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RESEARCH ARTICLE

The use of reed canary grass and giant miscanthus in the phytoremediation of municipal sewage sludge

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Abstract The application of municipal sewage sludge on energy crops is an alternative form of recycling nutrients, food materials, and organic matter from waste. Municipal sewage sludge constitutes a potential source of heavy metals in soil, which can be partially removed by the cultivation of energy crops. The aim of the research was to assess the effect of municipal sewage sludge on the uptake of heavy metals by monocotyledonous energy crops. Sewage sludge was applied at doses of 0, 10, 20, 40, and 60 Mg DM · ha^{-1} once, before the sowing of plants. In a 6-year field experiment, the effect of four levels of fertilisation with sewage sludge on the uptake of heavy metals by two species of energy crops, reed canary grass (*Phalaris arundinacea* L.) of 'Bamse' cultivar and giant miscanthus (*Miscanthus* × *giganteus* GREEF et DEU), was analysed. It was established that the increasing doses of sewage sludge had a considerable effect on the increase in biomass yield from the tested plants. Due to the increasing doses of sewage sludge, a significant increase in heavy metals content in the energy crops was recorded. The heavy metal uptake with the miscanthus yield was the highest at a dose of 20 Mg DM · ha^{-1}, and at a dose of 40 Mg DM · ha^{-1} in the case of reed canary grass. Research results indicate that on account of higher yields, higher bioaccumulation, and higher heavy metal uptake, miscanthus can be selected for the remediation of sewage sludge.

Keywords Reed canary grass · Giant miscanthus · Heavy metals · Content · Uptake · Phytoremediation · Sewage sludge

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Introduction

The wealth of organic matter and nutrients contained in sewage sludge is most often wasted on waste dumps or in incinerating plants, both of them not solving the issue completely (Otero et al. 2008). According to national regulations, as from 2016, dumping sewage sludge will not be permitted. Thereafter, other more effective ways of managing this kind of waste should be considered (Act on waste 2012). One such solution is to use sewage waste for the purposes of remediation or land reclamation (Suchkova et al. 2014). In Poland, the lands in need of reclamation (devastated, degraded) cover more than 62 thousand hectares (Environment 2015). Their presence has been, to a major extent, a result of the mining, power, or chemical industries. Because of this, these lands are not suitable for the cultivation of plants intended for consumption. Thus, earmarking these lands for the cultivation of plants used to generate energy is undoubtedly an advantageous way of managing them, because such plants can meet both the need for the provision of energy and reclamation (Directive 2009/28/CE).

The cultivation of grass plants, among which one can name reed canary grass (*Phalaris arundinacea* L.) and giant miscanthus (*Miscanthus* × *giganteus* GREEF et DEU), as a direction for the development of crop production is justified not only by the need for increasing the share of energy coming from renewable energy sources, but it would make it possible to safely and efficiently manage municipal sewage sludge (Aşık and Katkat 2010; Fischer et al. 2011; Lewandowski et al. 2003).

Energy crops can give much higher yields of biomass after the application of municipal sewage sludge which is a source of many valuable nutrients and organic matter (Lindvall et al. 2012; Kołodziej et al. 2015). Sewage sludge is often characterised by its fertiliser value, which is close to the fertiliser value of manure and organic fertilisers (Antonkiewicz 2014; Komilis and Ham 2004). Publications in the field indicate that sewage sludge contains more than 2 % N, ca. 1 % P, and trace amounts of K, and that the contents of these elements is comparable to those in manure (Casado-Vela et al. 2006; Singh and Agrawal 2008; Gondek 2012). Moreover, using municipal sewage sludge in the cultivation of crops not intended for consumption has a positive effect on the biological and physicochemical properties of the soil profile. Heavy metals introduced into soil along with a dose of sewage sludge may constitute an environmental problem (Barrera et al. 2001; Singh and Agrawal 2008). Plants cultivated for energy have high nutrient requirements which—together with the large absorbing surface of their roots—result in nutrients contained in sewage sludge being absorbed and not causing environmental problems. Additionally, the large surface area of the root systems allows the absorption of large quantities of heavy metals, thus excluding or minimising the risk of contaminating ground water (Korzeniowska and Stanisławska-Glubiak 2015).

The scientific literature proposes using various plant species for the phytoextraction of heavy metals from the soil (Kusznierewicz et al. 2012; Chaney et al. 1997; Hseu et al. 2010). Therefore, it is suggested to use industrial crops not only for energy purposes, but also in the phytoextraction of heavy metals from the soil. The objective of the presented study includes the evaluation of the potential for the phytoextraction of heavy metals by plants grown on sewage sludge for energy purposes. A substantial yield-forming potential, high nutrient requirements and resistance to diseases and pests speak in favour of using reed canary grass and giant miscanthus for phytoextraction. This will allow these species to be effectively used in the remediation of sewage sludge (Borkowska and Molas 2012; Sheaffer et al. 2008).

Material and research methods

Research on the effect of increasing doses of municipal sewage sludge on the phytoextraction of heavy metals by energy crops was conducted in the years 2008–2013 on an area belonging to the municipal wastewater treatment plant in Janów Lubelski (50°43′17.7″N 22°22′08.0″E), which is located in south-eastern Poland.

Soil and municipal sewage sludge

The soil on which the experiment was set up was classified as clay loam (CL), (Table 1), (Polish Soil Classification 2011; Soil Survey Staff 2014). The soil had a slightly acid reaction, the content of available phosphorus and potassium was at a low level, and the content of available magnesium was at a very low level. Heavy metal content in the soil did not exceed permissible values when municipal sewage sludge was used for reclamation (Regulation 2002, 2015).

Municipal sewage sludge catalogued as organic waste under catalogue number 19 08 05 (Catalogue of waste 2014) was stabilised and hygienised. Municipal sewage sludge was used once; it was mixed with the surface soil layer at a depth of 20 cm in late autumn 2007. Due to the low potassium content in the sewage sludge on all the plots, supplementary potassium fertilisation of 100 kg $K \cdot ha^{-1}$ was applied in the form of 40 % potassium salt (KCl). The potassium dose covered the requirement of tested plants. No phosphorus fertilising was applied because the phosphorus content in the municipal sewage sludge covered the requirement of energy crops plants for this element. The heavy metal content determined in the sewage sludge did not exceed permissible values when using municipal sewage sludge for reclamation (Regulation 2002, 2015). No microbiological pollutants were detected in the sewage sludge used in the experiment.

Scheme and conditions of the experiment

The experiment was established as a randomised complete block design with two treatment factors: sewage sludge application and variety on plots with an area of 14.4 m^2 (3 × 4.8 m) with three replications. A dose of municipal sewage sludge was the first experimental factor. The experimental design consisted of five treatments: 1–control; 2–10 Mg DM; 3–20 Mg DM; 4–40 Mg DM; and 5–60 Mg DM of municipal sewage sludge per 1 ha. Two species of energy crops: reed canary grass (*Phalaris arundinacea* L.) and giant miscanthus (*Miscanthus* × *giganteus* GREEF et DEU) were the second experimental factor.

Giant miscanthus was used in the form of in vitro propagated seedlings which were acquired from VitroGen company from Poland and seeds of Swedish variety 'Bamse' were used in the case of reed canary grass. On 26 June 2008, 15 $kg \cdot ha^{-1}$

Table 1 Selected physical and chemical properties of the soil before experiment establishment and chemical composition of municipal sewage sludge used

Parameter	Unit	Content in the soil layer		Content in sewage sludge
		0–20 cm	20–40 cm	
Fraction 2–0.05 mm	%	32	23	–
Fraction 0.05–0.002 mm		39	45	–
Fraction < 0.002 mm		29	32	–
pH_{KCl}		6.29	6.44	6.04
Organic matter	$g \cdot kg^{-1}$ DM	14.5	14.1	594.0
Available phosphorus (P)	$mg \cdot kg^{-1}$ DM	30.9	29.6	2.25
Available potassium (K)		91.3	60.6	Bdl
Available magnesium (Mg)		27.6	24.6	0.28
Total chromium (Cr)	$mg \cdot kg^{-1}$ DM	9.66	9.89	25.4
Total nickel (Ni)		6.39	6.31	14.8
Total copper (Cu)		3.20	3.60	111
Total zinc (Zn)		31.97	31.00	1005
Total cadmium (Cd)		<0.27	<0.27	2.35
Total lead (Pb)		13.67	13.63	42.9

Bdl below detection level

reed canary grass seeds were sown into rows 12 cm apart at a depth of 2 cm. While micropropagated seedlings of miscanthus were planted on 22 April 2008 with spacing 0.75 × 0.8 m. The above-mentioned energy crops were harvested once in late autumn.

The determination of dry matter yield and the heavy metal content and soil enzymatic activity

Each year, the energy crops were harvested in autumn, at the turn of October and November. Every year, after harvest, the plant material from each plot (replication) was dried at 70 °C for 72 h in a dryer with forced air circulation. After drying, the air-dry mass of energy crops, and then the yield of air-dry mass was determined, in accordance with the commonly adopted methodology of field experiments (Ostrowska et al. 1991). Samples of the analysed crops were subjected to dry mineralization in a muffle furnace at a temperature of 450 °C (Ostrowska et al. 1991; Kusznierewicz et al. 2012). After incineration of the organic matter and digestion in a mixture of HNO_3 and $HClO_4$ (3:2, *v*/*v*), the contents of elements determined in the soil and in the sewage sludge were similar to the total contents (Ostrowska et al. 1991). After the mineralization of the plant and soil material, contents of Cr, Ni, Cu, Zn, Cd, and Pb were determined using an ICP-OES emission spectrometer (Jones and Case 1990).

Soil pH in 1 mol dm^{-3} KCl was determined with potentiometric method, available P and K content was determined after the Egner-Riehm method, and available Mg content was determined according to the Schachtschabel method (Ostrowska et al. 1991). Each year, during the vegetation season, in May, soil samples were collected from each plot (each repetition) from 0.20 cm depth, using the Egner's soil probe sampler, in order to assess the enzymatic activity of the soil. Analyses of the soil also involved determinations of the activities of enzymes which play a key role in the stable mineralization of organic matter and in supplying nutrients to the roots of energy crops. The activity of the studied enzymes was determined using the following methods: of dehydrogenases with a TTC (triphenyl tetrazolium chloride) substrate using the Thalmann method (Thalmann 1968); of acid phosphatase and alkaline phosphatase using the Tabatabai and Bremner method (Tabatabai and Bremner 1969); of urease using the Zantua and Bremner method (Zantua and Bremner 1975); and of protease using the Ladd and Butler method (Ladd and Butler 1972). The activity of dehydrogenases was given in cm^3 H_2, necessary for reducing TTC to TFP (triphenyl phormosan); of phosphatases–in mmols of p-nitrophenol (PNP) produced from sodium 4-nitrophenylphosphate; urease–in mg $N\text{-}NH_4^+$ generated from hydrolyzed urea; protease–in mg tyrosine developed from sodium caseinate. The results of the analyses of enzymatic activity of the soil were presented in the paper as means for the 6 years of studies i.e. for the 2008–2013 period.

Analytical quality control

The ICP-OES Optima 7300 DV, atomic emission spectrometer from Perkin Elmer Company was used for the determination of heavy metals in plant and soil materials. Determinations in each of the analysed samples were carried out in three replications. For the data acquisition of the samples, a quantitative analysis mode was used. The scanning of each single sample was repeated three times to gather reasonably good results. During measurements, care was taken to avoid memory effect and therefore a wash-out time of 0.5 min was used. The accuracy of the analytical methods was verified based on certified reference materials: CRM IAEA/V–10 Hay (International Atomic Energy Agency), CRM–CD281–Rey Grass (Institute for Reference Materials and Measurements), CRM023-050–Trace Metals–Sandy Loam 7 (RT Corporation).

Calculations and statistical analysis of the results

Due to the cultivation of various plant species and the changeability of conditions in individual years, the heavy metal content in the total plant yield is presented as a weighted mean. The heavy metal uptake (U) was calculated as the product of dry matter yield (Y) and the nutrient content (C), according to the formula: $U = Y \cdot C$. The heavy metal balance (B) was calculated from the difference between the amount of metals introduced (I) with the dose of sewage sludge and the amount of metals uptake (U) with the plant yield, according to the formula: $B = I - U$. The simplified balance did not take into account the input of heavy metals with atmospheric precipitation, nor the leaching of heavy metals into the deeper layer of the soil profile. The recovery of heavy metals shown in the balance is the percentage uptake of heavy metals in relation to the amounts introduced into the soil along with the municipal sewage sludge.

The statistical analysis of the research results was conducted using a Microsoft Office Excel 2003 spreadsheet and Statistica package version 10 PL. The statistical evaluation of the result variability was conducted using the two-factor analysis of variance. The significance of differences among mean values was verified using *t* Tukey's test at the significance level $\alpha \leq 0.05$. For selected (parameters) relations, the value of the Pearson linear correlation index (r) was computed at a significance level of $p < 0.05$. A maximum 5 % level of dispersion between measurements in chemical analysis was adopted in the study.

Research results

Weather conditions during the experiment

Mean air temperature and precipitation during the experiment in 2008–2013 were obtained from the Agrometeorological Observatory of University of Life Sciences in Lublin. The mean air temperature during the vegetation period in 2008–2013 was 14.6 °C (higher than the average for a long-term period by 1.4 °C), while the total precipitation exceeded the average by 64.9 mm. Each year of the experiment was characterised by considerable variability. The lowest precipitation and men air temperature were found in the first year of the experiment, while the best conditions for growth and development of giant miscanthus and reed canary grass were recorded in 2010 and 2013. In June 2009, high precipitation but at the same time low air temperature were recorded, whereas in July and August drought periods were observed. What is more, a temperature 1.1 °C lower than in the multiannual period was recorded in October 2009, when there was severe hoarfrost, with extremely heavy snowfall (103.6 mm total precipitation). Extremely unfavourable weather conditions (high air temperature and insufficient precipitation especially from July to September) were recorded in the fifth year of the experiment (2012), while from February to June 2011 there was insufficient precipitation and the means of the multiannual period were exceeded two-fold in July.

Yield of plants

Mean yields of plant dry matter, obtained in the years 2008–2013, depending on the treatment, varied within the following range: 5.3–17.3 Mg $DM \cdot ha^{-1}$ (Table 2). The lowest yield of plants was obtained in the control treatment in which sewage sludge had not been applied. The multi-year research cycle showed that the single use of sewage sludge (in the amount of 10–60 Mg $DM \cdot ha^{-1}$) in the experiment brought about a significant increase in the yield of plants as compared with the control. Considerable variation in the amount of yield between plant species was also found. A dose of 10 Mg sewage sludge DM caused an increase in the mean yield of reed canary grass and giant miscanthus by, respectively, over 10 and 8 % in relation to the control. Doubling the dose of sewage sludge (20 Mg $DM \cdot ha^{-1}$) led to an increase in the yield of the species by, respectively, over 96 and 12 % compared with the control treatment. The subsequent doubling of the dose of sewage sludge to 40 Mg DM ha^{-1} influenced significantly the increase in the yield of only reed canary grass, by over one-and-a-half times as compared with the control treatment. A subsequent increase in the dose of sewage sludge to 60 Mg DM influenced the increase in the yield of reed canary grass by over 81 % as compared with the control. In the case of giant miscanthus, it was found that doses of 40 and 60 $Mg \cdot ha^{-1}$ sewage sludge DM caused a decrease in yield by, respectively, over 17 and 26 % as compared with the control. The research shows that in comparison to giant miscanthus, reed canary grass responded with a higher increase in yield under the influence of sewage sludge.

The yield of plant biomass obtained in the years 2008–2013 was varied (Table 2). The lowest yields were obtained in the first year of the research, which was a result of the late sowing of reed canary grass, the long period of miscanthus emergence, and the necessity for the plants to put down roots. Higher yields were obtained in the second year of the research

Table 2 Yield of energy crops after experiment (Mg dry matter · ha^{-1})

Treatments	Years						Mean for years
	2008	2009	2010	2011	2012	2013	
Phalaris arundinacea							
Sludge dose (Mg DM · ha^{-1})							
0	4.5	7.9	5.1	4.8	4.6	4.7	5.3
10	5.6	10.2	6.3	4.3	4.3	4.3	5.8
20	9.7	16.0	10.7	9.4	8.2	8.3	10.4
40	12.9	21.3	13.9	11.5	10.3	10.4	13.4
60	10.1	19.1	12.1	5.6	5.3	5.3	9.9
Mean	8.5	14.9	9.6	7.1	6.5	6.6	8.9
CV (%)	37.6	35.5	36.5	41.9	36.9	37.1	35.1
Miscanthus giganteus							
Sludge dose (Mg DM · ha^{-1})							
0	3.0	13.7	16.0	15.6	21.2	22.5	15.3
10	3.0	14.8	17.4	16.8	22.5	24.8	16.6
20	3.0	15.3	17.8	18.7	23.8	25.3	17.3
40	2.6	10.9	12.8	13.3	17.6	18.9	12.7
60	2.1	10.2	11.8	12.7	15.0	16.0	11.3
Mean	2.7	13.0	15.1	15.4	20.0	21.5	14.6
CV (%)	13.0	16.5	16.8	15.3	17.2	17.3	16.3
Mean for dose (of sewage sludge)							
Sludge dose (Mg DM · ha^{-1})							
0	3.7	10.8	10.5	10.2	12.9	13.6	10.3
10	4.3	12.5	11.9	10.6	13.4	14.5	11.2
20	6.3	15.6	14.2	14.1	16.0	16.8	13.8
40	7.8	16.1	13.3	12.4	13.9	14.6	13.0
60	6.1	14.7	11.9	9.1	10.1	10.6	10.4
LSD for dose	0.20	0.27	0.28	0.39	0.45	0.25	0.15
LSD for species	0.32	0.43	0.44	0.62	0.72	0.39	0.24
LSD for interaction	0.45	0.61	0.63	0.88	1.02	0.56	0.33

CV variability coefficient, *LSD* least significant differences

and in the years that followed. In the case of reed canary grass, the highest yields were obtained in the second and third year of the research. Yields of this species in the following research years remained at the same level. Yields of giant miscanthus also increased systematically. Depending on the treatment, the amount of miscanthus yield in 2013 was between 6.5 and 7.5 times higher than the yield obtained in the first vegetation year. Moreover, the author's own research shows that the mean yield of miscanthus in the control treatment was over 1.9-fold higher than the yield of reed canary grass. The increasing doses of sewage sludge made the differences in the amount of yield between plant species smaller. The conducted research shows that the highest yield-forming effect for reed canary grass was obtained in the treatment where 40 Mg · ha^{-1} sewage sludge DM had been applied, and in the case of giant miscanthus −20 Mg · ha^{-1} sewage sludge DM.

Heavy metal content in plants

Municipal sewage sludge was a potential source of heavy metals for energy crops (Table 1). The contents of Cr, Ni, Cu, Zn, Cd, and Pb detected in the sewage sludge were, respectively, over 1.6, 1.3, 33.6, 30.4, 7.7, and 2.1-fold higher than in the soil surface layer. The applied increasing doses of sewage sludge influenced an increase in the heavy metals content in the studied plants (Table 3). The lowest dose of sewage sludge (10 Mg DM · ha^{-1}) led to a significant increase in the content of the studied heavy metals in the plants as compared with the control treatment.

Subsequent doses of sewage sludge also caused a significant increase in the content of the studied heavy metals in the energy crops. The greatest increases in the content of these elements were recorded after the application of sewage sludge

Table 3 Weighted average of heavy metals content in energy crops ($mg \cdot kg^{-1}$ DM)

Treatments	Cr	Ni	Cu	Zn	Cd	Pb
Phalaris arundinacea						
Sludge dose (Mg DM · ha^{-1})						
0	0.93	1.04	2.56	26.76	0.07	0.46
10	1.07	1.29	3.12	32.82	0.11	0.48
20	1.32	1.45	4.21	38.33	0.13	0.57
40	1.46	1.91	5.35	42.76	0.17	0.71
60	1.70	2.24	6.34	48.26	0.27	0.85
Mean	1.30	1.58	4.32	37.79	0.15	0.61
CV (%)	22.2	28.2	33.4	20.7	46.1	24.7
Miscanthus giganteus						
Sludge dose (Mg DM · ha^{-1})						
0	0.91	0.58	1.17	14.14	0.09	0.45
10	1.05	0.70	2.89	23.69	0.13	0.51
20	1.16	0.80	4.33	28.83	0.19	0.58
40	1.38	0.95	4.69	37.01	0.22	0.67
60	1.55	1.29	6.01	39.68	0.28	0.83
Mean	1.21	0.86	3.82	28.67	0.18	0.61
CV (%)	20.0	29.2	45.0	33.5	36.3	22.8
Mean for dose (of sewage sludge)						
Sludge dose (Mg DM · ha^{-1})						
0	0.92	0.81	1.87	20.45	0.08	0.46
10	1.06	1.00	3.00	28.26	0.12	0.50
20	1.24	1.13	4.27	33.58	0.16	0.57
40	1.42	1.43	5.02	39.88	0.19	0.69
60	1.63	1.76	6.18	43.97	0.27	0.84
LSD for dose	0.033	0.017	0.094	0.903	0.007	0.014
LSD for species	0.052	0.026	0.148	1.429	0.012	0.022
LSD for interaction	0.074	0.037	0.209	2.020	0.017	0.032

CV variability coefficient, *LSD* least significant differences

in a dose of 60 Mg DM · ha^{-1}. In reed canary grass, they amounted to, respectively, 82 % for Cr, 84 % for Pb, and 285 % for Cd in relation to the control treatment. In the case of miscanthus biomass, the highest increase in heavy metal content (at the highest dose of sewage sludge) reached 413 % for Cu, 180 % for Zn, and 122 % for Ni in relation to the control treatment. The research shows that the content of Cu increased the most, followed by Cd, Zn, and Ni; the lowest increases in the content were recorded in the case of Cr and Pb.

Data presented in Table 3 indicate that reed canary grass accumulated more Cr, Ni, Cu, Zn, and Pb than miscanthus. Higher contents in miscanthus were found only in the case of Cd (in comparison to reed canary grass). Therefore, the author's own research shows that the increasing doses of sewage sludge diversify the content of heavy metals in plants.

Heavy metal uptake by plants

Heavy metal uptake by plants, as a sum of the entire research period (2008–2013), is presented in Table 4. The amount of elements collected from treatments depended on the amount of yield and on the content of a given element in the yield (Tables 2 and 3). The increasing doses of municipal sewage sludge led to the increased uptake of Cr, Ni, Cu, Zn, Cd, and Pb with the yield of the energy crops (Table 4). The heavy metal uptake with the miscanthus yield was the highest at a dose of 20 Mg DM · ha^{-1}. After the application of 20 Mg DM · ha^{-1} of sewage sludge, the uptake of Cr, Ni, Cu, Zn, Cd, and Pb by miscanthus was, respectively, over 44, 55, 316, 130, 125, and 43 % higher in comparison to the control treatment where plants without the additional sewage sludge were cultivated. In the case of reed canary grass, the highest uptake of Cr, Ni, Cu, Zn, and Pb was at a dose of 40 Mg DM · kg^{-1}, and of Cd—at a dose of 60 Mg DM · ha^{-1} (Table 4). At the

Table 4 Heavy metals uptake by energy crops ($g \cdot ha^{-1}$)

Treatments	Cr	Ni	Cu	Zn	Cd	Pb
Phalaris arundinacea						
Sludge dose (Mg DM · ha^{-1})						
0	29.3	32.8	81.1	846.8	2.3	14.6
10	37.4	45.2	109.1	1147.0	3.7	16.9
20	82.2	90.2	262.2	2384.5	8.2	35.3
40	117.5	152.9	429.5	3431.4	13.8	56.6
60	98.0	128.8	365.1	2777.5	15.3	48.9
Mean	72.9	90.0	249.4	2117.4	8.6	34.4
CV (%)	48.7	53.4	56.9	47.9	62.5	50.4
Miscanthus giganteus						
Sludge dose (Mg DM · ha^{-1})						
0	83.3	53.6	107.9	1300.6	8.7	41.6
10	104.2	69.7	286.6	2353.5	13.3	50.5
20	120.3	83.1	448.9	2993.9	19.5	59.7
40	104.6	72.3	356.8	2813.3	16.5	51.0
60	104.8	87.1	406.9	2686.8	18.7	56.1
Mean	103.4	73.1	321.4	2429.6	15.3	51.8
CV (%)	12.7	16.9	38.6	26.0	27.4	12.8
Mean for dose (of sewage sludge)						
Sludge dose (Mg DM · ha^{-1})						
0	56.3	43.2	94.5	1073.7	5.5	28.1
10	70.8	57.4	197.8	1750.2	8.5	33.7
20	101.3	86.7	355.5	2689.2	13.8	47.5
40	111.0	112.6	393.1	3122.3	15.1	53.8
60	101.4	107.9	386.0	2732.1	17.0	52.5
LSD for dose	3.35	1.86	6.09	79.71	0.66	1.42
LSD for species	5.30	2.94	9.64	126.04	1.04	2.25
LSD for interaction	7.50	4.16	13.63	178.24	1.47	3.18

CV variability coefficient, *LSD* least significant differences mentioned doses of sewage sludge, the uptake of Cr, Ni, Cu, Zn, Cd, and Pb was, respectively, over 301, 366, 429, 305, 554, and 288 % as compared with the control treatment.

The research shows that at a dose of 20 $Mg \cdot ha^{-1}$ sewage sludge DM, miscanthus took up significantly more Cr, Cu, Zn, Cd, and Pb than reed canary grass. At a dose of 40 $Mg \cdot ha^{-1}$ sewage sludge DM, reed canary grass took up significantly more Cr, Ni, Cu, Zn, and Pb than miscanthus. The research revealed that the lower the dose of sewage sludge, the lower the uptake of these elements with the yield of the energy crop biomass. The lowest heavy metal uptake was recorded in the control treatment, which was mainly connected with the lowest yield and a low content of these elements in the yield of plants (Tables 2 and 3). Despite the decrease in yielding of miscanthus at a dose of 40 $Mg \cdot ha^{-1}$sewage sludge DM, and of reed canary grass at a dose of 60 $Mg \cdot ha^{-1}$ sewage sludge DM, amounts of discharged heavy metals were higher as compared with the control treatment. It was a result of the increased concentration of these elements in the plant biomass (Table 3).

Simplified balance and phytoremediation of heavy metals

In addition, using municipal sewage sludge for the fertilisation of energy crops can possibly increase the accumulation of heavy metals in the soil and their introduction into plants. Understanding heavy metal circulation will allow for a better assessment of the risk connected with the fertiliser use of these organic wastes. Such an assessment can be done on an approximate basis, for instance basing on the simplified balance of heavy metals and on phytoremediation. Heavy metal balances in individual treatments were varied (Table 5).

This outcome was influenced by the dose and content of a metal in the sewage sludge, total metal uptake with the yield of plants. The balance of all the heavy metals in the control treatments where sewage sludge had not been applied was always negative as the amounts of metals in soils were exhausted. Additionally, neither the input of heavy metals with atmospheric precipitation nor the leaching of heavy metals into deeper layers of soil was considered in the balance.

The heavy metal balance in the treatments fertilised with sewage sludge in doses of 10–60 $Mg \cdot ha^{-1}$ was always positive. Municipal sewage sludge was the material that enriched the soil with trace elements to the greatest degree. Such a large excess of metals supplied with subsequent sewage sludge doses caused an increase in content of these metals in the soil, which explains the positive balance of the heavy metals.

Generally, the highest phytoremediation of heavy metals was recorded in the treatments where the lowest doses of sewage sludge i.e. 10 Mg $DM \cdot ha^{-1}$ were applied (Table 5). In addition, in comparison to reed canary grass, miscanthus phytoextracted a greater percentage of the heavy metals, which was associated with higher yielding and uptake of these elements. The lowest phytoremediation was recorded in the treatments where the highest dose of sewage sludge was applied, which was undoubtedly connected with the highest enrichment of the soil with heavy metals and with the lower yielding of the plants.

When comparing the percent of phytoremediation of heavy metals by miscanthus, regardless of the sewage sludge dose, one can establish a series in the following order (starting from the highest value): Cd, Ni, Cr, Cu, Zn, Pb. The mentioned series indicates that Cd was recovered to the greatest degree, in approximately 57 %, and Pb in the smallest degree, approximately 12 %.

Soil enzymatic activity

Soil enzymatic activity is an important parameter that shows the state of the natural environment and informs about the biochemical process taking place in it. It is a parameter which reflects the degree and extent of pollution occurring in this environment (Wang et al. 2006). The high total values of activities of all the enzymes in this experiment confirm the efficacy of using sewage sludge as an organic fertiliser in the cultivation of energy crops, as well as the positive effect of this fertilisation on soil microorganisms and on soil biological activity (Table 6).

The activity of the studied enzymes increased progressively with an increase of the dose of sewage sludge introduced into soils of all the experimental plots, which was associated with the quantity of carbon substrates available for microorganisms and enzymes. In the years 2008–2013, under conditions of application of the highest sewage sludge dose, dehydrogenase activity was approximately four-fold higher, and activities of the studied phosphatases as well as urease and protease were approximately three-fold higher than in the soil of the control treatments. This research confirms significant relationships between the activity of soil enzymes and the sewage sludge dose ($r = 0.8564$–0.9791).

Apart from biogenes, organic colloids, and soil microorganisms which act stimulatingly on soil enzymes, considerable amounts of heavy metals were also introduced into the soil profile with the sewage sludge (Table 5). The author's research shows, therefore, strong relationships between the activities of soil enzymes with the content of heavy metals in plants ($r = 0.4438$–0.9469) and the heavy metal uptake by plants ($r = 3955$–0.7529). The research shows that under the conditions of the conducted experiment soil enzymatic activity is strictly correlated with heavy metals. In their active centre, enzymes contain some heavy metals. That is why a small increase in the content of heavy metals in soil may

Table 5 Simplified balance of heavy metals after 6 years of research

Treatments	Introduced	Uptake g ha^{-1}	Balance	Recovery Percent	Uptake g ha^{-1}	Balance	Recovery Percent
		Phalaris arundinacea			*Miscanthus giganteus*		
Cr							
Sludge dose (Mg DM · ha^{-1})							
0	0	29	−29	0	83	−83	0
10	254	37	217	15	104	150	41
20	508	82	426	16	120	388	24
40	1016	118	898	12	105	911	10
60	1524	98	1426	6	105	1419	7
Ni							
Sludge dose (Mg DM · ha^{-1})							
0	0	33	−33	0	54	−54	0
10	148	45	103	31	70	78	47
20	296	90	206	30	83	213	28
40	592	153	439	26	72	520	12
60	888	129	759	14	87	801	10
Cu							
Sludge dose (Mg DM · ha^{-1})							
0	0	81	−81	0	108	−108	0
10	1110	109	1001	10	287	823	26
20	2220	262	1958	12	449	1771	20
40	4440	429	4011	10	357	4083	8
60	6660	365	6295	5	407	6253	6
Zn							
Sludge dose (Mg DM · ha^{-1})							
0	0	847	−847	0	1301	−1301	0
10	10,050	1147	8903	11	2353	7697	23
20	20,100	2385	17,715	12	2994	17,106	15
40	40,200	3431	36,769	9	2813	37,387	7
60	60,300	2778	57,522	5	2687	57,613	4
Cd							
Sludge dose (Mg DM · ha^{-1})							
0	0	2	−2	0	9	−9	0
10	24	4	20	16	13	10	57
20	47	8	39	17	20	27	42
40	94	14	80	15	16	78	18
60	141	15	126	11	19	122	13
Pb							
Sludge dose (Mg DM · ha^{-1})							
0	0	15	−15	0	42	−42	0
10	429	17	412	4	51	379	12
20	858	35	823	4	60	798	7
40	1716	57	1659	3	51	1665	3
60	2574	49	2525	2	56	2518	2

stimulate their activity. It indicates that the amount of heavy metals introduced into the soil with the sewage sludge did not have a negative effect on the soil's biological state.

Table 6 Soil enzymatic activity (average from 2008 to 2013)

Treatments	Dehydrogenases activity ($cm^3\ H_2 \cdot kg^{-1}\ day^{-1}$)	Urease activity ($mg\ N\text{-}NH_4^+ \cdot kg^{-1} \cdot h^{-1}$)	Protease activity ($mg\ of\ tyrosine \cdot kg^{-1} \cdot h^{-1}$)	Acid phosphatase activity ($mmol\ PNP \cdot kg^{-1} \cdot h^{-1}$)	Alkaline phosphatase activity ($mmol\ PNP \cdot kg^{-1} \cdot h^{-1}$)
Phalaris arundinacea					
Sludge dose ($Mg\ DM \cdot ha^{-1}$)					
0	4.0	8.3	10.5	27.5	19.9
10	4.8	10.5	12.6	29.1	21.0
20	5.4	12.9	17.5	41.7	25.8
40	7.1	13.1	25.8	83.5	61.5
60	15.4	22.7	33.4	76.2	53.9
Mean	7.3	13.5	20.0	51.6	36.4
CV (%)	58.8	39.4	44.7	47.6	50.8
Miscanthus giganteus					
Sludge dose ($Mg\ DM \cdot ha^{-1}$)					
0	4.4	7.7	11.8	29.2	20.1
10	6.0	9.0	14.2	30.4	20.3
20	7.5	10.3	19.2	40.4	27.2
40	10.2	13.9	27.1	103.6	66.7
60	17.3	26.5	37.1	95.5	61.8
Mean	9.1	13.5	21.9	59.8	39.2
CV (%)	52.3	52.7	44.4	59.4	58.1
Mean for dose (of sewage sludge)					
Sludge dose ($Mg\ DM \cdot ha^{-1}$)					
0	4.2	8.0	11.1	28.3	20.0
10	5.4	9.8	13.4	29.8	20.7
20	6.5	11.6	18.4	41.0	26.5
40	8.7	13.5	26.5	93.5	64.1
60	16.3	24.6	35.2	85.8	57.8
LSD for dose	0.52	1.04	1.3	6.85	5.37
LSD for species	0.82	1.64	2.0	10.84	8.49
LSD for interaction	1.16	2.32	2.9	15.33	12.01

CV variability coefficient, *LSD* least significant differences

Discussion

The chemical composition of energy crops does not always meets standards set for biofuels. That is why these crops can be recommended for the phytoextraction of heavy metals from organic waste or for the phytoremediation of chemically polluted soils (Collura et al. 2006; Monti et al. 2008; Regasa and Wortmann 2014; Pidlisnyuk et al. 2014).

Yield of energy crops

The size of obtained yields of energy crops was directly related to the level of fertilisation with municipal sewage sludge. The author's own research shows that the mean yield of miscanthus in the control treatment was over 1.9-fold higher than the yield of reed canary grass, and the increasing doses of sewage sludge made the difference in the yielding of these plants smaller. The results of the presented research suggest that giant miscanthus has a higher yielding potential in comparison to the size of yield of reed canary grass, which is of great importance in phytoextraction processes (Borkowska and Molas 2013; Li et al. 2014; Nsanganwimana et al. 2014). The yields of biomass of reed canary grass that were obtained in the author's own research were significantly higher than the ones described in the literature (Christian et al. 2006; Sahramaa and Jauhiainen 2003). They result from plantation fertilisation with sewage sludge that took place in the author's own research but was not taken into account in other authors' experiments. This is because sewage sludge is a source of valuable organic matter and nutrients (Fischer et al. 2011; Singh and Agrawal 2008). Research by Lindvall et al. (2012) also confirms that sewage sludge influences the increase in yields of biomass of reed canary grass and other energy crops. In our study, we have also found the effect of climatic conditions on the quantity of yield of energy crops again confirmed in the research by Borkowska and Molas (2012; 2013). The studies by Borkowska and Molas (2013) as well as by Kołodziej et al. (2015) provided evidence that the best effects on the growth and development of plants are exerted by nitrogen, phosphorus, and potassium. The sewage sludge used for fertilising energy crops is a perfect source of these elements, particularly of nitrogen and phosphorus (Gondek 2012; Kołodziej et al. 2015). However, sewage sludge contains too little potassium to meet the needs of energy crops, therefore it should be applied in mineral form after a prior evaluation of the soil resources of this component (Aşık and Katkat 2010; Gondek 2012).

Content of heavy metals

It is recommended to use plant species that accumulate substantial amounts of heavy metals (by extracting them from the subsoil) for the phytoextraction of heavy metals from the soil (Audet and Charest 2007; Meagher 2000; Pilon-Smits 2005). Many authors confirm that it is possible to use energy plants (grasses) for the phytoremediation of soils polluted with heavy metals (Korzeniowska and Stanisławska-Glubiak 2015; Li et al. 2014; Maestri et al. 2010). The application of miscanthus and reed canary grass for the phytoextraction of heavy metals from sewage sludge is substantiated on account of their considerable potential phytoextraction power. The author's own research indicates that reed canary grass accumulated more Cr, Ni, Cu, Zn, and Pb in the above-ground parts than miscanthus. That is why reed canary grass can be used as a good phytoextractor. On the other hand, miscanthus accumulated more Cd than reed canary grass, which may be of great importance in the phytoextraction of this toxic metal from organic waste. Similarly, research by Fernando et al. (2004) confirms that miscanthus is a plant with potential and can be used for the phytoextraction of heavy metals from sewage sludge. Research by Li et al. (2014) confirms that miscanthus is tolerant of heavy metals, especially to high concentrations of Zn and Cr in the environment. Another piece of research by Lindvall et al. (2015) confirms that reed canary grass may accumulate substantial amounts of Pb, Zn and Cd in its biomass, and which then are passed to ash. Again, studies by Kusznierewicz et al. (2012) indicate that the common cabbage which has a high yield potential can also be used for the phytoextraction of Cd from chemically contaminated soil.

The heavy metal content in soil can also be affected by the course of climatic conditions which can greatly modify soil properties and—by the same token—affect the size of plant yield and the levels of these elements in the plants (Borkowska and Molas 2013; Kołodziej et al. 2015). In the climatic conditions prevailing in Poland, the yield sizes of the studied energy crops were diversified, whereas the uptake of these elements into the biomass of plants increased systematically in particular years of vegetation.

Heavy metal uptake

Energy crops are average in terms of the quantities of metals accumulated in their tissues, but the total uptake of metals in a large yield of biomass can be even greater than the effects of hyperaccumulation plants (Antonkiewicz and Para 2015; Kusznierewicz et al. 2012). Research by Korzeniowska and Stanisławska-Glubiak (2015) as well as by Pogrzeba et al. (2011) showed that *Miscanthus × giganteus* is a weaker phytoextractor of heavy metals than *Spartina pectinata*. The author's research shows that giant miscanthus fertilised with 20 $Mg \cdot ha^{-1}$ sewage sludge DM had a higher uptake of Cr, Cu, Zn, Cd, and Pb than reed canary grass. Research by Barbu et al. (2010) showed that miscanthus uptakes Pb and Cd in quantities comparable to the ones recorded in this paper. Other authors' research studies (Vymazal et al. 2007) also confirm that reed canary grass accumulates substantial amounts of Cr, Ni, Cu, Zn, Cd, and Pb, and can be a good phytoextractor. In research carried out by Sidle et al. (1976), it was also shown that reed canary grass fertilised with municipal waste makes good use of nutrients and uptakes heavy metals. However, other studies by Nsanganwimana et al. (2014) and Antonkiewicz (2014) showed that excessive heavy metal contamination of sewage sludge can be a powerful factor limiting the biomass increment, and—as a consequence—reduces the uptake of heavy metals by plants.

Balance of heavy metals

In this research, a simplified balance and phytoremediation of heavy metals from municipal sewage sludge was made. However, it should be emphasised that the balance of heavy metals did not cover the influx of metals from other sources, including precipitation. The positive balance of heavy metals in soil is indicative of chemical pollution of soil which should be immediately subjected to phytoremediation and phytoextraction processes (Gardea-Torresdey et al. 2005; Padmavathiamma and Li 2007). What is more, a part of heavy metals can be leached deep into the soil profile (Page et al. 2014). Zn and Cu belong to microelements necessary for the proper increase and growth of plants, hence an increase in their content in soil as a result of using sewage sludge may have a positive character, but to certain limits. On the other hand, an increase in content of other heavy metals including Cd, Pb, Cr, and Ni may influence the chemical degradation of soil (Ahmed et al. 2010; Ahmad and Ashraf 2011; Fischer et al. 2011).

The author's research shows that among heavy metals zinc had the lowest recovery. This was due to the fact that, among other things, this metal was introduced in the largest amounts with the sewage sludge, and it is also strongly complexed by organic matter of sewage sludge and soil, which finds confirmation in the research by Padmavathiamma and Li (2007) and Pavel et al. (2014) and Milinovic et al. (2014). Research studies conducted by Březinová and Vymazal (2015), Liu et al. (2015), and Pavel et al. (2014) confirm that miscanthus and reed canary grass recover similar amounts of heavy metals from soil, sewage sludge, and from solutions. The intensive uptake of trace elements from the soil fertilised by sewage sludge and the accumulation of these elements in the biomass of energy plants create new environmental issues (Pidlisnyuk et al. 2014). The residual material after incineration or other methods of processing biomass can thus contain large quantities of metals which qualify them as environmentally hazardous waste. As so-called bio-ores they should be transferred to ore-processing plants in order to avoid their deposition in ash dumps of the power industry (Boominathan et al. 2004; Ghosh and Singh 2005).

Soil enzymatic activity

The ultimate goal of each remediation process is not only to remove contaminants, but also to restore soil functionality to the level of uncontaminated control soil (Audet and Charest 2007). The activity of soil microorganisms is also influenced by the level of heavy metals that, in many cases, has a negative effect on soil microflora and macrofauna (Epelde et al. 2009; McGrath et al. 1995). The direction and intensification of the observed changes depended on the enzyme type, which is associated both with the content of specific substrates for enzymatic responses in the soil as well as with the sensitivity and resistance of individual enzymes to environmental factors (Wang et al. 2006).

The author's research shows that increasing sewage sludge doses had a significant stimulating influence on the soil enzymatic activity, which is of great importance for the intensity of heavy metal uptake in phytoremediation processes (Epelde et al. 2009). In their research, Sastre et al. (1996) and Singh et al. (2011) also observed the positive effect of the increasing sewage sludge doses on the soil enzymatic activity. In their research, Fernández et al. (2009) also obtained the highest activity of the studied enzymes of dehydrogenase, urease, and protease in the treatments where the highest sewage sludge doses were applied. Similarly to the activity obtained by the author of this research, these authors obtained the lowest activity of the studied enzymes in the control plots where sewage sludge was not applied.

Conclusions

In comparison to giant miscanthus, reed canary grass responded with a higher increase in yield under application of sewage sludge, whereas giant miscanthus had a higher yielding potential than reed canary grass. The applied increasing doses of sewage sludge influenced an increase in heavy metal content in the crops. Reed canary grass accumulated more Cr, Ni, Cu, Zn, and Pb than miscanthus. In comparison to reed canary grass, higher contents in miscanthus were found only in the case of Cd. At a dose of 20 $Mg \cdot ha^{-1}$ sewage sludge DM, giant miscanthus took up significantly more Cr, Cu, Zn, Cd, and Pb than reed canary grass. At a dose of 40 $Mg \cdot ha^{-1}$ sewage sludge DM, reed canary grass took up significantly more Cr, Ni, Cu, Zn, and Pb than miscanthus. From the tested energy crops, miscanthus was characterised by the highest uptake of heavy metals from the sewage sludge, which was due to a substantial yielding potential of this crop. The highest percent of phytoremediation of heavy metals by miscanthus concerned Cd, followed by Ni, Cr, Cu, Zn, Pb. This series indicates that Cd was recovered to the greatest degree (in approximately 57 %), and Pb in the smallest degree (approximately 12 %). Based on uptake, the phytoremediation of heavy metals, one can choose miscanthus as a potential crop for the phytoremediation of municipal sewage sludge.

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