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INFLUENCE OF SEWAGE SLUDGE FERTILIZATION ON HEAVY METAL CONTENT IN BIOMASS OF SILVER GRASS DURING FIELD EXPERIMENT

The field experiment allowed us to compare the influence of sewage sludge fertilization applied at varied rates with that of mineral fertilizers (NPK) on heavy metal content (Pb, Cd, Cr, Co, Cu, Zn, and Ni) in stems and leaves of silver grass (*Miscanthus sacchariflorus*) harvested in the first year of experiment and in the whole biomass harvested in the second year. Higher concentration of heavy metals (except for zinc) was found in the leaves than in the stems of the grass. No cadmium was detected in the silver grass biomass in the first year, while large amounts of this element were recorded in the second year of the experiment. The doses of sewage sludge (10, 20, and 30 tha^{-1} d.m.) did not cause the excessive accumulation of heavy metals in the plant biomass in the first and the second vegetation seasons.

1. INTRODUCTION

The application of sewage sludge and other organic waste materials to plant production is the method of both utilizing their biogenic elements, mainly nitrogen and phosphorus, and decreasing concentration of other nutrient elements (including heavy metals) in the soil solution as a result of their uptake by plants and specific microorganisms found in soil and on plant roots and precipitation of elements in soil. Utilization of municipal wastes for fertilizing energetic plants may give measurable economic effects and prevent the environmental pollution [1]. These plants can be cultivated in the areas strongly polluted with heavy metals that cannot be used for other-purposes plant plantations [2].

The present experiment was aimed at evaluating the influence of fertilization with various doses of sewage sludge, and, for comparison, mineral fertilizer on heavy metal content in the biomass of silver grass (*Miscanthus sacchariflorus*) in the first and second cultivation years.

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2. MATERIAL AND METHODS

The experiment was set up in spring 2005 on light soil of granulometric composition of loamy sand (according to PN-R-04033), which was characterized by the following features: $\text{pH}_{\text{KCl}} = 6.60$, organic carbon content of 30.5 g kg^{-1} , total content of selected trace elements (mg kg^{-1} of soil): Pb – 63.99, Cd – 0.960, Cr – 8.95, Co – 4.62, Cu – 18.85, Zn – 279, Ni – 5.53. The total concentration of these elements was determined by means of ICP-AES technique after soil combustion in muffle furnace at 450°C . The plots of 2 m^2 area in three replications were selected in a completely randomized pattern. Silver grass (*Miscanthus sacchariflorus*) was the test plant.

In the experiment, the following types fertilization were applied:

- control (no fertilization);
- NPK nutrition (mineral nitrogen was introduced in a form of urea, according to nitrogen amount contained in 20 t ha^{-1} d.m of sludge);
- fertilization with sewage sludge at the rate of 10 t ha^{-1} d.m;
- fertilization with sewage sludge at the rate of 20 t ha^{-1} d.m;
- fertilization with sewage sludge at the rate of 30 t ha^{-1} d.m.

The experimental plots were fertilized with phosphorus (triple superphosphate) and potassium (potassium sulfate) fertilizers, maintaining the ratio of N:P:K as 1:0.8:1.2. Sewage sludge was produced by mechanical-biological sewage treatment plant in the town of Siedlce. A chemical composition of this sewage sludge proved to be appropriate for plant nutrition [3]; heavy metal content was at the following levels (mg kg^{-1} d.m): Pb – 98.7, Cd – 2.70, Cr – 20.5, Co – 4.49, Cu – 199, Zn – 2453, and Ni – 52.74. Before rhizome planting, the sludge was mixed with the soil to 25 cm depth. The biomass was harvested in December 2005 and 2006 after the first and the second year of cultivation. In the first year, the stem and leaf chemical compositions were analyzed separately, while in the second one – the whole biomass (stems + leaves) was examined. The plant material was ground to 0.25 mm particle size, 1-g samples were weighed out and poured into the stoneware crucibles, and then organic matter was ashed at 450°C in muffle furnace for 15 hours. Aliquots of 10 cm^3 of diluted HCl (1:1) were added into each crucible and evaporated on sand bath in orders to decompose carbonates and to separate silicates. After adding 5 cm^3 of 10% HCl, the content of each crucible was filtered through hard filter paper to a measure flask of a 100 cm^3 capacity and the volume was made up to the mark with distilled water. Such basic solution served to determine the total content of Pb, Cd, Cr, Co, Cu, Zn, and Ni by means of ICP-AES technique.

Taking into account the harvested crop and the content of the selected elements, their uptake was calculated. The data referring to the stem and leaf yields in the first and second years of silver grass cultivation were presented in other publications [4], [5]. The results achieved were statistically processed; the differences between mean values for plant's parts and the doses of fertilizers were estimated by means of Fisher-

Snedecor's test and in the case of their significance, LSD_{0.05} values were calculated according to Tukey's test.

3. RESULTS AND DISCUSSION

In the first year of silver grass cultivation, the chemical analyses revealed various content of heavy metals in its leaves and stems (table 1). Total concentrations of the elements of interest can be arranged in the following descending orders (mg · kg⁻¹):

- For leaves: Zn (62.86–34.82) > Ni (8.91–5.70) > Cu (2.06–0.520) > Pb (1.71–0.903) > Cr (0.670–0.472) > Co (0.339–0.108) > Cd – not detected;
- For stems: Zn (149.8–85.98) > Ni (5.04–2.50) > Cu (0.440–0.425) > Pb (0.672–0.00) > Co (0.167–0.00) > Cd – not detected.

Table 1
Total content of heavy metals (mg · kg⁻¹ d.m.) in biomass of *Miscanthus sacchariflorus*
in first year of field experiment

Fertilization	Leaves					
	Pb	Cr	Co	Cu	Zn	Ni
Control	0.91	0.544	0.213	1.41	62.86	5.70
NPK	1.56	0.472	0.253	1.08	45.89	6.60
10 t · ha ⁻¹	0.90	0.645	0.108	0.52	34.82	8.91
20 t · ha ⁻¹	1.65	0.652	0.339	2.06	49.95	6.91
30 t · ha ⁻¹	1.71	0.670	0.303	1.16	58.76	7.61
Mean	1.35	0.597	0.243	1.25	50.46	7.15
Stems						
Control	n.d.	0.082	n.d.	0.425	149.8	5.04
NPK	n.d.	0.051	n.d.	0.431	121.3	3.56
10 t · ha ⁻¹	n.d.	n.d.	n.d.	0.428	79.39	3.48
20 t · ha ⁻¹	0.672	0.060	0.167	0.440	129.6	2.50
30 t · ha ⁻¹	0.636	0.050	0.165	0.438	85.98	2.61
Mean	0.262	0.049	0.066	0.432	113.2	3.44
NIR _{0.05} for:						
A – plant parts	0.188	0.041	0.033	n.s.	4.34	0.369
B – fertilization	0.426	0.093	0.075	n.s.	9.86	0.837
A/B – interaction	n.s.	0.191	n.s.	n.s.	9.70	0.825
B/A – interaction	n.s.	0.131	n.s.	n.s.	13.92	1.18

n.d. – not detected Cd.

n.s. – insignificant.

In the silver grass leaves, the concentrations of Pb; Cr; Co; Cu and Ni were 5 times, 12 times, over 3 times and over 2 times higher, respectively, than in its stems, while the concentration of Zn in leaves was fairly lower than in stems (Cd not detected)

(table 1). In the biomass of the silver grass (*Miscanthus sacchariflorus*) harvested after the first year of cultivation, KRZYWY et al. [6] found higher content of Cu and lower content of Zn in comparison with our results. The control plants revealed the highest zinc accumulation both in leaves and stems as well as Cr and Ni in stems, in relation to the plants fertilized with minerals and various doses of sludge. Sewage sludge caused an increase in Ni and Cr concentrations in grass leaves compared with mineral fertilization. Zn and Co were detected only in the stems of silver grass fertilized with the highest rates of sewage sludge (20 and 30 t · ha⁻¹). A significant influence of fertilization on the majority of the elements' concentrations (except for Cu) in the leaves and stems of test plant was observed.

Table 2

Total content of heavy metals (mg · kg⁻¹ d.m.) in biomass of *Miscanthus sacchariflorus* in second year of field experiment

Fertilization	Pb	Cd	Cr	Co	Cu	Zn	Ni
Control	1.11	6.54	0.361	0.356	2.46	36.86	3.52
NPK	1.77	7.22	0.341	0.158	2.87	51.32	3.13
10 t · ha ⁻¹	1.34	8.99	0.311	0.187	4.12	39.04	2.94
20 t · ha ⁻¹	1.68	7.03	0.319	0.185	3.01	41.52	2.21
30 t · ha ⁻¹	3.02	8.72	0.358	0.138	2.72	55.47	3.06
Mean	1.78	7.70	0.338	0.205	3.04	44.84	2.97
LSD _{0.05}	1.60	n.s.	n.s.	0.011	n.s.	12.01	n.s.

n.s. – not significant.

In the second year of the experiment, mean contents of the heavy metals varied in the whole silver grass biomass (leaves + stems) and they could be arranged in the following descending order (mg · kg⁻¹): Zn (55.47–36.88) > Cd (8.99–6.54) > Cu (4.12–2.46) > Ni (3.52–2.21) > Pb (3.02–1.11) > Cr (0.361–0.311) > Co (0.356–0.138). Relatively high concentrations of Cd (7.70 mg · kg⁻¹), higher Cu concentration (3.04 mg · kg⁻¹), and slightly higher of Pb (1.78 mg · kg⁻¹) were found just after fertilization with the sewage sludge in comparison with those in the first year. In the biomass of *Miscanthus sacchariflorus* fertilized with sewage sludge and harvested in the second year of cultivation, KRZYWY et al. [6] measured similar content of Cu and Zn. Fertilization significantly differentiated the concentrations of Pb, Co, and Zn; for other elements no considerable influence was observed.

High doses of the sewage sludge applied to fertilizing the silver grass did not cause any excessive accumulation of the majority of the heavy metals, either in direct or consequent action. Significant cadmium bioaccumulation was recorded in the second year of the experiment. Many authors place grasses among plants that show remarkable tolerance towards high levels of this element in the environment [7], [8].

Table 3

Concentration of heavy metals ($\text{g} \cdot \text{ha}^{-1}$) in *Miscanthus sacchariflorus* leaves and stems in first year of field experiment

Fertilization	Leaves					
	Pb	Cr	Co	Cu	Zn	Ni
Control	1.02	0.61	0.239	1.58	70.40	6.34
NPK	2.45	0.74	0.397	1.70	72.05	10.36
$10 \text{ t} \cdot \text{ha}^{-1}$	1.41	1.01	0.169	0.81	54.32	13.89
$20 \text{ t} \cdot \text{ha}^{-1}$	2.64	1.04	0.542	3.29	79.92	11.06
$30 \text{ t} \cdot \text{ha}^{-1}$	2.62	1.03	0.464	1.78	89.90	11.64
Mean	2.03	0.89	0.362	1.83	73.32	10.68
Stems						
Control	n.d.	0.057	n.d.	0.293	103.4	3.48
NPK	n.d.	0.056	n.d.	0.474	13.34	3.92
$10 \text{ t} \cdot \text{ha}^{-1}$	n.d.	n.d.	n.d.	0.419	77.80	3.41
$20 \text{ t} \cdot \text{ha}^{-1}$	0.773	0.069	0.192	0.506	149.0	2.88
$30 \text{ t} \cdot \text{ha}^{-1}$	0.623	0.049	0.114	0.429	84.26	2.56
Mean	0.279	0.046	0.061	0.424	109.6	3.25
Total						
Control	1.02	0.67	0.239	1.87	17.38	9.82
NPK	2.45	0.80	0.397	2.17	205.5	14.28
$10 \text{ t} \cdot \text{ha}^{-1}$	1.41	1.01	0.169	1.23	132.1	17.30
$20 \text{ t} \cdot \text{ha}^{-1}$	3.41	1.11	0.734	3.79	228.9	13.94
$30 \text{ t} \cdot \text{ha}^{-1}$	3.24	1.08	0.578	2.21	174.2	14.20
Mean	2.31	0.94	0.423	2.25	182.9	13.91

n.d. – not detected.

Table 4

Concentration of heavy metals ($\text{g} \cdot \text{ha}^{-1}$) in *Miscanthus sacchariflorus* leaves and stems in second year of field experiment

Fertilization	Pb	Cd	Cr	Co	Cu	Zn	Ni
Control	0.18	10.79	0.596	0.587	4.06	60.82	5.81
NPK	3.45	14.08	0.665	0.308	5.59	100.1	6.10
$10 \text{ t} \cdot \text{ha}^{-1}$	2.24	15.01	0.519	0.312	6.88	65.19	4.91
$20 \text{ t} \cdot \text{ha}^{-1}$	2.87	12.02	0.546	0.316	5.15	70.99	3.78
$30 \text{ t} \cdot \text{ha}^{-1}$	4.77	13.78	0.566	0.218	4.29	76.68	5.08
Mean	3.03	13.14	0.578	0.348	5.20	61.73	5.14

In the first year, the silver grass accumulated more Cr, Co, Zn, and Ni in terms of the total yield (leaves + stems), while less Pb and Cu compared with the second year of experiment (tables 3 and 4). These values can be arranged in the following descending orders for the particular harvest dates:

1st year: Zn > Ni > Pb > Cu > Cr > Co;
 2nd year: Zn > Cd > Cu > Ni > Pb > Cr > Co.

The ability to accumulate heavy metals depends on the spectrum of soil environment factors [9]. Our results were confirmed by other authors' observations, because heavy metal content in the biomass of silver grass fertilized with minerals and sewage sludge was generally similar, which indicates that the latter may be used for the plant fertilizing. It is possible that organic matter along with sewage sludge caused the complexation of heavy metals and their reduced uptake by plants.

4. CONCLUSIONS

1. Fresh sewage sludge used as the fertilizer of the silver grass did not cause excessive accumulation of the heavy metals studied, either in direct or consequent action.
2. In the first year of the silver grass cultivation, much higher contents of the heavy metals (except for zinc) were found in leaves than in stems.
3. In the second year of experiment, average concentrations of the heavy metals varied in comparison with those found in the first year. Relatively high concentrations of cadmium, higher of copper, and slightly higher of lead were found that year.

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**WPŁYW NAWOŻENIA OSADEM ŚCIEKOWYM NA ZAWARTOŚĆ METALI CIĘŻKICH
W BIOMASIE TRAWY *MISCANTHUS SACCHARIFLORUS* W DOŚWIADCZENIU POLOWYM**

W doświadczeniu polowym badano wpływ nawożenia osadem ściekowym, zastosowanym w różnych dawkach, oraz dla porównania nawozami mineralnymi (NPK) na zawartość metali ciężkich: Pb, Cd, Cr, Co, Cu, Zn i Ni w łodygach i liściach trawy *Miscanthus sacchariflorus* w I roku eksperymentu oraz w całej biomasie zebranej w II roku. Stwierdzono, że badana trawa gromadzi więcej metali ciężkich (oprócz cynku) w liściach niż w łodygach. W I roku badań nie wykryto kadmu w biomasie miskanta, a w II roku zanotowano znaczne jego stężenie. Zastosowane dawki osadu ściekowego (10, 20 i 30 tha^{-1} s.m.) nie spowodowały nadmiernej bioakumulacji badanych metali ciężkich w biomasie testowanej rośliny ani w pierwszym, ani drugim okresie wegetacji.