A; C/A; A/C; C/B; B/C – interaction	A = n.s. B = n.s. C = 0.567			B/A = n.s. C/A = 0.507 A/B = n.s.			A/C = 0,359 C/B = n.s. B/C = n.s.										
53	0.725	0.698	0.712	1.67	1.14	1.41	2.19	1.17	1.68	2.23	3.43	2.83	2.42	2.98	2.70	1.85	1.88
63	0.985	1.120	1.050	2.42	1.54	1.98	2.17	2.15	2.16	1.41	1.67	1.54	2.05	1.86	1.96	1.81	1.67
POL	0.836	0.808	0.822	2.25	1.11	1.68	2.07	1.72	1.90	2.30	1.77	2.04	2.38	2.16	2.27	1.97	1.51
Mean for triploid g enotypes	0.849	0.875	0.862	2.11	1.26	1.69	2.14	1.68	1.91	1.98	2.29	2.14	2.28	2.33	2.31	1.87	1.69
Mean	0.852	0.862	0.857	2.19	1.07	1.63	1.53	1.86	1.70	1.99	1.96	1.98	1.84	1.83	1.84	1.68	1.52
$LSD_{0,05}$ for: A – fertilization B – genotypes C – month A/B; B/A; C/A; A/C; C/B; B/C – interaction	A = n.s. B = n.s. C = 0.466			B/A = n.s. C/A = 0.511 A/B = n.s.			A/C = 0.363 C/B = 0.808 B/C = 0.690										

n.s. – not significant difference

Table 3

Total content of nickel [mg · kg^{-1} d.m.] in the biomass of *Miscanthus* grass at different development stages

Genotypes	Month																
	June			July			August			September			October			Mean	
	0	NPK	mean	0	NPK	mean	0	NPK	mean	0	NPK	mean	0	NPK	mean	0	NPK
1	2.56	2.79	2.68	12.00	7.06	9.53	3.25	3.02	3.14	0.650	5.80	3.23	0.945	1.25	1.10	3.83	3.98
19	2.86	2.82	2.84	12.21	7.53	9.87	1.93	2.75	2.34	3.73	5.20	4.47	1.94	2.99	2.47	4.53	4.26
Mean for diploid genotypes	2.71	2.81	2.76	12.11	7.30	9.71	2.59	2.89	2.74	2.19	5.50	3.85	1.44	2.12	1.78	4.21	4.12
$LSD_{0,05}$ for: A – fertilization B – genotypes C – month A/B; B/A; C/A; A/C; C/B; B/C – interaction	A = 0.213 B = n.s. C = 0.475			B/A = n.s. C/A = 0.425 A/B = n.s.			A/C = 0.301 C/B = 0.672 B/C = 0.476										
53	3.23	3.48	3.36	11.40	5.76	8.58	2.47	3.19	2.86	4.33	13.41	8.87	3.04	3.99	3.52	4.89	5.97
63	2.51	2.65	2.58	19.60	9.05	14.33	1.53	2.08	1.81	3.02	4.71	3.87	4.02	3.96	3.99	6.14	4.49
POL	3.45	3.67	3.56	23.01	13.03	18.02	2.96	1.66	2.31	5.91	7.12	6.52	3.98	3.87	3.93	7.86	5.87
Mean for triploid genotypes	3.06	3.26	3.16	17.82	9.28	13.55	2.34	2.31	2.33	4.42	8.41	6.42	3.68	3.45	3.57	6.30	5.44
Mean	2.89	3.04	2.97	14.97	8.29	11.63	2.47	2.60	2.54	3.31	6.96	5.14	2.56	3.94	3.25	5.26	4.78
$LSD_{0,05}$ for: A – fertilization B – genotypes C – month A/B; B/A; C/A; A/C; C/B; B/C – interaction	A = 0.217 B = 0.320 C = 0.483			B/A = 0.453 C/A = 0.530 A/B = 0.377			A/C = 0.377 C/B = 0.837 B/C = 0.716										

n.s. – not significant difference

Total nickel concentration [mg · kg^{-1}] in biomass of five *Miscanthus* genotypes during the second year of its cultivation can be lined up in the following sequence (means for study dates and fertilization):

– June: POL (3.56) > No. 53 (3.36) > No. 19 (2.84) > No. 1 (2.68) > No. 63 (2.58);

– July: POL (18.02) > No. 63 (14.33) > No. 19 (9.87) > No. 1 (9.53) > No. 53 (8.58);

– August: No. 1 (3.14) > No. 53 (2.86) > No. 19 (2.34) > POL (2.31) > No. 63 (1.81);

– September: No. 53 (8.87) > POL (6.52) > No. 19 (4.47) > No. 63 (3.87) > No. 1 (3.23);

– October: No. 63 (3.99) > POL (3.93) > No. 53 (3.52) > No. 19 (2.47) > No. 1 (1.10).

Contents of examined heavy metals in *Miscanthus* biomass were much lower than those recorded by Kaniuczak et al [5] and Niedźwiecki et al [6].

Conclusions

1. Biomass of triploid *Miscanthus* genotypes in the second year of the field experiment was characterized by higher cadmium, lead, and nickel bioaccumulation than that of diploid ones.
2. Total contents of all studied heavy metals in the biomass of five *Miscanthus* genotypes could be lined up in the following sequence [mg · kg^{-1}]: Ni (0.650–23.01) > Pb (0.684–3.43) > Cd (0.039–0.141).
3. Chemical analyses revealed that cadmium, lead, and nickel amounts in the biomass of tested grass varied depending on the study date. The highest total cadmium level was recorded at the beginning of June, while lead at the beginning of September and October, and nickel at the beginning of July.
4. Mineral NPK nutrition significantly differentiated cadmium content in the biomass of diploid, whereas that of nickel – in diploid and triploid genotypes of *Miscanthus*.

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ZAWARTOŚĆ KADMU, OŁOWIU I NIKLU W RÓŻNYCH OKRESACH ROZWOJU WYBRANYCH GENOTYPÓW TRAWY *Miscanthus*

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Abstrakt: Badano bioakumulację trzech metali ciężkich (Cd, Pb i Ni) w drugim roku uprawy trawy *Miscanthus sinenis* (2 genotypy diploidalne) i *Miscanthus sinensis x gigantheus* (3 genotypy triploidalne) na obiektach bez nawożenia i nawożonych mineralnie NPK, w pięciu terminach. Stwierdzono (średnio) większą zawartość analizowanych metali ciężkich w biomasie genotypów triploidalnych niż diploidalnych. Nawożenie mineralne znacznie wpłynęło na zawartość kadmu w biomasie genotypów diploidalnych oraz niklu we wszystkich badanych genotypach. Stwierdzono największą zawartość Cd w biomasie miskanta w pierwszej dekadzie czerwca, Pb w pierwszej dekadzie września i października, a Ni w pierwszej dekadzie lipca.

Słowa kluczowe: trawa *Miscanthus,* genotypy di- i triploidalne, kadm, ołów, nikiel, nawożenie mineralne