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Effect of unconventional fertilization on heavy metal content in the biomass of giant *miscanthus*

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ABSTRACT

The article presents the results of the studies on the effects of fertilization with (1) sewage sludge, (2) compost produced from the mixture of sewage sludge and forestry waste and (3) compost from green waste produced with Dano technology on bioaccumulation of heavy metals in *Miscanthus giganteus* biomass. The results were obtained from the pot experiment in semi-natural conditions during the period of April 2007 to November 2010. The testing presents the studies concerning the third year of the experiment (the plant crop of November 2010). The soil used for cultivation of *M. giganteus* was sampled from the area in close vicinity to the steel works. The effects of sewage sludge, compost and mineral fertilizers on the concentration of Cd, Zn, Pb and Ni were compared in the above-ground parts and roots of *M. giganteus*. These treatments resulted in an increase in the concentration of zinc, cadmium, lead and nickel in the soil in comparison to the control. The highest increase in the concentration of metals was observed in soil fertilized with sewage sludge at the lowest dose of 40 t/ha. The results obtained in the third year of the experiment indicate that the *M. giganteus* has a tendency to accumulate zinc and cadmium in the above-ground parts, and lead in the roots. Depending on the treatment, the concentration of the investigated metals in the above-ground parts of *M. giganteus* was in the range of 57.0–62.5 mg/kg for Zn, 0.88–1.18 mg/kg for Cd, 3.70–3.90 mg/kg for Pb and 3.15–3.90 mg/kg for Ni. The lowest concentrations of Zn, Cd, Pb and Ni in the above-ground biomass of the plant was observed for mineral fertilization and the control. The highest concentrations of zinc, cadmium and lead were observed in biomass of plants grown on soils fertilized with sewage sludge at the dose of 20 t/ha, whereas the highest concentrations of nickel were observed for soils fertilized with the compost produced with Dano technology. However, fertilization of soils with sewage sludge and compost had no impact on the concentration of zinc, cadmium, lead and nickel in the biomass of *M. giganteus*. The concentrations of Cd, Zn, Pd and Ni in the roots were affected by the type of fertilization, and in most cases, the concentration was the highest when sewage sludge at dose of 20 and 40 t/ha was applied. Furthermore, some significant differences regarding bioaccumulation indicators for the above-ground parts and roots of the plant and selected metals were observed.

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Keywords: Miscanthus giganteus; Soil; Heavy metals; Compost; Sewage sludge

1. Introduction

Among renewable energy sources, biomass plays a very important role. The principles of the European Union's agricultural policy and environmental protection policy point out that as the generally accessible waste biomass resources are depleted, an intensive increase in energy plant growing will follow [1–3].

Among many energy plants, giant *miscanthus* (*Miscanthus* × *giganteus*) was selected for investigation due to its good adaptation to the Polish soil-climatic conditions. This plant belongs to perennial plants; its plantation life is estimated at 15–25 years [4–6]. With adequate fertilization, it has a high cropping capability of around 15–20 t/ha, and its calorific value is comparable to that of wood [7,8]. Research results show that *miscanthus* plant has bioremediation properties [6].

It should be emphasized, however, that using biomass for energy generation purposes only makes sense when the energy expenditures on the production of biomass are much lower than the energy generated from it [9]. Therefore, using various types of wastes as a source of nutrients for plants of this type is justified economically and ecologically. To fertilize the soil for the conducted experiment, sludge and composts (characterized in the rest of the work) are used due to their high-fertilizing properties. Fertilization of soils with this type of substrates is not only recommended from the economic point of view, but also advisable for preserving and restoring the ecological balance of soils, especially those poor in organic matter [10–15]. Fertilizing soils with sludge and compost usually involves some enrichment of the soil in heavy metals. The results of research on the effect of fertilization with sewage sludge and compost on the cropping of giant *miscanthus* were reported by the authors in their earlier publications [16]. The purpose of the investigation described in the present paper is to assess the effect of fertilizing affected regional soils with sewage sludge and compost on the heavy metal bioaccumulation in giant *miscanthus* biomass.

2. Material and methods

2.1. Description of the experiment

A pot experiment was conducted in the years 2007–2011. The soil on which giant *miscanthus* was grown was taken from an area neighbouring on the Czestochowa Steel Mill premises. About 300 kg of soil was taken for the experiment from several dozen points situated within an area of 20,000 m^2 from a 0–25-cm-deep layer. The soil material was taken following the applicable procedure so that it would reflect the condition of the examined area as precisely as possible [17]. The pot experiment was prepared in accordance with the scheme shown in Table 1.

Six samples and one control sample fertilization combinations were used in the experiment, each in three repetitions. In total, 21 pots were set up, each with 12-kg soil. Sewage sludge and compost were used for plant growing due to their very good fertilizing properties. The sewage sludge used for the experiment originated from the municipal sewage-treatment plant. In addition to the sewage sludge, compost produced from small municipal treatment plant sewage sludge was also used for fertilization. Moreover, fertilization with the Dano technology compost from municipal waste was used. The applied sewage sludge and

Table 1
The fertilization combinations investigated in the pot experiment

Fertilization combination	Type and dose of fertilizer
0	Control—soil without fertilization
1	Soil + sewage sludge in a dose of 10 t d.m./ha
2	Soil + sewage sludge in a dose of 20 t d.m./ha
3	Soil + sewage sludge in a dose of 40 t d.m./ha
4	Soil + DANO technology compost from municipal waste, in a dose of 20 t d.m./ha
5	Soil + compost from sewage sludge and forest waste in a dose of 20 t d.m./ha
6	Soil + NPK (carbamide, potash salt and superphosphate), in a dose of N— 120 kg/ha, P_2O_5—80 kg/ha, K_2O—100 kg/ha

compost were characterized by good fertilizing properties due to their contents of nitrogen, phosphorus, potassium, etc. Their total contents of heavy metals were relatively low, which allowed them to be used for energy plant crops [18]. Fertilization was applied once before planting crops.

2.2. Analytical methods

The investigation included samples soil and plant. Applied research methods:

(1) the heavy metal contents of the soil and plant biomass was assayed on a Thermo ICP-AES plasma spectrophotometer according to PN-ISO 11047:2001, after the material having been previously mineralized in concentrated nitric acid using a Plasmotronic UniClever microwave mineralizer,
(2) the presence of the ova of the intestinal parasites *Ascaris* sp., *Trichuris* sp., *Toxocara* sp. was determined in accordance with Standard PN-Z-19000-4:2001. *Salomonella* were identified in accordance with Standard PN-ISO 6579:1998,
(3) the overall Kjeldahl nitrogen quantity was determined in accordance with PN-ISO 11261:2002 using a BUCHI 426 mineralizer and a BUCHI 323 distilling apparatus,
(4) the overall phosphorus (in the phosphate from) was determined by the molybdate method in accordance with PN-EN 1189-2000,
(5) the potassium was determined by the Egner-Rieham method (PN-R-04023:1996) by extracting it with a calcium lactate solution and determining the colour of the phosphomolybdate complex on an HACH spectrophotometer for a wavelength of $L = 660$ nm,
(6) pH in H_2O—the measurement was made by the potentiometric method in accordance with PN-ISO-10390:1997,
(7) the organic carbon content was determined by the modified Tiurin colorimetric method in accordance with PN-ISO 14235:2003,
(8) the bioaccumulation factor (BCF) was calculated from the formula:

$$\mathrm{BCF} = \frac{C_B}{C_G} \tag{1}$$

where C_B—metal concentration in the tissues of the plant overground/underground organs (mg/kg d.m.); C_G—metal concentration in the soil at the beginning of the process (mg/kg d.m.),

(9) immobilization factors (I_f) were calculated:

$$I_f = \frac{C_O^G - C_K^G}{C_O^P - C_K^P} \tag{2}$$

where C_O^G—initial element concentration in soil (mg/kg d.m.); C_K^G—final element concentration in soil (mg/kg d.m.); C_O^P—initial element concentration in fertilized soil (mg/kg d.m.); C_K^P—final element concentration in fertilized soil (mg/kg d.m.).

The obtained results were subjected to statistical analysis by the variance analysis and single-factor regression methods. The detailed analysis of the significance of differences between the results of individual fertilization combinations, as against the control, was made using the Student test at a significance level of $p = 0.05$.

2.2.1. Description of the soil used for the experiment

A grain size analysis showed that the soil used for the experiment belongs to light soils. It was characterized by slight contamination with zinc (acc. to the IUNG scale) and increased contents of cadmium and lead (Table 2). The contents of copper, nickel and chromium were at the level of natural contents. The amount of heavy metals in the soil does not exceed the permissible content of the metals specified in the Regulation of the Minister of the Environment of 13 July 2010 in the matter of municipal sewage sludge.

Stabilized, dehydrated sewage sludge with a slightly acid reaction, a high organic matter content and a small amount of heavy metals were used for fertilizing. The sewage sludge was taken from small municipal sewage. The characteristics of the substrates and soil are given in Table 2. The quantity of heavy metals in the sludge and compost did not exceed the permissible metal content limits applicable to their use for crops intended for consumption, in conformance with the Regulation of the Minister of the Environment of 13 July 2010 on municipal sewage sludge and the Regulation of the Minister of Agriculture 21 December 2009 on the implementation of certain provisions of the Act on fertilizers and fertilization. The examined composts and sewage sludge met the requirements of the Regulation [18], thus were eligible for being used for soil reclamation.

Table 2
Characteristics of substrates and soil

Designation	Sewage sludge	Compost from sewage and waste from forestry	Compost (technology DANO)	The soil characteristic
$C_{org.}$ (% s.m)	25.06	18.80	19.00	1.38
N (% s.m)	4.24	2.50	2.28	0.11
P (% s.m)	2.50	1.20	0.98	1.9
K (% s.m)	0.50	1.05	1.1	2.2
Ca (mg/kg)	28,000.00	8,500.00	1,540.00	0.87
Mg (mg/kg)	4,600.00	1,000.00	580.00	0.82
Pb (mg/kg)	40.00	25.00	52.00	39.0
Cd (mg/kg)	2.40	1.20	1.40	1.18
Zn (mg/kg)	1,000.00	270.00	242.00	122.2
Cu (mg/kg)	160.00	48.00	40.00	8.3
Ni (mg/kg)	18.00	19.00	14.00	7.0
Cr (mg/kg)	22.00	16.00	15.00	13.0
Salmonella	Lack	Lack	Lack	–
Ascaris sp., *Trichuris* sp., *Toxocara* sp., szt./kg s.m.	3	Lack	Lack	–
pH w H_2O	6.5	6.80	7.80	6.1

3. Results and discussion

3.1. *The effect of fertilization on the metal contents of the soil and giant* miscanthus *biomass*

The total cadmium content of the unfertilized soil (control) was 1.18-mg/kg soil. In accordance with the guidelines for soil contamination with heavy metals developed [19], the soil concerned should be classified as low cadmium-contaminated soil—II^0. As a result of fertilization, the overall cadmium quantity in individual fertilization combinations increased as against the control, which was due to the introduction of cadmium with the fertilizers. The highest cadmium concentration was found for fertilizing with sewage sludge in a dose of 40 t/ha; it amounted to 1.23 mg/kg soil. In contrast, fertilizing with the lowest sewage sludge dose of 10 t/ha, the forest waste compost and sewage sludge, and the DANO urban greenery compost resulted in an increase in the cadmium content of soil by 0.01 mg/kg (Table 3).

The total zinc content of the unfertilized soil was 122.2 mg/kg, which classified the soil as low zinc-contaminated soil—II^0. As a result of fertilization, the quantity of zinc in individual fertilization combinations was higher compared to the control, which is associated with the zinc content of the applied fertilizers. The highest zinc content increase of 10.2 mg/kg was found for the soil fertilized with sewage sludge in the highest dose of 40 t/ha, while the lowest of 0.60 mg/kg, for mineral fertilization, as against the control (Table 4).

Table 3
The content of heavy metals in soil, mg/kg d.m.

Samples	Cd	Zn	Pb	Ni
0	1.18	122.2	39.00	9.20
1	1.19	124.3	39.15	9.40
2	1.20	126.4	39.26	9.51
3	1.23	130.0	40.01	9.58
4	1.19	122.6	39.60	9.40
5	1.19	123.4	38.50	9.45
6	1.19	122.8	39.19	9.18

The total lead content of the unfertilized soil was 39.0 mg/kg, which classified the soil as the one of the increased lead contents—I^0. As a result of fertilization,

Table 4
The heavy metal content in the aerial parts of *Miscanthus*, mg/kg d.m.

Samples	Cd	Zn	Pb	Ni
0	1.10	58.0	3.80	3.75
1	1.12*	60.0*	3.80	3.70*
2	1.18*	62.5*	3.90*	3.75
3	1.09	60.5*	3.85*	3.75
4	1.13*	59.0*	3.80	3.90*
5	1.14*	60.5*	3.82*	3.70*
6	0.88*	57.0	3.70*	3.15*

Significance at a confidence level: $^*p = 0.05$.

the quantity of lead in individual fertilization combinations (except for fertilizing with the five compost) increased, as compared to the control. The highest lead content increase was found for fertilization with the sewage sludge in the highest dose of 40 t/ha (the difference being statistically significant at the level of $p < 0.01$), amounting to 1.01 mg/kg as against the control. Fertilization with the sewage sludge in the lowest dose (10 t/ha) and with six samples resulted in an increase in the total lead content by 0.15 mg/kg, while with the DANO compost, by 0.6 mg/kg soil, as against the control (Table 3).

The total content of nickel of the unfertilized soil was 9.20 mg/kg, which suggests the natural content of this element. As a result of fertilization, the quantity of nickel rose, with the exception of the minerally fertilized soil. The highest nickel content was found for fertilizing with sewage sludge in the highest dose of 40 t/ha; it amounted to 9.29-mg/kg soil (Table 3).

The accumulation of heavy metals in plants also depends largely on the species, or even variety. The testing results for the heavy metal contents of *miscanthus* biomass (Tables 4 and 5) concerning the third year of the experiment (the plant crop of November 2010). It should be noted that the biomass in the first and the second year of the experiment characterized by the lower content of metals in comparison to harvest three. Studies show that *Miscanthus giganteus* only in the third year of the experiment was characterized by good capacity of zinc and cadmium accumulation in the aerial parts and roots of lead accumulation, which, given the relatively high yields allows one to use it in the process of phytoremediation. The test results given in Table 5 indicate that the metal contents of *miscanthus* biomass was, depending on the fertilization, up to: 62.5 mg/kg Zn, 3.9 mg/kg Ni, 3.9 mg/kg Pb and 1.18 mg/kg Cd. Studies by other authors suggest that the concentration of metals in *miscanthus* straw may reach the level of, respectively: 70 mg/kg Zn, 15 mg/kg Cu, 4 mg/kg Ni, 4 mg/kg Pb and 1.5 mg/kg Cd [7,20,21]. The increase in the contents of some metals of *miscanthus* biomass is, however, always tantamount to a greater phytoremediation effect, as with high metal contents of substrates, some researchers noted simultaneously lower biomass crops [20,21].

Table 5
The heavy metal content in the roots of *Miscanthus*, mg/kg d.m.

Fertilization combination	Cd	Zn	Pb	Ni
0	1.80	89.0	28.4	13.5
1	1.82	97.0*	35.0*	14.6
2	1.90*	110.0*	38.0*	16.2*
3	1.95*	112.0*	37.5*	16.8*
4	1.90*	105.2*	30.7*	16.0*
5	1.85*	110.5*	32.8*	17.0*
6	1.65*	100.0*	30.7	15.2*

Significance at a confidence level: $^*p = 0.05$.

It should be emphasized that the concentration of heavy metals in *miscanthus* biomass was dependent to some extent on the type of soil fertilization. The heavy metal contents of biomass was not uniquely dependent on the metal contents of soil. The *miscanthus* grown on substrates enriched with sewage sludge in a dose of 10 and 20 t/ha, respectively, and with the compost collected and accumulated greater quantities of cadmium, compared with the control substrate. The largest quantity of cadmium was introduced to the soil with the highest sewage sludge dose of 40 t/ha, but the plants absorbed less metal with this fertilization than with fertilization using sewage sludge in a dose of 10 and 20 t/ha and the compost. The high organic matter content and the associated improvement in sorption conditions might have reduced the cadmium migration from the soil to the plant [13,22–24].

Similarly, as for cadmium, the *miscanthus* content of zinc was lower for fertilization with the highest sewage sludge dose (40 t/ha) that introduced the largest quantity of the metal to the soil, compared to the zinc content of plants fertilized with sewage sludge in a dose of 20 t/ha. It should be stressed, however, that the soil fertilization had no significant impact on the zinc content of the plants (Table 4).

The analysis of the results (Table 4) shows that the lead and nickel contents of *miscanthus* biomass were very similar for all types of fertilization. Introducing the sewage sludge and compost to the soil caused a significant increase in the contents of lead and nickel of the soil, but did not have any big effect on the contents of these metals of plant biomass.

The content of Cd, Zn, Pn and Ni of *miscanthus* roots was associated with the type of fertilization and was generally the highest on the substrates fertilized with sewage sludge in a dose of 20 and 40 t/ha. Considerably, higher contents of all examined metals of the roots of plants, as compared with their above-ground part, were found. The largest differences were observed for Pb. The lead content of the roots was even 10 times as high as that of the above-ground biomass.

3.2. Bioaccumulation of metals

The BFC, determined as the quotient of the content of a given metal of plant to its content of soil, defines the ability of the plant to accumulate heavy metals while allowing for their initial soil content. The higher the values that it takes on, the higher the concentration of the element is found in the plant biomass as against its initial substrate content. As a basis for the assessment of bioaccumulation, a four-degree scale (as shown in Table 6) was adopted.

It should be noted that the accumulation factors for all the examined metals were considerably higher for roots than for above-ground parts (Figs. 1 and 2). The giant *miscanthus* roots accumulated Cd and Ni at a high level with small differences between individual soil improvement types, while Zn and Pb at a medium level. The above-ground parts accumulated Cd, Zn and Ni at a medium level and Pb at a low level. The BCF factors calculated for the above-ground parts, similar to their counterparts for the roots, indicate a small effect of fertilization on the accumulation of metals in plants. Among the investigated metals, BCFs characterized by the highest cadmium. In the above-ground parts, a very low lead accumulation was found, which was about 8–10 times lower than in the roots, depending on the fertilization.

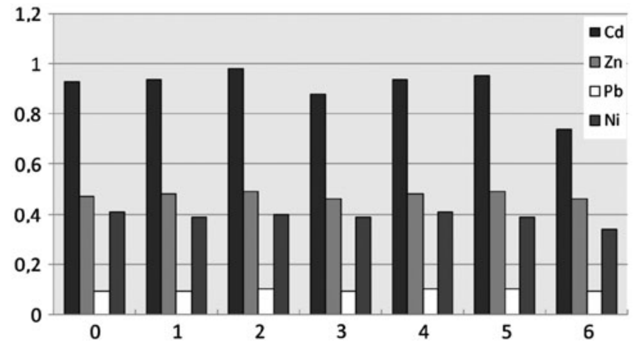

Fig. 1. The BCF for the ground *Miscanthus*.

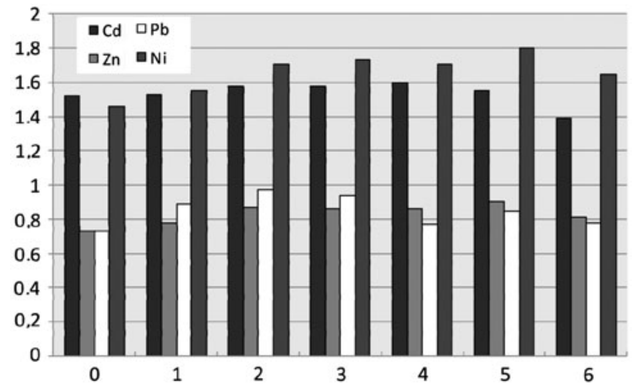

Fig. 2. The BCF for the roots of *Miscanthus*.

3.3. Immobilization of metals in the substrates

To estimate the degree of immobilization of metals in the substrates, the immobilization factors, I_f, were calculated. After completion of the three-year experiments, the heavy metal contents of the soil on which the *miscanthus* was grown were determined for different fertilization types. The obtained results were compared with the initial concentration in order to determine the degree of metals immobilization in the soil. The calculated immobilization factors are summarized in Table 7.

The factor value below 1 indicates that the applied fertilization has had the effect of moving heavy metals in the soil, while the one above 1 indicated that the introduced fertilizers contributed to the immobilization of the heavy metals.

The obtained I_f factor values indicate that fertilization with sewage sludge in a dose of 20 t/ha and with the Dano compost technology contributed to a certain extent to the immobilization of the investigated metals. The remaining fertilization modes had a neutral effect or, in rare cases, mobilized Zn and Cd only to a small extent. It should be noted that immobilization factor values generally oscillated around 1, which indicates a small effect of fertilization on changing the mobility of metals.

Table 6
The plant metal BCF [25]

Bioaccumulation factor	Accumulation degree
0.001–0.01	None
0.01–0.1	Low
0.1–1.0	Medium
1.0–10.0	High

Table 7
Immobilization factor of metals

Samples	Cd	Zn	Pb	Ni
1	0.92	0.90	1.00	1.10
2	0.80	0.84	0.85	0.90
2	1.12	1.00	1.00	0.93
4	0.98	0.87	0.98	0.90
5	0.75	1.00	1.05	0.85
6	1.10	1.05	1.15	1.12

4. Conclusions

The obtained investigation results have provided the basis for formulating the following findings:

(1) the heavy metal content in the aerial parts of *miscanthus* in the third year of the experiment depending on the fertilization ranged between 57.0 and 62.5 mg Zn/kg, Cd 1.65 and 1.95 mg/kg, Pb 28.4 and 38.0 mg/kg, Ni 13.5 and 17.0 mg/kg,

(2) the fertilization of soils with sewage sludge and compost had no significant influence on the contents of zinc, cadmium, lead and nickel in giant *miscanthus* biomass,

(3) considerably, higher contents of all examined metals of the roots of plants, as compared with their above-ground part, were found. The contents of Cd, Zn, Pb and Ni of *miscanthus* roots were associated with the type of fertilization and were generally the highest on the substrates fertilized with sewage sludge in a dose of 20 and 40 t/ha.

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