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Original Research

Using Jerusalem Artichoke to Extract Heavy Metals from Municipal Sewage Sludge Amended Soil

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Abstract

The aim of our research was to assess the effectiveness of phytoextraction of heavy metals from sewage sludge by 'Rubik' and 'Albik' varieties of Jerusalem artichoke. The 6-year field experiment involved four levels of fertilization with sewage sludge at doses of 0, 10, 20, 40, and 60 Mg DM sludge · ha^{-1}. The research evaluated the amount of Jerusalem artichoke yield as well as the uptake and use of heavy metals by the Jerusalem artichoke varieties.

It was established that increasing doses of sewage sludge had significantly increased the yield of the Jerusalem artichoke varieties. Increasing doses of sewage sludge also had a significant effect on the increase in the content of heavy metals in aboveground parts of plants. The highest heavy metal uptake with the yield of the Jerusalem artichoke varieties was observed at a dose of 60 Mg DM · ha^{-1}. Among the tested varieties of Jerusalem artichoke, Albik had higher yield, higher content and uptake of heavy metals, and greater recovery of these elements as compared to Rubik. Therefore, based on the obtained research results, Albik can be recommended for phytosequestration of heavy metals from sewage sludge amended soil.

Keywords: Helianthus tuberosus, heavy metals, phytoremediation, sewage sludge, varieties

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Introduction

Some plants have a special tolerance to high concentrations of heavy metals in tissues, without harming growth and development [1-2]. They belong to the so-called hyperaccumulators, which have been found to accumulate large amounts of heavy metals [3-4]. It is recommended that phytoremediation of soils contaminated with heavy metals be carried out using plant species of different applications, including grasses and energy crops [5-7].

Currently, Jerusalem artichoke (*Helianthus tuberosus* L.), also known as topinambur, is becoming increasingly popular among researchers of phytoremediation plants. This plant comes from North America and belongs to the Asteraceae family [8]. Jerusalem artichoke is a species that can be used in various branches of the food, pharmaceutical, chemical, and power industries [9-10]. Topinambour biomass is used as a raw material for production of biogas and biodiesel [11-12]. The Jerusalem artichoke, also called earth apple, is a perfect source of fructose and a raw material for ethanol production [13].

A high-yield potential, easy cultivation, low cost of setting up a plantation, and a substantial ability to adapt to climatic conditions speak in favor of adopting Jerusalem artichoke cultivation in Poland and in the world. The viable possibility of obtaining high yields is a reason for studying this plant, also with respect to phytoremediation [8, 14]. Our previous research also showed that Jerusalem artichoke is suitable for phytoremediation of soils contaminated with heavy metals [15].

It is permitted to use municipal sewage sludge in agriculture, in reclamation of landfills and post-industrial areas, provided that environmental standards are met [16-17]. When using municipal sewage sludge and other organic waste in reclaiming post-industrial areas, we introduce not only nutrients, but also heavy metals that can constitute a substantial ecological and economic problem [18-19].

In the case of soil contamination with heavy metals by using large doses of sewage sludge, it is rational to introduce vegetation with a high phytoextraction potential [4, 20]. Plants with a high phytoextraction potential include, among other things, Jerusalem artichoke, miscanthus, and spartina [21-22]. Using the process of phytoextraction of heavy metals from sewage sludge is an alternative method of soil cleaning [2, 5, 23]. Scientific literature indicates that there is little research on assessing the applicability of different Jerusalem artichoke varieties for phytoextraction of heavy metals from municipal sewage sludge used on landfills or in reclaiming post-industrial areas.

The aim of our research was to verify (in field conditions) the efficacy of phytoremediation of soils from heavy metals that have been introduced with doses of municipal sewage sludge. Two varieties of Jerusalem artichoke, 'Rubik' and 'Albik,' were used in the experiment. They were evaluated with respect to the amount of Jerusalem artichoke yield as well as the uptake and use of heavy metals from sewage sludge. The effect of municipal sewage sludge on biological activity of the soil on which the Jerusalem artichoke varieties were cultivated was also assessed. Based on the above-mentioned parameters, a variety for phytoextraction of heavy metals from soil amended with sewage sludge was designated.

Such a way of using Jerusalem artichoke varieties in phytoextraction may bring a positive ecological and economic effect, especially in post-industrial areas and landfills. Jerusalem artichoke plants, on account of their high yield, can purify soil from heavy metals and other pollutants. They also make use of nutrients that can be found in sewage sludge. It is worth underlining that the obtained Jerusalem artichoke biomass was used only for energy purposes.

Material and Methods

Research on the effect of increasing doses of municipal sewage sludge on phytoextraction of heavy metals by Jerusalem artichoke varieties was conducted in 2008-13. The research was conducted in an area that belongs to the municipal sewage treatment plant in Janów Lubelski [50°43'17.7"N 22°22'08.0"E] in southeastern Poland. Municipal sewage sludge originated from mechanical and biological sewage treatment plants. The experiment was part of long-term research on using sewage sludge in cultivating different species of energy crops. The research was aimed at selecting species that would be the most useful for phytoremediation and at the same time have favorable energy parameters.

Soil and Municipal Sewage Sludge

The soil on which the experiment was set up was classified as clay loam (CL), (Table 1) [24-25]. According to WRB classification, the soil is included in Cambisols/Inceptisols [26].

The soil had a slightly acidic reaction, and the content of available phosphorus and potassium was at a low level, whereas magnesium content was at a very low level. Heavy metal content in the soil did not exceed acceptable values for using municipal sewage sludge for reclamation [16, 27].

Municipal sewage sludge, as organic waste with catalogue number 19 08 05 [28], had been stabilized and hygienized before use. It came from the sewage treatment plant in Janów Lubelski. The municipal sewage sludge was applied once and mixed with soil surface layer at a depth of 20 cm in late autumn 2007. Due to the low potassium content in the sewage sludge, supplemental potassium fertilization (100 kg K · ha^{-1}, in the form of 40% potassium chloride (KCl) was applied on all plots. The potassium dose met the nutrient demand of the tested varieties. Phosphorus fertilization was not applied because it had been calculated that the amount of phosphorus present

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

Parameter	Unit	Content in the soil layer		Content in
		0-20 cm	20-40 cm	sewage sludge
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
$CaCO_3$	%	<1	<1	-
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

*Clay loam (CL) **Below detection level

in the sewage sludge was sufficient to meet the plant demand for this component (depending on the sewage sludge dose, from 125 to 750 kg · ha^{-1} of total phosphorus was applied). The heavy metal content determined in the sewage sludge did not exceed acceptable values for using municipal sewage sludge for reclamation (Table 1) [16, 27]. Sewage sludge used in the experiment contained no pathogenic bacteria of the genus Salmonella and live eggs of intestinal parasites Ascaris sp., Trichuris sp., and Toxocara sp. [16].

Scheme and Conditions of Conducting the Field Experiment

The two-factor field experiment was set up in the split-plot system in triplicate. The area of the plot was 14.4 m^2. The dose of municipal sewage sludge was the first experimental factor. The experimental design comprised 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 hectare. Two varieties of Jerusalem artichoke (*Helianthus tuberosus* L.) – Rubik (purple tubers) and Albik (yellow tubers) – were the second experimental factor. Jerusalem artichoke tubers were planted in May 2008, with spacing 28 × 75 cm (68 tubers of Jerusalem artichoke were planted at one time in each experimental plot). Cultivation of the Jerusalem artichoke varieties with varied doses of sewage sludge was conducted in 2008-13.

In the first year of vegetation, agricultural practices (manual weeding, soil scarification using a hoe) were performed several times, depending on need. In the second and later years of vegetation, these practices were not necessary owing to strong shadowing of soil. Every year at the end of plant vegetation (at the turn of October and November), aboveground parts (stems and leaves) were harvested. Aboveground parts of Jerusalem artichoke plants were harvested from tubers planted in the first year of their vegetation.

Weather Conditions

Mean air temperature and rainfall during the experiment in 2008-13 were based on data from the Agrometeorological Observatory of University of Life Sciences in Lublin. The average air temperature during six years of Jerusalem artichoke vegetation was 14.6ºC (being higher than the average of the long-term period by 1.4ºC), while total precipitation exceeded the average by 36 mm. However, each of the years of the experiment were characterized by considerable variability. The complete characteristics of weather conditions were described in our previous paper [29].

Determining Yield, Heavy Metal Content, and Soil Enzymatic Activity

Each year, varieties of Jerusalem artichoke were harvested using hand tools in autumn, at the turn of

October and November. After the harvest the plant material from each plot (replication) was dried and all aerial biomass from a plot was chopped in a Bear Cat 70080 s-8HP chipper shredder (Colorado, USA). Then, 500-g subsamples from each plot were taken for chemical analysis and dried at 105ºC 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 [30]. Each year, samples of the analyzed crops were subjected to dry mineralization in a muffle furnace at 550ºC [30-31]. 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 [30]. Each year, after mineralization of the plant and soil material, contents of Cr, Ni, Cu, Zn, Cd, and Pb were determined using an ICP-OES emission spectrometer [32].

Soil pH in 1 mol · dm^{-3} KCl was determined using the 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 [30]. Available phosphorus and potassium compounds were extracted from the soil using a solution of calcium lactate acidified with hydrochloric acid to pH 3.55. Available magnesium was extracted from the soil using a solution of calcium chloride. In the soil filtrate, phosphorus was determined colorimetrically, while potassium and magnesium were measured by ASA method. Each year during the vegetation season, in May, soil samples were collected from each plot (each repetition) from 0-20 cm depth, using Egner's soil probe sampler, in order to assess the enzymatic activity of the soil. Analyses of the soil also involved determining 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 [33], of acid phosphatase and alkaline phosphatase using the Tabatabai and Bremner method [34], of urease using the Zantua and Bremner method [35], and of protease using the Ladd and Butler method [36]. 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; and 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 six years of studies, i.e., for the 2008-13 period.

Analytical Quality Control

The ICP-OES Optima 7300 DV atomic emission spectrometer from Perkin Elmer Company was used for determining heavy metals in plant and soil materials. Determinations in each of the analyzed samples were carried out in three replications. A quantitative analysis mode was used for the data acquisition of the samples. The scanning of each single sample was repeated three times to gather reasonably good results. During measurements, care was taken to avoid the memory effect and therefore a washout 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), and CRM023-050 – Trace Metals – Sandy Loam 7 (RT Corporation).

Computations and Statistical Analysis of the Results

Heavy metal content in the yield of the above-ground parts of Jerusalem artichoke, due to cultivation of two varieties and variability of conditions in individual years, is presented in the form of a multi-year (2008-13) weighted mean. Heavy metal uptake (U) was calculated annually, as the product of dry matter yield (Y) and heavy metal content (C), according to the formula: U = Y · C. Heavy metal uptake was expressed in g · ha^{-1}. Heavy metal uptake is presented as the total from the entire research period (2008-13). The balance (B) of heavy metals was calculated from the difference between the amount of metals applied (A) with the dose of sewage sludge in 2008, and the amount of metals uptaken (U) with plant yield, according to the formula: B = A-U. The simplified balance did not take into account the inflow of metals with precipitation or leaching of heavy metals deep into the soil profile. Recovery of heavy metals presented in the balance is the percentage share of the uptake of heavy metals by the varieties in relation to the amount introduced to soil along with sewage sludge.

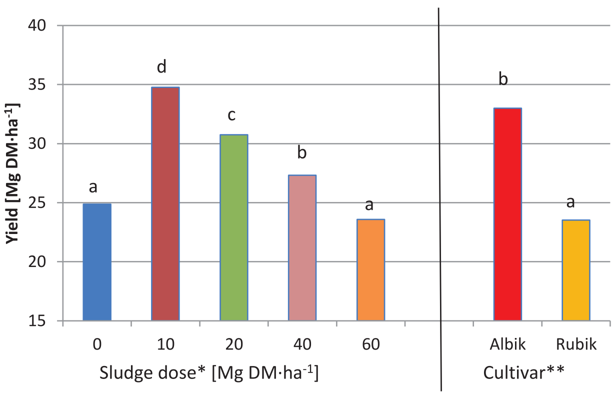

Fig. 1. The yield of underground biomass of Jerusalem artichokes (on average, from the research years 2008-13) under the experimental factors of sewage sludge dose and cultivar).
*The sludge dose's means denoted by different letters differ statistically at a level of p = 0.05.
**The cultivar's means denoted by different letters differ statistically at a level of p = 0.05.

The statistical analysis of the research results was carried out using the Statistica software package. Statistical significance of the analyzed sources of variability was assessed using a two-factor analysis of variance. Significance of differences between mean values was verified using t-Tukey's test at a significance level of $p = 0.05$. For selected parameters, values of Pearson's linear correlation coefficients (r) were computed, at a significance level of $p \leq 0.05$. 5% was adopted as maximum dispersion between measurements in chemical analysis.

Results

Crop Yield

The yield of Jerusalem artichoke grown on municipal sewage sludge varied and depended on variety, sewage sludge dose, and vegetation seasons (Fig. 1, Table 2).

Fig. 1 shows the mean yield of two Jerusalem artichoke varieties obtained in the multi-year period 2008-13, which, depending on the dose of sewage sludge, ranged between 20.1 and 41.3 Mg DM · ha^{-1}. The yield of Albik, depending on the dose of sewage sludge, was 24% to 56%

Table 2. Yield of varieties of Jerusalem artichoke tested in the following years of the study (Mg DM · ha^{-1}).

Treatments	Year					
	2008	2009	2010	2011	2012	2013
Sludge dose (Mg DM · ha^{-1})	'Rubik'					
0	12.5	20.5	15.5	29.4	19.6	35.6
10	13.2	20.0	18.1	44.0	31.6	42.6
20	15.4	19.6	17.3	33.5	27.6	41.3
40	10.0	17.9	16.0	22.9	20.8	40.4
60	9.4	17.3	12.2	22.7	24.9	34.1
Mean	12.1	19.1	15.8	30.5	24.9	38.8
CV (%)*	19.6	8.0	14.2	30.4	19.2	11.4
Sludge dose (Mg DM · ha^{-1})	'Albik'					
0	11.6	27.6	17.5	38.9	30.6	39.1
10	11.3	24.7	19.9	62.0	67.5	62.3
20	14.2	23.5	25.7	54.0	35.0	61.8
40	16.8	21.7	22.2	57.6	30.6	51.0
60	13.3	21.6	18.7	40.4	28.0	40.3
Mean	13.5	23.8	20.8	50.6	38.3	50.9
CV (%)*	16.6	10.5	15.5	25.5	42.0	21.7
Sludge dose (Mg DM · ha^{-1})	Mean for dose of the sewage sludge					
0	12.0	24.1	16.5	34.1	25.1	37.4
10	12.3	22.4	19.0	53.0	49.5	52.4
20	14.8	21.5	21.5	43.7	31.3	51.6
40	13.4	19.8	19.1	40.3	25.7	45.7
60	11.4	19.5	15.5	31.6	26.4	37.2
LSD for dose**	1.2	1.4	1.0	9.0	4.9	7.1
LSD for species	0.8	1.0	0.8	7.6	3.9	2.3
LSD for interaction	1.7	n.s.***	1,8	n.s.	8.6	7.9

*Variability coefficient
**Least significant differences
***Not significant

Table 3. Weighted average of heavy metals content in varieties of Jerusalem artichoke (mg · kg^{-1} DM).

Treatments	Cr	Ni	Cu	Zn	Cd	Pb
Sludge dose (Mg DM · ha^{-1})	‘Rubik’					
0	0.17	0.23	1.10	15.27	0.08	0.29
10	0.22	0.28	1.77	18.27	0.10	0.34
20	0.30	0.52	2.54	21.08	0.13	0.37
40	0.34	0.77	3.68	23.11	0.15	0.45
60	0.41	1.03	4.33	26.90	0.25	0.56
Mean	0.29	0.57	2.68	20.92	0.14	0.40
CV (%)*	32.7	56.1	48.0	21.1	46.0	26.9
Sludge dose (Mg DM · ha^{-1})	‘Albik’					
0	0.13	0.25	1.23	14.67	0.09	0.33
10	0.16	0.37	1.59	18.02	0.10	0.40
20	0.22	0.56	2.09	20.63	0.14	0.41
40	0.31	0.72	3.32	22.66	0.18	0.49
60	0.40	0.88	3.93	43.89	0.27	0.65
Mean	0.24	0.56	2.43	23.97	0.15	0.45
CV (%)*	42.5	42.8	44.7	45.9	44.8	26.9
Sludge dose (Mg DM · ha^{-1})	Mean for dose of the sewage sludge					
0	0.15	0.24	1.16	14.97	0.08	0.31
10	0.19	0.32	1.68	18.14	0.10	0.37
20	0.26	0.54	2.31	20.85	0.13	0.39
40	0.32	0.75	3.50	22.89	0.16	0.47
60	0.40	0.95	4.13	35.39	0.26	0.60
LSD for dose**	0.01	0.05	0.38	2.25	0.02	0.02
LSD for species	0.01	n.s.	0.12	1.00	0.01	0.01
LSD for interaction	0.02	0.07	0.42	2.75	n.s.***	n.s.

*Variability coefficient
**Least significant differences
***Not significant

higher than that of Rubik. The highest yield of the tested varieties of Jerusalem artichoke was obtained in the treatment where 10 Mg DM · ha^{-1} sewage sludge had been applied, and the lowest yield was obtained at the highest dose of sewage sludge, i.e., 60 Mg DM · ha^{-1}. The dose of 10 Mg DM · ha^{-1} sewage sludge caused an increase in the mean yield of the Rubik and Albik varieties by more than 27% and 49%, respectively, in relation to the control treatment (Fig. 1). The research shows that, compared to Rubik, Albik responded with a higher increase in yield under the influence of sewage sludge.

The analysis of the correlation coefficient value showed important correlations between the dose of sewage sludge and the yield of Rubik ($r_{(p = 0.022)}$ = -0.58), whereas for Albik such a correlation was not observed. The amount of metals (Cr, Ni, Cu, Zn, Cd, and Pb) introduced with the sewage sludge dose was also significantly correlated with the mean yield of Rubik ($r_{(p = 0.022)}$ = -0.58). In the case of Albik such a correlation was not observed.

The sewage sludge (applied once) exerted a significant effect on the amount of yield of the Jerusalem artichoke varieties in subsequent years of research (Table 2).

The lowest yields of the studied varieties were obtained in the first year of the research, which resulted mainly from the shorter vegetation period and the necessity for the plant to adapt and put down roots. Also, over time, the sewage sludge used has a positive effect on plants by releasing nutrients and improving the soil structure.

Higher yields of the tested Jerusalem artichoke varieties were obtained in the second and successive years of research as compared to the first vegetation year.

The highest yields of Rubik were obtained in the sixth year of research. Depending on the dose of sewage sludge, the amount of yield of this variety was from 2.7 to 4.0 times higher in 2013 than the yield obtained in the first year of vegetation.

Yields of Albik also increased gradually in successive years of vegetation. Generally, the highest yields of this variety were obtained in 2011-13. Depending on the dose of sewage sludge, the amount of yield of Albik was from 3.0 to 5.5 times higher in 2013 than the yield obtained in the first year of vegetation.

The conducted research confirms the significant correlations between sewage sludge dose (and heavy metals introduced with it) and the yield of the tested Jerusalem artichoke varieties only in 2009 ($r_{(p = 0.003)}$ = -0.52). The above-mentioned correlation was not observed in the remaining years of Jerusalem artichoke cultivation.

Heavy Metal Content in the Jerusalem Artichoke Varieties

The contents of Cr, Ni, Cu, Zn, Cd, and Pb in the municipal sewage sludge were, respectively, more than 1.6, 1.3, 33.6, 30.4, 7.7, and 2.1 times higher as compared to the soil surface layer (Table 1). The chemical composition of the sewage sludge indicates that this waste was a potential source of heavy metals in the soil for the Jerusalem artichoke varieties.

It has been shown that, apart from the doses of sewage sludge, properties of the varieties were a factor that significantly modified the heavy metal content in the above-ground parts of this species (Table 3). Under control conditions, there were significant differences in the content of heavy metals between the tested varieties of Jerusalem artichoke. The control, where sewage sludge was not applied, was found to have the lowest content of the studied metals in the tested varieties, as compared to the treatments where sewage sludge was applied (Table 3). Application of increasing doses of sewage sludge in the amount of 10 to 60 Mg DM · ha^{-1} had a significant effect on the increase in heavy metal content in the tested varieties. At the highest dose of sewage sludge, increases in metal content in Rubik amounted to: 147% for Cr, 347% for Ni, 295% for Cu, 76% for Zn, 205% for Cd, and 94% for Pb in relation to the control. The highest increase in the amount of heavy metals in Albik reached, respectively: 194% for Cr, 249% for Ni, 219% for Cu, 199% for Zn, 206% for Cd, and 97% for Pb in relation to the control.

In terms of increasing content, the metals were arranged in the following order for Rubik: Ni>Cu>Cd>Cr>Pb>Zn. In the case of Albik, the order was as follows: Ni>Cu>Cd>Zn>Cr>Pb.

In the case of the control treatment, there were no significant differences in the heavy metal content in the tested varieties of Jerusalem artichoke. The applied doses of sewage sludge, in the amount of 10-60 Mg DM · ha^{-1}, brought about diversity in the content of heavy metals in the tested varieties of Jerusalem artichoke. At the highest dose of sewage sludge (60 Mg DM · ha^{-1}), Rubik had a higher content of Cu and Ni than Albik, which was found to have a higher content of Zn, Cd, and Pb. Cr content in both tested varieties of Jerusalem artichoke was not significantly varied. The research shows that increasing doses of sewage sludge differentiated the content of heavy metals in the tested Jerusalem artichoke varieties.

The linear correlation analysis indicates strong correlations between the load of metals (Cr, Ni, Cu, Zn, Cd, Pb) introduced with the dose of sewage sludge and the content of these metals in the varieties ($r_{(p<0.000)}$ = 0.81-0.96). The correlation analysis also indicated a significant relationship between yield and Cr content in the Jerusalem artichoke varieties ($r_{(p = 0.019)}$ = -0.43). No significant correlation between yield and Ni, Cu, Zn, Cd, Pb content in the Jerusalem artichoke varieties were observed.

Significant relationships between the mean yield of Rubik and Cd and Ni content in this variety ($r_{(p = 0.030)}$ = -0.56 and $r_{(p = 0.015)}$ = -0.62 respectively) were observed. However, the linear correlation analysis did not show significant relationships between the mean yield of Albik and the content of heavy metals in this variety.

Uptake of Heavy Metals by the Jerusalem Artichoke Varieties

The uptake of heavy metals by Jerusalem artichoke was calculated annually as the product of dry matter yield and heavy metal content in these varieties. However, heavy metal uptakes are presented as the total (sum) from the entire research period, that is from 2008-13 (Table 4). The amount of heavy metals taken up from the control and treatments fertilized with sewage sludge depended on the yield (Table 2) and the content of metals in the Jerusalem artichoke varieties in the following years of the experiment. In the control treatment (not fertilized with sewage sludge), considerable diversity in Ni, Cu, Zn, Cd, and Pb uptake by Jerusalem artichoke varieties was observed. It was established that Albik took up more of these metals than Rubik. No significant diversity in the amount of Cr taken up by tested varieties was observed (Table 4). Compared to the control, applying increasing doses of sewage sludge had a significant effect on the increase in metal uptake by the yield of Jerusalem artichoke varieties. Research shows that at the highest dose of sewage sludge Albik took up significantly more metals than Rubik.

The highest Cr, Ni, Zn, Cd, and Pb uptake with the yield of Albik was observed at the highest dose of sewage sludge (60 Mg DM · ha^{-1}), and Cu at the dose of 40 Mg DM · ha^{-1}. Increases in the uptake of the above-mentioned metals from sewage sludge by Albik were, respectively, more than 189%, 243%, 193%, 200%, 94%, and (for Cu) 213% higher compared to the control treatment. Increases in the uptake of Cr, Ni, Cu, Zn, Cd, and Pb by Rubik,

Table 4. Heavy metals uptake by varieties of Jerusalem artichoke (g · ha^{-1}).

Treatments	Cr	Ni	Cu	Zn	Cd	Pb
Sludge dose (Mg DM · ha^{-1})	'Rubik'					
0	22.1	30.6	146.2	2,036.5	11.0	38.2
10	37.7	46.8	299.8	3,100.5	16.2	58.5
20	46.9	81.3	395.4	3,268.0	19.9	57.0
40	43.2	98.9	470.6	2,952.6	19.3	57.8
60	49.4	123.7	521.7	3,240.5	30.2	67.0
Mean	39.9	76.3	366.7	2,919.6	19.3	55.7
CV (%)*	28.4	47.3	40.2	18.7	36.5	21.8
Sludge dose (Mg DM · ha^{-1})	'Albik'					
0	22.3	41.4	203.2	2,422.0	14.4	54.0
10	39.1	90.3	391.3	4,440.3	25.0	99.0
20	46.8	121.0	448.1	4,431.4	29.0	88.2
40	62.3	143.8	665.2	4,538.5	35.7	97.5
60	64.5	142.3	637.1	7,108.4	43.4	104.9
Mean	47.0	107.8	469.0	4,588.1	29.5	88.7
CV (%)*	35.8	37.4	38.7	35.2	36.0	24.4
Sludge dose (Mg DM · ha^{-1})	Mean for dose of the sewage sludge					
0	22.2	36.0	174.7	2,229.3	12.7	46.1
10	38.4	68.5	345.5	3,770.4	20.6	78.8
20	46.9	101.1	421.7	3,849.7	24.5	72.6
40	52.8	121.3	567.9	3,745.6	27.5	77.6
60	56.9	133.0	579.4	5,174.4	36.8	85.9
LSD for dose**	4.0	8.7	64.0	341.7	3.2	7.0
LSD for species	2.2	3.0	23.6	265.1	1.7	5.5
LSD for interaction	5.3	9.9	74.0	592.7	4.2	n.s.***

*Variability coefficient
**Least significant differences
***Not significant

at the highest dose of sewage sludge, amounted to, respectively, over 123%, 304%, 256%, 59%, 174%, and 75% compared to the control treatment. Analysis of the percentage increase in heavy metal uptake indicates that Albik, under the influence of increasing doses of sewage sludge, took up more Cr, Zn, Cd, and Pb than Rubik. Rubik responded with a higher percentage increase in the uptake of Ni and Cu than Albik.

When analyzing the amount of extracted metals, at the highest dose of sewage sludge (60 Mg DM · ha^{-1}), it was established that Albik took up significantly more Cr, Ni, Cu, Zn, Cd, and Pb than Rubik.

Depending on treatment, Albik took up more Cr (between 0.6 and 30%) than Rubik. In the case of the other metals, Albik took up more of these elements (15-92% for Ni, 13-41% for Cu, 18-119% for Zn, 31-85% for Cd, and 41-69% for Pb) as compared to Rubik. Larger amounts of heavy metals taken up by Albik were associated with a higher yield of this variety and a higher content of these elements compared to Rubik (Tables 2-3).

The research shows that the greatest differences in the amount of heavy metals taken up by the Jerusalem artichoke varieties were observed at doses of 40 Mg DM · ha^{-1} for Cr and Cd, 10 Mg DM · ha^{-1} for Ni and Pb, for Cu in the control treatment, and for Zn at a dose of 60 Mg DM · ha^{-1}.

The correlation coefficient analysis showed significant correlations between the uptake of heavy metals by the

Jerusalem artichoke varieties and the load of heavy metals introduced with the sewage sludge dose (from $r_{(p = 0.016)}$ = 0.44 to $r_{(p<0.000)}$ = 0.83). The mean yield of the Jerusalem artichoke varieties was also correlated with the uptake of Zn and Pb by these varieties ($r_{(p = 0.024)}$ = 0.41 and $r_{(p<0.000)}$ = 0.66, respectively). The experiment revealed significant correlations between the content of heavy metals in the Jerusalem artichoke varieties and the uptake of these heavy metals by the plants (from $r_{(p = 0.081)}$ = 0.32 to $r_{(p<0.000)}$ = 0.89). Comparing the amount of heavy metals taken up by Albik to the other plants, this variety can be classified as a hyperaccumulator.

Simplified Balance of Heavy Metals

By using municipal sewage sludge, considerable amounts of heavy metals were introduced to the soil. These heavy metals can be taken up by plants and pose a risk for the cultivation of edible plants. Cultivation of selected varieties of Jerusalem artichoke (as an energy crop with a high phytoextraction potential) may contribute to a considerable elimination of harmful elements from the substrate. Understanding the circulation of heavy metals in the soil-plant system, through a simplified balance, will allow us to assess the effectiveness of phytoextraction by using Jerusalem artichoke varieties.

The outcome of the balance of heavy metals depended on the amount of metals introduced with the sewage sludge and on the metal uptake with the yield of the varieties (Table 5). In control treatments where sewage sludge was not applied, the balance of all the heavy metals was negative. The negative value of the balance resulted from a lack of influx of metals to the substrate.

Application of heavy metals into the soil (with sewage sludge doses of 10, 20, 40, and 60 Mg DM · ha^{-1}) led to a positive balance. Such high influx of metals supplied with increasing doses of sewage sludge caused an increase in the amount of these metals in the soil. Uptake of heavy metals by the Jerusalem artichoke varieties was lower compared to amounts introduced with the sewage sludge.

The analysis of Cd balance for Albik, in the treatment with the lowest sewage sludge dose (10 Mg DM · ha^{-1}), showed a negative value. This balance indicates that cadmium was taken up by this variety in higher quantities compared to the quantity introduced with the organic waste dose (10 Mg DM · ha^{-1}). The greatest balance difference was observed in treatments where the highest dose of sewage sludge was applied (60 Mg DM · ha^{-1}). This difference resulted from the amount of heavy metals introduced to the soil.

Phytoremediation enables assessment of plant suitability for removal of metals from the substrate. The highest phytoremediation of metals was observed in treatments where the lowest dose of sewage sludge (i.e., 10 Mg DM · ha^{-1}) was applied (Table 5). Subsequent doses of sewage sludge had an effect on the percentage reduction in phytoremediation of metals from the soil. The lowest phytoextraction was observed in treatments where the highest dose of sewage sludge was applied (60 Mg DM · ha^{-1}). The lowest phytoremediation was associated with the highest soil enrichment in heavy metals as well as with yielding and uptake of these metals by the Jerusalem artichoke varieties.

When assessing the phytoextraction effectiveness of the Jerusalem artichoke varieties, it was established that regardless of treatment, Albik phytoremediated more Ni, Cu, Zn, Cd, and Pb than Rubik. The higher phytoremediation of metals resulted from the higher yield and uptake of these elements by Albik. When analyzing phytoremediation of Cr, it was established that the tested Jerusalem artichoke varieties used this element equally only at 10 and 20 Mg DM · $ha^{-1}$of this sludge. It was established that Albik, at higher doses of sewage sludge (40 and 60 Mg DM · ha^{-1}), also used more of this element from the soil.

When comparing the percentage phytoremediation of heavy metals by Albik at the lowest dose of sewage sludge (10 Mg DM · ha^{-1}), the following order (from highest to lowest values) can be established: Cd (106%), Ni (61%), Zn (44%), Cu (35%), Pb (23%), and Cr (15%). The order shows that Albik recovered Cd to the greatest extent, and Cr to the smallest.

Soil Enzymatic Activity

Soil enzymatic activity can be a sensitive parameter providing information about the state of chemical environmental pollution. A significant effect of the increasing doses of sewage sludge on the soil enzymatic activity was observed in the field experiment (Table 6). Control treatments were found to have the lowest soil enzymatic activity compared to treatments where sewage sludge was applied. Increasing doses of sewage sludge had a significant effect on the increase in the activity of the studied soil enzymes, which was associated with the amount of carbon substrates available for microorganisms and enzymes.

In treatments with Rubik at the highest dose of sewage sludge (60 Mg DM · ha^{-1}), the increase in soil enzymatic activity in the case of dehydrogenase, urease, proteases, phosphatase acid, and alkaline was, respectively, more than 3.1, 2.3, 2.2, 2.7, and 2.6 times higher than in the control treatment. In treatments with Albik the increase in soil enzymatic activity was, respectively, more than 3.0, 2.0, 2.1, 2.6, and 2.7 times higher than the control treatment. In the treatment with the highest sewage sludge dose, regardless of Jerusalem artichoke variety, dehydrogenase activity increased the most. Maximum doses of sewage sludge were least effective in stimulating the increase in urease and protease activities.

From the tested Jerusalem artichoke varieties, it was shown that Albik stimulated the activity of the studied enzymes more than Rubik. This probably resulted from the higher yield, content, and uptake of heavy metals from the soil.

The conducted research has also confirmed significant correlations between the activity of soil enzymes and the

Table 5. Simplified balance of heavy metals after six years of research.

Treatments	Introduced	Uptake	Balance	Recovery	Uptake	Balance	Recovery
		g · ha^{-1}		%	g · ha^{-1}		%
		'Rubik'			'Albik'		
Sludge dose (Mg DM · ha^{-1})	Cr						
0	0	22	-22	0	22	-22	0
10	254	38	216	15	39	215	15
20	508	47	461	9	47	461	9
40	1,016	43	973	4	62	954	6
60	1,524	49	1,475	3	65	1,459	4
Sludge dose (Mg DM · ha^{-1})	Ni						
0	0	31	-31	0	41	-41	0
10	148	47	101	32	90	58	61
20	296	81	215	27	121	175	41
40	592	99	493	17	144	448	24
60	888	124	764	14	142	746	16
Sludge dose (Mg DM · ha^{-1})	Cu						
0	0	146	-146	0	203	-203	0
10	1,110	300	810	27	391	719	35
20	2,220	395	1,825	18	448	1,772	20
40	4,440	471	3,969	11	665	3,775	15
60	6,660	522	6,138	8	637	6,023	10
Sludge dose (Mg DM · ha^{-1})	Zn						
0	0	2,036	-2,036	0	2,422	-2,422	0
10	10,050	3,100	6,950	31	4,440	5,610	44
20	20,100	3,268	16,832	16	4,431	15,669	22
40	40,200	2,953	37,247	7	4,539	35,661	11
60	60,300	3,240	57,060	5	7,108	53,192	12
Sludge dose (Mg DM · ha^{-1})	Cd						
0	0	11	-11	0	14	-14	0
10	24	16	7	69	25	-1	106
20	47	20	27	42	29	18	62
40	94	19	75	20	36	58	38
60	141	30	111	21	43	98	31
Sludge dose (Mg DM · ha^{-1})	Pb						
0	0	38	-38	0	54	-54	0
10	429	59	370	14	99	330	23
20	858	57	801	7	88	770	10
40	1,716	58	1,658	3	97	1,619	6
60	2,574	67	2,507	3	105	2,469	4

Table 6. Soil enzymatic activity (average from 2008-13).

Treatments	Dehydrogenase activity	Urease activity	Protease activity	Acid phosphatase activity	Alcaline phosphatase activity
	(cm^3 H_2 · kg^{-1} · d^{-1})	(mg N-NH_4^+ · kg^{-1} · h^{-1})	(mg of tyrosine · kg^{-1} · h^{-1})	(mmol PNP · kg^{-1} · h^{-1})	(mmol PNP · kg^{-1} · h^{-1})
Sludge dose (Mg DM · ha^{-1})	'Rubik'				
0	4.2	7.0	10.8	27.8	19.2
10	5.2	10.9	13.5	33.2	21.8
20	8.0	11.8	18.2	43.1	28.4
40	11.5	16.0	26.8	101.6	67.9
60	17.3	23.2	34.6	102.1	68.4
Mean	9.2	13.8	20.8	61.6	41.1
CV (%)*	53.9	42.1	44.4	57.7	57.6
Sludge dose (Mg DM · ha^{-1})	'Albik'				
0	4.4	8.1	11.7	29.0	20.3
10	5.4	11.7	14.2	35.2	23.4
20	8.9	13.0	19.4	44.3	30.4
40	12.3	17.4	28.1	105.3	69.5
60	17.6	24.5	35.8	105.7	74.9
Mean	9.7	14.9	21.8	63.9	43.7
CV (%)*	51.5	39.5	43.0	57.9	58.3
Sludge dose (Mg DM · ha^{-1})	Mean for dose of the sewage sludge				
0	4.3	7.5	11.3	28.4	19.7
10	5.3	11.3	13.9	34.2	22.6
20	8.4	12.4	18.8	43.7	29.4
40	11.9	16.7	27.5	103.5	68.7
60	17.4	23.9	35.2	103.9	71.6
LSD for dose**	1.6	1.6	3.3	19.3	13.2
LSD for species	0.2	0.5	0.3	1.8	1.6
LSD for interaction	1.7	n.s.***	n.s.	n.s.	n.s.

*Variability coefficient
**Least significant differences
***Not significant

load of heavy metals introduced to soil with the sewage sludge dose ($r_{(p<0.000)}$ = 0.91-0.98).

Aside from biogenes, organic colloids and soil microorganisms that influence soil enzymatic activity, substantial quantities of heavy metals were supplied to the soil profile with municipal sewage sludge (Table 5). The experiment showed strong correlations between the activity of soil enzymes and heavy metal content in plants ($r_{(p<0.000)}$ = 0.65-0.95), and heavy metal uptake by plants (from $r_{(p = 0,056)}$ = 0.35 to $r_{(p<0.000)}$ = 0.82). The research shows that soil enzymatic activity is strictly correlated with heavy metal content in the soil and in the plant, which is of considerable importance in phytoremediation processes.

Discussion

Owing to its high yield potential and resistance as well as tolerance to pollutants, Jerusalem artichoke can be used as a potential phytoextractor of heavy metals from soil [14-15, 37]. Jerusalem artichoke has been used at industrial waste landfills and mining areas as a remedial plant [14-15]. However, there is little information in scientific literature regarding research on using specific varieties of this species for phytoextraction of heavy metals from municipal sewage sludge.

Yield of the Jerusalem Artichoke Varieties

The high yield of Jerusalem artichoke presented in this research resulted from the fertilizer effect of municipal sewage sludge. The average yield of tested Jerusalem artichoke varieties obtained from the multi-year period from 20.1 to 41.3 Mg DM · ha^{-1}. Such high yields of Jerusalem artichoke varieties are evidence of a positive response of this species to the applied sewage sludge, which finds confirmation in scientific literature [38-39]. The amount of yield obtained in the authors own research was comparable to the amount of yield obtained by Baldini et al. [10]. Scientific literature and the authors' own research indicate that the amount of Jerusalem artichoke yield depends on variety, soil, and climatic conditions, and on agrotechnical measures [10, 40]. Other studies have also shown that Jerusalem artichoke yield can be high and can reach even 30 Mg · ha^{-1} [41]. The study conducted by Pakarinen et al. [11] showed that the amount of Jerusalem artichoke yield varied from 7 to 16 Mg DM · ha^{-1} and was much lower than yields obtained in this research. The distinct diversity of the amount of yield of the Jerusalem artichoke varieties in respective years which was determined in this research finds confirmation in literature [42-43]. Our own research showed that the highest doses of sewage sludge (60 Mg DM · ha^{-1}) decreased the yield of the Jerusalem artichoke varieties, and this resulted, among other things, from a large load of heavy metals. This research confirms that, under the influence of a high heavy metal content, Jerusalem artichoke growth and development were inhibited [14].

Heavy Metal Content in Jerusalem Artichoke Varieties

Chemical composition of Jerusalem artichoke depends on numerous factors, such as the date of harvest, variety, soil conditions, reaction, and type of pollutant [3, 14, 44]. Our own research showed that increasing doses of municipal sewage sludge had a considerable effect on the increase of heavy metal content in the tested varieties. It was also shown that properties of the varieties significantly modified the heavy metal content in the aboveground parts of this species. Our own research showed that Albik had higher Zn, Cd, and Pb contents than Rubik, whereas Rubik had a higher Cu and Ni contents. Other studies [14] have shown that Rubik grown on soil polluted with heavy metals accumulates substantial quantities of Ni, Mn, and Zn in the aboveground parts. Research conducted by Zhang et al. [37] confirmed that Jerusalem artichoke, among other reclamation crops, is characterized by a substantial ability to accumulate heavy metals from degraded soils. Research conducted by Zhang et al. [37] showed that Jerusalem artichoke is extremely suitable for Cd phytoremediation from chemically polluted soil. Further studies confirm that there is varietal diversity of Jerusalem artichoke in terms of Cd accumulation in tubers and stems [21, 45]. Research conducted by Chen et al. [21] suggests that Jerusalem artichoke can be grown at high Cd concentrations in the substrate. On account of intensive Cd transfer from the substrate to the aboveground parts, Jerusalem artichoke can be a potential candidate for phytoremediation of soils polluted with this metal [21]. Other studies [43] confirm that there are varietal differences in chemical composition under the influence of mineral fertilization of Jerusalem artichoke. Varied doses of mineral fertilization not only diversify the chemical composition of Jerusalem artichoke varieties, but they also influence the chemical composition of tubers of other plants, including potato [46]. Other studies [47-48] point to the possibility of using energy crops, plantations for eliminating pollutants from sewage or sewage sludge, and from chemically polluted soils.

Uptake of Heavy Metals by the Aboveground Parts of Jerusalem Artichoke

Larger amounts of heavy metals extracted by Albik can be explained by higher yielding and higher content of these metals, which is of great importance when selecting varieties for the process of phytoextraction of metals from organic waste [14]. Research conducted by Borkowska et al. [20] confirms that Jerusalem artichoke, among other energy crops, is suitable for phytoextraction of heavy metals from sewage sludge. The research conducted by Borkowska et al. [20] showed that among heavy metals, Cd was taken up by Jerusalem artichoke in significantly larger amounts compared to the amounts obtained in this research. Research conducted by Long et al. [49] also showed that there are differences in phytoextraction potential between Jerusalem artichoke varieties. For example NY5 variety had a higher phytoextraction potential than NY2 variety [49].

Research on the suitability of willow for remediation of sewage sludge showed that there are differences in heavy metal uptake between clones of the same species [21, 47], which is why not only selection of appropriate plant species but also selection of proper variety or clone appear to be important in heavy metals phytoextraction [21, 23, 47, 49].

Balance

Negative value of the balance in control treatments resulted from the simplified balance in which the supply of metals from precipitation was not taken into account. Phytoremediation can be used to assess the effectiveness of removing metals from the substrate. The research shows that, compared to Rubik, Albik cleaned the substrate more effectively. Research conducted by Laidlaw et al. [23] confirms that energy crops, including willow, can be used for phytoextraction of heavy metals from substrate polluted with heavy metals. The cited authors [23] showed that willow, out of all heavy metals, extracts cadmium from the substrate most efficiently. Our own research also shows that Jerusalem artichoke, as an energy crop, also efficiently extracts Cd from the substrate. Other research [3] confirms that Jerusalem artichoke, next to other plants, is a potential hyperaccumulator of heavy metals from chemically polluted soils. The use of a proper Jerusalem artichoke variety as a plant with substantial phytoextraction potential may improve the balance of heavy metals in the environment [3, 21, 45].

Soil Enzymatic Activity

Soil enzymatic activity is a sensitive indicator of stress conditions in plants, and it serves for the assessment of the influence environmental factors have on plants [50]. Our own research confirms the stimulating effect of sewage sludge on soil enzymatic activity [51]. The increase in soil enzymatic activity can be explained primarily by the increase in the content of readily available carbon and nitrogen, and also by improvement of soil physicochemical properties [52-53]. On the other hand, research conducted by Yang et al. [54] showed that Jerusalem artichoke roots reduce the negative effects of salinization, increasing diversity and the number of microorganisms, and thereby increasing soil enzymatic activity. The cited authors explain the increase in enzymatic activity of the soil on which Jerusalem artichoke was grown by the presence of inulin from tubers of these plants [54]. Inulin, together with pectins and cellulose, binds a large number of unnecessary and harmful compounds such as heavy metals, which can be used in phytoremediation processes [46]. Research conducted by Sas-Nowosielska et al. [48] confirms the beneficial effect of Jerusalem artichoke on the increase in diversity of microorganisms in soil.

The high yield potential, great ability to adapt to soil conditions, and high phytoextraction potential (especially in the case of Albik), speak in favor of using Jerusalem artichoke for phytoremediation of chemically polluted soils [14, 49, 55].

Conclusions

1. The highest Jerusalem artichoke yield was obtained in the treatment where 10 and 20 Mg DM sewage sludge per hectare had been applied. Albik had a higher yield than Rubik.
2. Increasing doses of sewage sludge had a considerable effect on the increase in heavy metal content in the tested Jerusalem artichoke varieties. At the highest dose of sewage sludge, Albik had a higher content of Zn, Cd, and Pb than Rubik, whereas Rubik had a higher content of Cu and Ni than Albik.
3. Compared to Rubik, Albik extracted more heavy metals from the sewage sludge.
4. When assessing the Jerusalem artichoke's phytoextraction potential, it was established that Albik phytoremediated metals to a higher degree than Rubik.
5. From the point of view of efficiency of phytoextraction of heavy metals from municipal sewage sludge, Albik (due to higher yield, content, and uptake of heavy metals) can be recommended for phytoremediation of this waste.

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