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**ORIGINAL PAPER**

# RESEARCH ON THE UPTAKE AND USE OF TRACE ELEMENTS FROM MUNICIPAL SEWAGE SLUDGE BY MULTIFLORA ROSE AND VIRGINIA FANPETALS*

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### Abstract

The aim of the research was to assess the uptake and use of trace elements from municipal sewage sludge by the multiflora rose (*Rosa multiflora* Thunb. ex Murr) cultivar Jatar and Virginia fanpetals (*Sida hermaphrodita* Rusby). A six-year field experiment involved four levels of fertilization with sewage sludge at doses of 0, 10, 20, 40, 60 Mg DM ha$^{-1}$. The sewage sludge was applied once before planting energy crops. Due to the low potassium content in the sewage sludge, supplemental potassium fertilization (100 kg K ha$^{-1}$ in the form of 40% potash salt KCl) was applied once on all plots. Fertilization with nitrogen, phosphorus and with microelements was not performed during the crop cultivation. The research evaluated the size of yields, and the content, uptake and utilization of Al, Mn, Fe, Co and Mo from municipal sewage sludge by energy crops. It was established that increasing doses of sewage sludge significantly increased the yield of multiflora rose and Virginia fanpetals biomass. The yield of Virginia fanpetals was one and a half times higher than that of multiflora rose. Increasing doses of sewage sludge significantly increased the content and uptake of Al, Mn, Fe, Co and Mo by the tested species. It was established that Virginia fanpetals had a higher content of Al, Mn, Co and Mo compared to multiflora rose. Multiflora rose had a higher content of Fe compared to Virginia fanpetals. It was determined that Virginia fanpetals took up more trace elements compared

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to multiflora rose. The highest trace elements' uptake with the yield of the energy crops was observed at the dose of 60 Mg DM ha$^{-1}$. The highest phytoremediation of the studied trace elements was observed at the dose of 10 Mg DM ha$^{-1}$. Based on the research, it was concluded that Virginia fanpetals utilized trace elements from municipal sewage sludge more efficiently than multiflora rose.

**Keywords**: multiflora rose, Virginia fanpetals, Al, Mn, Fe, Co, Mo, phytoextraction, municipal sewage sludge.

## INTRODUCTION

With its load of macronutrients, organic matter, heavy metals and dioxins, municipal sewage sludge can be also a potential source of microelements and trace elements that are essential for the plant growth and development (Gworek et al. 2018, Lee et al. 2018*a*). Microelements and trace elements in sewage sludge come from different technological processes, particularly in industrial sewage (Lee et al. 2018*b*). Municipal sewage sludge contains many trace elements that come from uncontrolled inflow sources. Application of sewage sludge may lead to soil contamination and to an increase in the content of pollutants that poses an environmental risk (*Regulation* ... 2016). Thus, knowing the content of trace elements in sewage sludge will allow one to determine appropriate doses of this waste for the nourishment of plants that will prevent contamination (Asik et al. 2015). In the case of high trace element content in sewage sludge, it is important to use appropriate doses so as not to deteriorate the soil quality (R*egulation* ... 2016).

Sewage treatment processes often use aluminum compounds to precipitate phosphorus and other pollutants, hence the aluminum content in sewage sludge can be high and consititute an abundant source of this element for plants (Alvarenga et al. 2017). Other trace elements present in sewage sludge make up a small share in the chemical composition of this waste (Milik et al. 2017, Pi et al. 2018).

It seems reasonable to assume that the use of sewage sludge in cultivation of energy crops with high nutrient requirements can be an alternative to mineral fertilizers enriched with microelements (Asik et al. 2015, Kołodziej et al. 2016, Ozdemir et al. 2018). Moreover, using sewage sludge, particularly in large doses, causes soil acidification, and nutrients become more available to plants (Dede et al. 2015, Wieczorek et al. 2017).

The aim of the research was to verify (under field experiment conditions) which species takes up and utilizes trace elements (Al, Mn, Fe, Co and Mo) from municipal sewage sludge more effectively. The phytoextraction potential of the tested energy crops was assessed taking into account the amount of yield, content, uptake, balance, as well as the utilization of these trace elements.

In research on the uptake and utilization of trace elements from organic waste, it is important to choose an energy crop with great phytoextraction potential (Korzeniowska, Stanisławska-Glubiak 2018, Ozdemir et al. 2018). Choosing the species with respect to the most efficient use of microelements and trace elements from sewage sludge can help in the management of marginal lands, which are low in nutrients, including trace elements (Nahm, Morhart 2018, Schröder et al. 2018).

# MATERIAL AND METHODS

The study on the effect of increasing doses of municipal sewage sludge on the use of trace elements by multiflora rose and Virginia fanpetals was carried out in 2008-2013 in a municipal wastewater treatment plant in Janów Lubelski (50°43′17.7″ N 22°22′08,0″ E) located in south-eastern Poland. This study is a continuation of the research on the extraction of heavy metals from municipal sewage sludge by the aforementioned plant species (Antonkiewicz et al. 2017).

## Soil and municipal sewage sludge

The soil on which the experiment was set up is classified as clay loam (CL) – Table 1 (Polish Soil Classification 2011). The soil had slightly acidic pH, low available phosphorus and potassium content, and very low magne-

Table 1

Selected physical and chemical properties of the soil before the experiment and the chemical composition of municipal sewage sludge

| Parameter | Unit | Content in the soil layer | | Content in |
|---|---|---|---|---|
| | | 0-20 cm | 20-40 cm | sewage sludge |
| Fraction 2-0.05 mm | (%) | 32 | 23 | nd* |
| Fraction 0.05-0.002 mm | | 39 | 45 | nd |
| Fraction <0.002 mm | | 29 | 32 | nd |
| $pH_{KCl}$ | | 6.29 | 6.44 | 6.04 |
| Organic matter | (g kg$^{-1}$ DM) | 14.5 | 14.1 | 594.0 |
| Available phosphorus (P) | (mg 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 aluminum (Al) | (mg kg$^{-1}$ DM) | 10256 | nd | 6458 |
| Total manganese (Mn) | | 465 | nd | 750 |
| Total iron (Fe) | | 12588 | nd | 1200 |
| Total cobalt (Co) | | 3.6 | nd | 10.3 |
| Total molybdenum (Mo) | | 0.25 | nd | 7.2 |

* nd – not detected, ** Bdl – below detection level

sium content. The content of Cr, Ni, Cu, Zn, Cd, and Pb in the soil did not exceed the limit values set for the reclamation of municipal sewage sludge (*Regulation* ... 2016, Antonkiewicz et al. 2017). The surface layer of soil had an average content of Al, Mn, Fe, Co and Mo in Polish soils (Pasieczna 2012, Korzeniowska, Stanisławska-glubiak 2015) – Table 1.

The municipal sewage sludge came from the municipal wastewater treatment plant in Janów Lubelski and this organic waste, catalogue number 19 08 05, was stabilized and sanitized before use. The municipal sewage sludge was applied once in late autumn 2007 – it was mixed with a 20 cm surface layer of soil. Due to the low potassium content in sewage sludge, a single supplementary fertilization treatment with 100 kg K $ha^{-1}$ in the form of 40% potassium salt (KCl) was applied on each plot. No phosphorus fertilization was used in the field experiment because the element content in the municipal sewage sludge satisfied the energy plants' demand for this component. The determined content of Cr, Ni, Cu, Zn, Cd, Pb, and Hg in the municipal sewage sludge did not exceed the limit values for the reclamation of this waste (*Regulation* ... 2015). No microbiological contamination was detected in the sewage sludge used in the experiment.

**Design and conditions of the experiment**

A two-factor field experiment was set up using the randomized block method on 14.4 $m^2$ plots, in three replicates. The first experimental factor was a dose of municipal sewage sludge. The experimental design consisted of 5 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. The second experimental factor comprised two species of energy plants: the multiflora rose var. Jatar (*Rosa multiflora* Thunb. ex Murr.) and Virginia fanpetals (*Sida hermaphrodita* Rusby).

Woody cuttings (25 cm long) of multiflora rose and sections of roots (8-12 cm long with several buds) of Virginia fanpetals were planted on 22 April 2008 at a spacing of 0.75 x 0.8 m and 0.75 x 0.4 m, respectively.

**Determination of dry matter yields and micronutrient content in the soil, sewage sludge and plants**

Every year (2008-2013), the tested plants were collected in autumn, at the turn of October and November. Every year, after harvest, the plant material from each plot was dried at 70°C in a dryer with forced air circulation; and then the amount of air-dry mass yield was determined.

After microwave mineralization with *aqua regia* (HCl and $HNO_3$ 3:1, *v/v*), the total content of the elements (Al, Mn, Fe, Co and Mo) was determined in the air-dry soil samples, sewage sludge and plant material (Ostrowska et al. 1991). After mineralization of the soil material, sewage sludge and plant material, the content of the above mentioned elements was determined using an ICP-OES spectrometer.

### Determination of soil's enzymatic activity

Analyses of the soil also involved determinations pertaining to 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: the activity of dehydrogenases with a TTC (triphenyl tetrazolium chloride) substrate using the THALMANN'S method (1968); the activity of acid phosphatase and alkaline phosphatase using the TABATABAI and BREMNER'S method (1969); the activity of urease using the ZANTUA and BREMNER'S method (1975); the activity of protease using the LADD and BUTLER'S method (1972). The activity of dehydrogenases was given in $cm^3$ $H_2$, necessary for reducing TTC to TFP (triphenyl phormazan); 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 pertaining to the 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

An optical emission spectrometer from Perkin Elmer Company, model ICP-OES Optima 7300 DV, was used for the determination of trace elements in plant and soil materials. Determinations in each of the analyzed samples were carried out in three replications. The 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 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).

### Computations and statistical analysis

Due to the cultivation of various plant species and the variability of conditions in individual years, the content of Al, Mn, Fe, Co and Mo in the total plant yield is presented as the weighted average from 2008-2013. The trace element uptake (U) was computed by multiplying the dry matter yield (Y) and trace element content (C) according to the formula: $U = Y \cdot C$. The elements' balance (B) was computed as a difference between the amount of elements introduced (I) with a sewage sludge dose and the amount of element uptake (U) with the plant yield, according to the formula: $B = I - U$. The simplified balance computation did not include the supply of trace elements from atmospheric precipitation, mineralization of organic matter, and leaching of elements into the soil profile. The phytoremediation of trace elements presented in the balance constitutes the percentage of elements take up by plants in relation to the amounts introduced into the soil with municipal sewage sludge.

The statistical analysis of the results was carried out using the Microsoft Office Excel 2003 spreadsheet and Statistica v. 10 PL package. The statistical evaluation of the variability of the results was carried out using a two-way analysis of variance. The significance of differences between mean values was verified based on the Tukey's *t*-test at the significance level of $\alpha \leq 0.05$. The value of the Pearson's linear correlation coefficient ($r$) was calculated for some relations (parameters) at $p \leq 0.05$. The maximum 5% dispersion of measurements in a chemical analysis was assumed in the study.

## RESULTS

### Plant yield

The dry mass yields of multiflora rose and Virginia fanpetals obtained in individual years of the investigations were presented in an earlier publication (Antonkiewicz et al. 2017).

Plant yielding was an important indicator of the plant's response to the sewage sludge applied. The average yield of energy crops obtained from several years (2008-2013) ranged from 3.42 to 14.78 Mg DM ha$^{-1}$ and depended on a sewage sludge dose and plant species (Figure 1). Close correlations between the dose of sewage sludge and the average yield of energy crops ($r$ = 0.771) were demonstrated in the field experiment.

During the multi-year research period, it was shown that a single application of 10-60 Mg DM ha$^{-1}$ of sewage sludge significantly increased plant yields compared to the control. The application of the largest dose of sewage sludge (60 Mg DM ha$^{-1}$) resulted in an increase in the average yield of multiflora rose and Virginia fanpetals by over 264% and 90%, respectively, in relation to the control (Figure 1). The study showed that multiflora rose responded to the application of sewage sludge with a higher yield increase compared to Virginia fanpetals.

On the other hand, when comparing the yield of energy crops, one could notice that the yield potential of Virginia fanpetals was greater than that of the multiflora rose. The greatest difference in plant yielding was noted in the control treatment, where neither mineral nor organic fertilization was applied. The difference between the species in the control treatment was over 128%. Subsequent doses of sewage sludge decreased the differences in yields between the tested species (for the highest dose of sewage sludge, the difference between these species was over 18%). The study showed that the largest yield-forming effect for multiflora rose and Virginia fanpetals was achieved in the treatment where sewage sludge at a dose of 60 Mg ha$^{-1}$ DM was applied (Figure 1).

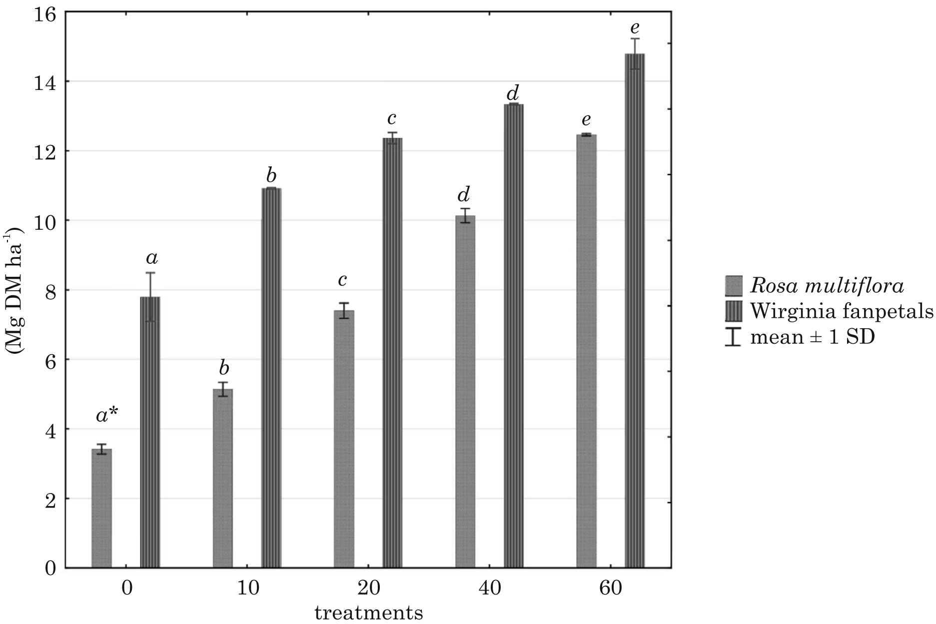


Fig. 1. The average yield of the energy crops

## Content of trace elements in the plants

The research shows that increasing doses of sewage sludge had a considerable effect on the increase in the content of elements in the tested energy crops (Table 2). These results were also confirmed by a linear correlation analysis, which showed close relationships between the dose of sewage sludge and the content of trace elements in the tested plants ($r$ = 0.365-0.791). Regardless of a treatment (sewage sludge doses), Virginia fanpetals had a higher content of Al, Mn, Co and Mo compared to multiflora rose. Multiflora rose had a higher Fe content compared to Virginia fanpetals. At the highest dose of sewage sludge (60 Mg ha$^{-1}$ DM), the increase in the content of Al, Mn, Co and Mo in Virginia fanpetals amounted to 26.9, 61.8, 43.5, 21.4%, respectively, in relation to the control. An increase in the Fe content in the multiflora rose biomass in the treatment with the highest dose of sewage sludge was more than 73% in relation to the control treatment (Table 2). Analysis of the chemical composition of energy crops showed the highest increase in the Fe content, followed by Mn, Co and Al, and the lowest increase in the Mo content. The linear correlation analysis also implicated close relationships between yield and content of trace elements in the plant biomass ($r$ = 0.405-0.956). The above correlation confirms that sewage sludge constituted a potential source of trace elements for the tested plants.

Table 2

Weighted average content of trace elements in energy crops (mg $kg^{-1}$ DM)

| Treatments | Al | Mn | Fe | Co | Mo |
|---|---|---|---|---|---|
| Sludge dose (Mg DM $ha^{-1}$) | *Rosa multiflora* | | | | |
| 0 | 18.9 ±0.4*** | 24.9 ±2.4 | 81.1 ±13.8 | 0.20 ±0.01 | 0.31 ±0.01 |
| 10 | 19.8 ±0.4 | 28.2 ±3.2 | 85.5 ±6.8 | 0.21 ±0.01 | 0.32 ±0.01 |
| 20 | 21.0 ±0.3 | 34.6 ±4.5 | 98.7 ±17.7 | 0.23 ±0.01 | 0.34 ±0.01 |
| 40 | 22.1 ±0.9 | 41.5 ±1.7 | 102.7 ±13.8 | 0.25 ±0.01 | 0.36 ±0.01 |
| 60 | 23.7 ±1.0 | 44.2 ±0.9 | 140.9 ±13.4 | 0.27 ±0.01 | 0.37 ±0.01 |
| Mean | 21.1 | 34.7 | 101.8 | 0.23 | 0.34 |
| CV (%)* | 8.7 | 23.2 | 24.2 | 11.8 | 7.0 |
| Sludge dose (Mg DM $ha^{-1}$) | *Sida hermaphrodita* | | | | |
| 0 | 22.6 ±1.8 | 38.8 ± 1.2 | 65.8 ±8.8 | 0.23 ±0.01 | 0.42 ±0.01 |
| 10 | 24.1 ±1.8 | 42.4 ±3.0 | 74.2 ±7.6 | 0.26 ±0.01 | 0.45 ±0.02 |
| 20 | 25.1 ±1.6 | 48.9 ±2.0 | 83.3 ±7.6 | 0.28 ±0.01 | 0.47 ±0.01 |
| 40 | 26.8 ±0.9 | 54.7 ±1.7 | 99.7 ±10.2 | 0.30 ±0.01 | 0.49 ±0.01 |
| 60 | 28.7 ±0.8 | 62.8 ±3.1 | 106.1 ±10.1 | 0.33 ±0.01 | 0.51 ±0.01 |
| Mean | 25.5 | 49.5 | 85.8 | 0.28 | 0.47 |
| CV (%)* | 9.3 | 18.4 | 20.3 | 11.9 | 7.2 |
| Sludge dose (Mg DM $ha^{-1}$) | mean for a dose of sewage sludge | | | | |
| 0 | 20.7 ±2.2 | 31.8 ±7.8 | 73.5 ±13.3 | 0.22 ±0.02 | 0.36 ±0.06 |
| 10 | 21.9 ±2.5 | 35.3 ±8.3 | 79.9 ±8.9 | 0.23 ±0.03 | 0.38 ±0.07 |
| 20 | 23.1 ±2.4 | 41.8 ±8.4 | 91.0 ±14.5 | 0.26 ±0.03 | 0.40 ±0.07 |
| 40 | 24.5 ±2.7 | 48.1 ±7.4 | 101.2 ±11.0 | 0.27 ±0.03 | 0.42 ±0.07 |
| 60 | 26.2 ±2.8 | 53.5 ±10.4 | 123.5 ±21.8 | 0.30 ±0.03 | 0.44 ±0.08 |
| LSD for dose** | 0.67 | 1.96 | 8.66 | 0.004 | 0.008 |
| LSD for species | 1.06 | 3.09 | 13.70 | 0.007 | 0.013 |
| LSD for interaction | 1.49 | 4.38 | 19.37 | 0.009 | 0.018 |

* CV – variability coefficient, ** LSD – least significant differences, *** SD – standard deviation

## Uptake of trace elements by plants

The increasing doses of sewage sludge raised the amount of elements taken up by energy crops (Table 3). With respect to the quantity of absorbed trace elements, it was established that Virginia fanpetals took up much higher quantities of these elements compared to multiflora rose. The greatest differences in the uptake of trace elements by the tested species was observed in the control treatments, whereas the smallest ones appeared in the plants from the treatments with the highest doses of sewage sludge. In the control treatment, the amounts of Al, Mn, Fe, Co and Mo taken up by Virginia fanpetals were higher by over 171%, 255%, 84%, 187%, 219%,

Table 3

Total uptake of trace elements by energy crops, as the sum of uptakes for the entire research period from 2008 to 2013 (g ha$^{-1}$)

| Treatments | Al | Mn | Fe | Co | Mo |
|---|---|---|---|---|---|
| Sludge dose (Mg DM ha$^{-1}$) | *Rosa multiflora* | | | | |
| 0 | 388 ±10*** | 510 ±43 | 1657 ±226 | 3.9 ±0.1 | 6.2 ±0.2 |
| 10 | 610 ±20 | 869 ±114 | 2634 ±230 | 6.5 ±0.2 | 10.0 ±0.2 |
| 20 | 933 ±17 | 1534 ±154 | 4368 ±633 | 10.2 ±0.3 | 15.1 ±0.5 |
| 40 | 1346 ±70 | 2526 ±140 | 6251 ±934 | 15.0 ±0.6 | 21.6 ±0.6 |
| 60 | 1772 ±68 | 3302 ±69 | 10525 ±969 | 20.3 ±0.7 | 27.5 ±0.4 |
| Mean | 1010 | 1748 | 5087 | 11.2 | 16.1 |
| CV (%)* | 51.3 | 61.6 | 64.8 | 54.7 | 49.8 |
| Sludge dose (Mg DM ha$^{-1}$) | *Sida hermaphrodita* | | | | |
| 0 | 1052 ±38 | 1812 ±152 | 3053 ±163 | 11.2 ±0.3 | 19.7 ±1.6 |
| 10 | 1577 ±88 | 2777 ±200 | 4862 ±509 | 16.8 ±0.2 | 29.2 ±1.5 |
| 20 | 1863 ±112 | 3629 ±140 | 6187 ±636 | 20.9 ±0.3 | 34.8 ±0.4 |
| 40 | 2144 ±55 | 4375 ±125 | 7973 ±798 | 24.1 ±0.2 | 39.0 ±0.6 |
| 60 | 2546 ±18 | 5562 ±109 | 9396 ±610 | 28.9 ±1.2 | 45.1 ±1.7 |
| Mean | 1836 | 3631 | 6294 | 20.4 | 33.6 |
| CV (%)* | 28.7 | 36.9 | 37.6 | 30.9 | 26.9 |
| Sludge dose (Mg DM ha$^{-1}$) | mean for a dose of sewage sludge | | | | |
| 0 | 720 ±365 | 1161 ±720 | 2355 ±785 | 7.5 ±4.0 | 12.9 ±7.5 |
| 10 | 1094 ±533 | 1823 ±1055 | 3748 ±1270 | 11.6 ±5.6 | 19.6 ±10.6 |
| 20 | 1398 ±515 | 2581 ±1155 | 5278 ±1146 | 15.6 ±5.9 | 24.9 ±10.8 |
| 40 | 1745 ±440 | 3451 ±1020 | 7112 ±1222 | 19.6 ±5.0 | 30.3 ±9.6 |
| 60 | 2159 ±426 | 4432 ±1240 | 9961 ±952 | 24.6 ±4.8 | 36.3 ±9.7 |
| LSD for dose** | 45.3 | 100.1 | 482.6 | 0.40 | 0.72 |
| LSD for species | 71.5 | 158.3 | 763.0 | 0.63 | 1.13 |
| LSD for interaction | 101.2 | 223.8 | 1079.1 | 0.88 | 1.60 |

* CV – variability coefficient, ** LSD – least significant differences, *** SD – standard deviation

respectively, compared to the amounts taken up by multiflora rose. At the highest dose of sewage sludge (60 Mg ha$^{-1}$ DM), the amount of Al, Mn, Co and Mo taken up by Virginia fanpetals was higher by over 43%, 68%, 42%, 63%, respectively, in relation to the amounts taken up by multiflora rose. In the case of Fe, it was established that multiflora rose took up over 12% more of this element compared to the amounts taken up by Virginia fanpetals.

When analyzing the dynamics of the increase in the quantities of trace elements taken up by the plants, it was established that multiflora rose took up elements more intensively than Virginia fanpetals did. Compared to the control, the increase in quantities of trace elements taken up by Virginia fanpetals at the highest sewage sludge dose was as follows: 142% Al, 206% Mn, 207% Fe, 158% Co and 129% Mo. Compared to the control, the increase in quantities of the above elements taken up by multiflora rose was higher, namely 356% Al, 548% Mn, 535% Fe, 420% Co and 346% Mo.

The linear correlation analysis showed close relationships between the dose of sewage sludge and the uptake of trace elements by the plants ($r$ = 0.663-0.933). Then, significant relationships between yield and uptake of trace elements by the plants were shown ($r$ = 0.878-0.983). Close relationships between the content and the uptake of trace elements by energy crops were also demonstrated ($r$ = 0.388-0.985).

**Simplified balance and phytoremediation of trace elements**

Calculating the balance of trace elements in the field experiment allowed us to to assess the cycling of elements from the soil environment to plants. In the control treatment, trace elements were not introduced to the soil because sewage sludge was not applied (Table 4). The increasing doses of sewage sludge (10-60 Mg ha$^{-1}$ DM) raised the amounts of trace elements in the soil for the tested plants. The balance of elements in the control treatment was negative, which was due to the lack of element supply with organic waste. In the treatments where the increasing doses of sewage sludge (10-60 Mg ha$^{-1}$ DM) were applied, the balance of elements was positive. The presented balance data suggest that the amounts of trace elements taken up by the plants were lower than the amounts introduced with sewage sludge.

Phytoremediation is an important measure, providing a possibility of recycling elements from sewage sludge by plants. Data from Table 4 suggest that the highest phytoremediation of trace elements was observed in the treatment with the lowest dose of sewage sludge (10 Mg ha$^{-1}$ DM), and the lowest phytoremediation was recorded in the treatment with the highest dose of sewage sludge (60 Mg ha$^{-1}$ DM). Calculations indicate that Virginia fanpetals recovered (utilized) Al, Mn, Fe, Co and Mo from sewage sludge to a higher degree than multiflora rose did. At the lowest dose of sewage sludge (10 Mg ha$^{-1}$ DM), the recovery of Al, Mn, Fe, Co and Mo by Virginia fanpetals reached 2.4%, 37%, 41%, 16%, and 41%, respectively, in relation to the quantities introduced with sewage sludge. The research shows that Virginia fanpetals and multiflora rose recovered Fe and Mo to the greatest extent, whereas the recovery of Al was the weakest. The percentage phytoremediation of micronutrients from sewage sludge was determined mostly by the volume of yield and quantities of elements taken up by the plants, and also by the amounts of elements introduced with a dose of sewage sludge.

Table 4

Simplified balance of trace elements after six years of research

| Treatments | Introduced | Uptake | Balance | Recovery | Uptake | Balance | Recovery |
|---|---|---|---|---|---|---|---|
| | (g ha$^{-1}$) | | | % | (g ha$^{-1}$) | | % |
| | *Rosa multiflora* | | | | *Sida hermaphrodita* | | |
| Sludge dose (Mg DM ha$^{-1}$) | Al | | | | | | |
| 0 | 0 | 388 | -388 | - | 1052 | -1052 | - |
| 10 | 64 580 | 610 | 63 970 | 0.9 | 1577 | 63 003 | 2.4 |
| 20 | 129 160 | 933 | 128 227 | 0.7 | 1863 | 127 297 | 1.4 |
| 40 | 258 320 | 1346 | 256 974 | 0.5 | 2144 | 256 176 | 0.8 |
| 60 | 387 480 | 1772 | 385 708 | 0.5 | 2546 | 384 934 | 0.7 |
| Sludge dose (Mg DM ha$^{-1}$) | Mn | | | | | | |
| 0 | 0 | 510 | -510 | - | 1812 | -1812 | - |
| 10 | 7 500 | 869 | 6 631 | 12 | 2777 | 4 723 | 37 |
| 20 | 15 000 | 1534 | 13 466 | 10 | 3629 | 11 371 | 24 |
| 40 | 30 000 | 2526 | 27 474 | 8 | 4375 | 25 625 | 15 |
| 60 | 45 000 | 3302 | 41 698 | 7 | 5562 | 39 438 | 12 |
| Sludge dose (Mg DM ha$^{-1}$) | Fe | | | | | | |
| 0 | 0 | 1657 | -1657 | - | 3053 | -3053 | - |
| 10 | 12 000 | 2634 | 9 366 | 22 | 4862 | 7138 | 41 |
| 20 | 24 000 | 4368 | 19 632 | 18 | 6187 | 17813 | 26 |
| 40 | 48 000 | 6251 | 41 749 | 13 | 7973 | 40027 | 17 |
| 60 | 72 000 | 10 525 | 61 475 | 15 | 9396 | 62604 | 13 |
| Sludge dose (Mg DM ha$^{-1}$) | Co | | | | | | |
| 0 | 0 | 3,9 | -4 | - | 11.2 | -11 | - |
| 10 | 103 | 6,5 | 96 | 6 | 16.8 | 86 | 16 |
| 20 | 205 | 10,2 | 195 | 5 | 20.9 | 184 | 10 |
| 40 | 410 | 15,0 | 395 | 4 | 24.1 | 386 | 6 |
| 60 | 615 | 20,3 | 595 | 3 | 28.9 | 586 | 5 |
| Sludge dose (Mg DM ha$^{-1}$) | Mo | | | | | | |
| 0 | 0 | 6.2 | -6 | - | 19.7 | -20 | - |
| 10 | 72 | 10.0 | 62 | 14 | 29.2 | 42 | 41 |
| 20 | 143 | 15.1 | 128 | 11 | 34.8 | 108 | 24 |
| 40 | 286 | 21.6 | 264 | 8 | 39.0 | 247 | 14 |
| 60 | 429 | 27.5 | 401 | 6 | 45.1 | 384 | 11 |

## Soil enzymatic activity

An earlier paper, concerning phytoextraction of heavy metals, describes a significant effect of the increasing doses of sewage sludge on soil microorganisms and their enzymatic activity – Table 5 (ANTONKIEWICZ et al. 2017).

Table 5

Soil enzymatic activity (average from 2008-2013)

| Treatments | Dehydrogenases activity | Urease activity | Protease activity | Acid phosphatase activity | Alkaline 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 $ha^{-1}$) | *Rosa multiflora* | | | | |
| 0 | 4.7 ±0.4*** | 7.9 ±0.9 | 10.2 ±0.9 | 23.1 ±0.9 | 17.3 ±1.9 |
| 10 | 4.8 ±0.1 | 9.7 ±0.7 | 10.8 ±0.8 | 24.2 ±0.3 | 17.6 ±1.9 |
| 20 | 5.6 ±0.5 | 11.7 ±1.2 | 16.4 ±1.2 | 35.1 ±2.7 | 22.2 ±2.0 |
| 40 | 8.4 ±0.9 | 16.7 ±1.0 | 24.4 ±2.2 | 72.2 ±1.7 | 51.7 ±4.4 |
| 60 | 17.0 ±0.4 | 24.1 ±1.0 | 32.5 ±0.8 | 69.4 ±2.6 | 43.3 ±2.9 |
| Mean | 8.1 | 14.0 | 18.9 | 44.8 | 30.4 |
| CV (%)* | 59.8 | 43.4 | 47.1 | 50.1 | 49.3 |
| Sludge dose (Mg DM $ha^{-1}$) | *Sida hermaphrodita* | | | | |
| 0 | 4.3 ±0.2 | 7.5 ±0.5 | 10.5 ±0.7 | 26.3 ±3.3 | 18.1 ±1.8 |
| 10 | 5.4 ±0.5 | 9.3 ±1.3 | 13.1 ±0.8 | 31.8 ±1.5 | 20.6 ±1.4 |
| 20 | 7.6 ±1.3 | 12.1 ±0.4 | 17.5 ±1.2 | 43.4 ±6.7 | 28.4 ±4.0 |
| 40 | 11.7 ±0.8 | 16.3 ±1.1 | 27.0 ±4.2 | 98.2 ±21.2 | 66.6 ±15.5 |
| 60 | 16.9 ±0.9 | 22.2 ±1.7 | 32.6 ±2.4 | 95.7 ±22.5 | 63.0 ±15.7 |
| Mean | 9.2 | 13.5 | 20.2 | 59.1 | 39.3 |
| CV (%)* | 52.6 | 41.1 | 44.0 | 58.7 | 59.6 |
| Sludge dose (Mg DM $ha^{-1}$) | mean for dose of the sewage sludge | | | | |
| 0 | 4.5 ±0.3 | 7.7 ±0.7 | 10.4 ±0.7 | 24.7 ±2.8 | 17.7 ±1.7 |
| 10 | 5.1 ±0.4 | 9.5 ±0.9 | 12.0 ±1.5 | 28.0 ±4.3 | 19.1 ±2.2 |
| 20 | 6.6 ±1.4 | 11.9 ±0.8 | 17.0 ±1.2 | 39.2 ±6.5 | 25.3 ±4.4 |
| 40 | 10.0 ±2.0 | 16.5 ±1.0 | 25.7 ±3.3 | 85.2 ±19.6 | 59.1 ±13.0 |
| 60 | 16.9 ±0.6 | 23.2 ±1.6 | 32.6 ±1.6 | 82.5 ±20.3 | 53.1 ±14.8 |
| LSD for dose** | 0.52 | 0.80 | 1.40 | 7.74 | 5.64 |
| LSD for species | 0.82 | 1.26 | 2.22 | 12.23 | 8.92 |
| LSD for interaction | 1.16 | 1.79 | 3.14 | 17.30 | 12.62 |

* CV – variability coefficient, ** LSD – least significant differences, *** SD – standard deviation

Application of increasing doses of sewage sludge to soil enhanced the bioavailability of trace elements and biogens, not only to plants, but also to soil microorganisms. The activity of the tested soil enzymes increased significantly (progressively) with the increasing sewage sludge doses, which was associated with the amount of carbon substrates available for microorganisms and enzymes. At the highest sewage sludge dose applied in the cultivation of multiflora rose, there was an over 3.6-, 3.0-, 3.1-, 3.0-, and 2.5-fold increase in dehydrogenase, urease, protease, acid phosphatase, and alkaline phosphatase, respectively, compared with the control. In turn, the soil enzymatic activity in Virginia fanpetals was over 3.9-, 2.9-, 3.1-, 3.6-, and 3.4-fold higher than in the control. The investigations demonstrated that, at the highest sewage sludge dose, dehydrogenase activity attained the highest increase and alkaline phosphatase activity was characterised by the lowest increase, irrespective of the plant species. The field experiment showed that Virginia fanpetals had a stronger stimulating effect on dehydrogenase, protease, and acid and alkaline phosphatase activity than multiflora rose. In turn, multiflora rose stimulated urease activity more than Virginia fanpetals did. The research revealed close correlations between soil enzymatic activity and trace elements' content in plants ($r$ = 0.436-0.798), and elements' uptake by plants ($r$ = 0.606-0.923). These correlations prove that soil enzymatic activity stimulates the uptake of trace elements from sewage sludge by energy crops.

## DISCUSSION

Management of municipal sewage sludge, especially on marginal lands or municipal waste landfills, is an alternative form of recycling of this waste (Nahm, Morhart 2018). Municipal sewage sludge contains many valuable microelements and trace elements, which can be recovered through its application to soil, under plants with great yield-forming potential and high nutritive demands (Wierzbowska et al. 2016, Antonkiewicz et al. 2018).

### Yield

Our research showed a significant effect of sewage sludge on the increase in the amount of yield of the Virginia fanpetals and multiflora rose. Other research confirms that municipal sewage sludge increases plant yield and efficiency of energy crop biomass (Kołodziej et al. 2016, Antonkiewicz et al. 2018, Ozdemir et al. 2018).

### Content of trace elements

Our research showed that the applied municipal sewage sludge (aerobically stabilized) constitutes an available source of micronutrients for plants with high demand for microelements and trace elements. Our research showed that the content of Al, Mn, Co and Mo was higher in Virginia fanpetals than in multiflora rose. Compared to Virginia fanpetals, multiflora rose had a greater capacity to accumulate Fe. Other studies indicate that multiflora rose is a plant which contains substantial quantities of iron (Hayashi 1998, Kowalczyk-Juśko 2016). Research conducted by Asik et al. (2015) showed that application of sewage sludge to soil increases the availability of elements to plants. An increase in the trace elemental content in energy crops results primarily from acidification of the soil environment and mineralization of organic matter (Kalembasa, Symanowicz 2009, Dede et al. 2015, Wieczorek et al. 2017).

In addition, an increase in the content of trace elements in plants is due to the high uptake by the root system of plants, follwoed by their translocation of these elements to aerial parts (Rutkowska et al. 2014, Schröder et al. 2018).

Currently, it is increasingly common to recommend sewage sludge thermal processing to biochar, owing to the stability of organic compounds in the latter (Ren et al. 2018). In terms of using microelements and trace elements from sewage sludge, thermal processing of sewage sludge is unfavourable because, as shown in the research conducted by Pi et al. (2018), thermal processing of sewage sludge decreases availability of microelements to plants (Pi et al. 2018).

**Uptake of trace elements**

A significant effect of sewage sludge on the uptake of trace elements by the energy crops was observed. Intensive uptake of trace elements from soil and sewage sludge by energy crops results primarily from these plants' high nutrient requirements and great yield-forming potential (Monti et al. 2008, Ozdemir et al. 2018). A study conducted by Ozdemir et al. (2018) confirms that a supply of trace elements with sewage sludge will allow to remove them from soil during consecutive harvests of the biomass of energy crops, including Miscanthus.

Our research showed that, in quantitative terms, Virginia fanpetals took up more trace elements than multiflora rose did. These results are supported by Wierzbowska et al. (2015, 2016), who showed that Virginia fanpetals, apart from heavy metals, takes up significant quantities of microelements and trace elements from sewage sludge. Another study also confirms that, compared to other energy crops, Virginia fanpetals has great yield-forming potential and absorbs intensively not only heavy metals but also microelements and trace elements (Borkowska, Molas 2012, Krzywy-Gawrońska 2012).

Wierzbowska et al. (2016) demonstrated that the uptake of trace elements (Fe, Mn, Al) from sewage sludge depends on soil conditions, including pH and mineralization of organic matter, because these elements occur in an oxide form or are bound to organic matter. Other research demonstrated that aluminum and iron oxides (occurring, for example, in sewage sludge biochar) are capable of stabilizing and binding trace elements, and thereby limiting the availability of these nutrients to plants (Shen et al. 2018).

**Balance**

The highest permissible content of substances posing an environmental risk to group IV lands (i.e. industrial areas) is 50 for Co and 30 for Mo. The content of Co and Mo in the soil on which the experiment was set up was below the threshold values for industrial soils (*Regulation* ... 2016). The determined content of Co and Mo in the municipal sewage sludge was 1.86 and 27.8 times higher, respectively, compared to the content in the soil. Also, the Mn content in the sewage sludge was over 61-fold higher compared to the content in the soil. The Al and Fe content in the soil was over 58 and 949% higher compared to the content in the sewage sludge. The research showed that municipal sewage sludge was the main source of trace elements, especially Mo and Co, for plants. The remaining elements (Al, Fe and Mn) might have been taken up from the soil and sewage sludge, which is confirmed in the study by Wierzbowska et al. (2016).

Application of sewage sludge in energy crop cultivation makes it possible to use nutrients from sewage sludge, and it is an alternative method of minimizing the amount of disposed organic waste (Antonkiewicz et al. 2018, Ozdemir et al. 2018).

Both ours and other authors' studies indicate that cultivation of energy crops with great yielding potential and with substantial 'strength' of element phytoextraction must last from ten to twenty or even tens of years so as to recover nutrients introduced with sewage sludge in their entirety (Antonkiewicz et al. 2017, 2018, Korzeniowska, Stanislawska-Glubiak 2018). Of the tested species, Virginia fanpetals turned out to be more efficient in recovering trace elements from sewage sludge compared to multiflora rose. Studies by Krzywy-Gawrońska (2012) and Wierzbowska et al. (2016) confirm that Virginia fanpetals has great phytoextraction potential. A study by Kocoń and Jurga (2017) showed that, compared to Virginia fanpetals, miscanthus was more efficient at phytoextraction of elements from heavy metal contaminated soils.

**Enzymatic activity**

Our research indicates that increasing doses of sewage sludge increased the enzymatic activity. This research finding is confirmed by other authors who state that sewage sludge applied to soil increase soil enzymatic activity (Kalembasa, Symanowicz 2012). In their research, Symanowicz et al. (2014) showed that soil enriched with nutrients is characterized by higher enzymatic activity. Our research revealed significant correlations between soil enzymatic activity and trace elements content and uptake by energy crops. This strict relationship is confirmed by other researchers who point to a significant effect of elements on soil fertility (Lisetskii et al. 2015, Bartkowiak et al. 2017).

Studies by Bartkowiak et al. (2017) and Pi et al. (2018) indicate that even thermal processing of sewage sludge was not toxic to soil bacteria. In their studies, the former authors even observed some stimulation of enzymatic activity in thermally treated sewage sludge (Pi et al. 2018).

This research points to significant relationships between trace elements in sewage sludge and their uptake by the plants, which were stimulated by soil enzymatic activity.

## CONCLUSIONS

1. It was determined that, in terms of volumes of yield, Virginia fanpetals had a greater yielding potential than multiflora rose.

2. Virginia fanpetals had a higher content of Al, Mn, Co and Mo compared to multiflora rose. Multiflora rose had a higher Fe content compared to Virginia fanpetals.

3. Concerning amounts of nutrients taken up by the plants, it was found that Virginia fanpetals took up more of the studied trace elements compared to multiflora rose.

4. It was shown that of the two tested species Virginia fanpetals recovered more trace elements than multiflora rose did. At the lowest dose of sewage sludge, Virginia fanpetals recovered more trace elements compared to multiflora rose and the difference was: Al – 1.5%, Mn – 25%, Fe – 19%, Co – 10%, Mo – 27%.

5. Based on the volume of yields, the content and uptake of micronutrients, Virginia fanpetals can be regarded as a more ‘efficient’ plant for phytoremediation of these nutrients from sewage sludge than multiflora rose.

### Conflict of interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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