PRELIMINARY RESULTS ON GROWING SECOND GENERATION BIOFUEL CROP *MISCANTHUS X GIGANTEUS* AT THE POLLUTED MILITARY SITE IN UKRAINE

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Abstract: The semi-field research on using second-generation biofuel crop *Miscanthus x giganteus* for restoration of former military site in Kamenetz-Podilsky, Ukraine was carried out during two vegetation seasons. Despite high metal pollution of soil, in particular, by Fe, Mn, Ti, and Zr, no growth inhibition was observed. The concentrations followed pattern soil > roots > stems > leaves. Accumulation of particular metals in roots was different: Fe, Mn and Ti were accumulated rather palpably after the first vegetation season and less tangible after the second one. Cu, Pb and Zn were less accumulative in both vegetation seasons, and for As and Pb the accumulative concentrations were very small. Accumulations in the above-ground parts of the plant in comparison to roots were significantly lower in case of Fe, Ti, Mn, Cu, Zn, Sr and even statistically comparable to zero in case of As, Pb and Zr. Calculated translocation ratio of metals in the plant's parts preferably indicated lack of metals' hyper accumulation. Generally, no correlations were observed between concentrations of metals in the soil and in the upper plant's parts. The research confirmed the ability of *Miscanthus x giganteus* to grow on the military soils predominantly contaminated by metals.

Key words: *Miscanthus x giganteus,* phytotechnology, restoration of military contaminated soil, translocation ratio

1. Introduction

One of the significant environmental problems in the Eastern European countries including Ukraine are abandoned military contaminated sites widely dispersed and intensively polluted by metals, oil, and products of their decomposition. They constantly pose health risks and negatively affect soil and water resources as well as biodiversity. Some former military sites may be used for agriculture, increasing risks of toxicity, while others are without plants and lack proper land management, allowing soil and water erosion. Revitalization of those sites is an important task for the region's sustainable development.

The use of bioenergy plants for the restoration of polluted soils is an innovative strategy to derive additional benefits (i.e. phytoproducts) from those remediation

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activities (GOMES, 2012; MOSA *et al.*, 2016; TRIPATHI *et al.,* 2016). Plants applicable for bioenergy and phytoremediation of contaminated soils have to be fast growing and showing high biomass, possessing an extensive root system, easy to harvest and tolerate (WENZEL, 2009; GULDANOVA *et al.*, 2010; KULAKOW and PIDLISNYUK, 2010; PRASAD, 2016). Synergistic bonding by using potential energy crops in phytoremediation programs would be useful to generate new bioenergy resources along with remediation of contaminated soil (PANDEY *et al.*, 2016).

Among the bioenergy crops second generation biofuel crop *Miscanthus x giganteus* is considered to be attractive and useful for the goals of phytoremediation (NSANGANWIMANA *et al.*, 2014; MASTERS *et al., 2016*) or for combination of phytoremediation process with biomass production (PIDLISNYUK *et al.*, 2014a; KOŁODZIEJ *et al.*, 2016). *M. x giganteus* was introduced in Europe and exhibited good production properties while used at the brownfield sites, former mining sites and contaminated agricultural lands (KAHLE *et al.*, 2001; KOCON and MATYKA, 2012; NSANGANWIMANA *et al.*, 2015; MOSA *et al.*, 2016). This plant is a C-4 perennial grass, has a high biomass productivity accompanied by good water use efficiency and low nutrient demands (PIDLISNYUK *et al.*, 2014a). *M. x giganteus* has the potential to improve soil quality by adding organic matter to soil, while as a perennial grass, it has advantages to reduce water and wind erosion (USDA, 2011). The plant can be harvested and used in place for combustion heating including in a pellet form, or processed to liquid biofuels (NSANGANWIMANA *et al.,* 2014).

We have initiated investigation on possibility to use *M. x giganteus* for restoration of former military sites (PIDLISNYUK, 2012; PIDLISNYUK and STEFANOVSKA, 2013; DAVIS *et al.*, 2014). The plant showed the good ability to grow at the model soil, artificially contaminated by heavy metals (PIDLISNYUK *et al.*, 2014b). The next step was to test ability of *Miscanthus x giganteus* to grow at the abandoned military soil collected directly from the contaminated site and to explore translocation coefficients. Experiment was done using moderately contaminated soil taken from abandoned military site located in Kamenetz-Podilsky, Ukraine during two vegetation seasons, 2014 and 2015, as a greenhouse pot experiment.

2. Materials and methods

The contaminated research site was located in city Kamenetz-Podilsky, Ukraine and had the following coordinates: Latitude-48.680910N; Longitude-26.58025E. The contaminated site was used as a military storage of former Soviet Union Army and it is placed close to the Central City Park. The soil was collected on May 20th, 2014. Soil sampling was done in accordance with the standard approach (GOST, 1984). Briefly, one testing square was selected at the site with the size of 5m × 5m; from the square five samples were taken from the depth of 0-0.3 m using ”envelope methods”, mixed and used in the pot experiment. General agronomic characteristics of the soil were determined using standard procedures (Table 1).

Rhizomes of *M. x giganteus* were obtained from the Agricultural Station of the Institute of Bioenergy and Sugar Beetroot, Ukraine and were one year old at the time of planting. In each pot two rhizomes of *M. x giganteus* were planted. The vegetation season in 2014 started on June 6th and finished on November 19th when stems and leaves withered and were cut down; the pieces of rhizomes were sampled from each pot for the analysis. For the winter season pots with rhizomes were stored in the dark, dry conditions. The second year of the experiment started on April 25th, 2015, when the first sprouts appeared. That day pots were taken back to the light in the greenhouse. The growing year ended on October 15th, 2015 when stems and leaves withered.

Table 1. Agronomic data of the soil from the research site

Parameter	**Value**	**Method**
pH	6.90 ± 0.15	DSTU ISO 10390-2001
N-NO_3^- [mg/kg]	11.6 ± 2.3	DSTU 4729-2007
N-NH_4^+ [mg/kg]	35.2 ± 1.8	DSTU 4729-2007
Humus [%]	2.84 ± 0.16	DSTU 4289-2004

Preparation of soil samples for analysis was done in accordance with the standard ISO 11464-2001. The soil sample was dried at 105°C to a constant mass. The dry sample was put on the clean sheet of paper, and small stones, plant particles and other inclusions were removed. Bigger soil clods were ground in a porcelain mortar and mixed with the main part of the soil sample. Then average soil sample was prepared for the analysis using the following approach. Thoroughly mixed soil was put on the clean paper in the form of a square and divided in four equal parts by spatula. Two opposite parts were removed, and two others were combined, mixed again and taken further in the analysis. This average sample was additionally sieved (0.25 mm pore size). The bigger particles were milled if necessary. Preparation of roots, stems and leaves samples for analysis was done in accordance with the standard DSTU ISO 11464-2001 and DSTU ISO 11465 -2001. The samples of roots were carefully cleaned by distilled water and first dried in the open air and after dried at 105 °C to a constant mass, cooled in desiccators for 1 hour and weighed. For further Roentgen-fluorescence analysis that examples were combusted at 450 °C, 4 hours, cooled during 1 hour in desiccators and weighed.

Analysis of heavy metals in the soil, roots, stems and leaves were carried out by Roentgen-fluorescence analysis using analyser Expert-3L (INAM, Ukraine, http://inam.kiev.us/contact-information).

Statistical evaluation of data was carried out using Microsoft Excel and Statistica software pack at the significance level $\alpha = 0.05$. Extreme values were excluded using the inner-fence test (ALTMAN, 1990).

3. Results and discussion

The research soil, likely due to former intensive army activities, was contaminated with metals (Table 2), especially iron, manganese, strontium, titanium and zirconium. Concentrations of As, Cu, Pb and Zn were also elevated compared to the inherent soil in the area (MEDVEDEV, 2001). The variability of metal concentrations in soils was not high, maximal relative deviation was ± 33% around average. Also with contribution of this low variability the correlations between metal concentrations in soil and in aboveground plant parts were either insignificant (As, Fe, Mn, Sr, Ti, Zr) or occasional (Cu, Pb, Zn) (supplementary material Tables S1a-i). This enabled to consider all variants labelled 1 to 5 as equal and to compare them together in order to increase significance of statistical comparisons.

Table 2. Concentrations of selected metals in soil samplings (1-5) taken from the research site (in mg/kg dwt- dry weight).

	c [mg/kg dwt]				
	1	2	3	4	5
As	75±5	165±85	115±35	70±0	75±5
Cu	180±10	120±20	125±25	155±5	255±45
Fe	140 955 ±5 715	135 140 ±14 580	139 010 ±13 870	131 530 ±8 570	136 115 ±1 515
Mn	5 020±1 580	5 210±40	5 835±115	4 305±375	7 205±1 245
Pb	395±85	185±85	150±50	230±10	450±50
Sr	795±25	935±65	700±10	655±115	1 055±135
Ti	19 815±1 475	17 640±1 370	19 160±1 960	20 265±1 115	19 755±775
Zn	560±30	540±0	515±15	505±15	585±15
Zr	1 910±140	1 515±235	1 165±65	1 070±230	1 115±145

Despite high concentrations of metals pollution in the soil the growth of *M. x giganteus* seemed not affected (supplementary material, Fig. S1a and S1b) and plant height was comparable to regular plantation growing at the clean agricultural land with similar climates (HANZHENKO *et al.*, 2015). Two years observation confirmed high adaptability of *M. x giganteus* for growth on marginal and metal-contaminated soils. These preliminary results therefore extend list of possible non-agricultural sites needing reclamation which are suitable for plantation of *M. x giganteus*.

Accumulation of metals in the plants took place predominantly in the roots; translocation to upper parts was order of magnitude lower (Tables 3 and 4). It has to be stressed that accumulation in roots was different: Fe, Mn and Ti were accumulated rather palpably after the first vegetation season and less tangible after the second one. Cu, Pb, Zn were less accumulative in both vegetation seasons, and for As and Pb the accumulative concentrations were very small. Accumulations in the above-ground parts in comparison to roots were significantly lower in case of Fe, Ti, Mn, Cu, Zn, Sr and statistically comparable to zero in case of As, Pb and Zr. These phenomena may be attributed to annual accumulation of nutrients in rhizomes, since *M. x giganteus* above grounds parts fade at the end of each season. Shoot/root coefficients were however significantly lower than 1 (with exception of Zn in year 1) indicating no hyperaccumulation of metals in the plant.

The absolute totals of metals accumulated in roots, stems and leaves were higher in year 1 than 2; however overall the values were statistically comparable (sign test, $P>0.05$). Individually significant decrease of concentrations between year 1 and 2 was detected for Fe and Ti (in stems and leaves), and Mn and Sr (in stems only). Contrary, concentrations of Cu (stems and leaves) and Zn (stems) significantly increased between year 1 and 2. For As and Pb, the concentrations in above-ground parts were statistically comparable to zero (t-test, $\alpha = 0.05$); the same phenomena was observed for concentrations of Zr in stems.

Table 3. Accumulation of metals in the different parts of *M. x giganteus* at the end of first and second vegetation seasons (average ± std. deviation, n = 10). Letters indicate overlapping of confidence intervals based on mutual comparisons ($\alpha = 0.05$), i.e. values with the same letters can be considered comparable; bolded values indicate values significantly higher than zero (t-test, $\alpha = 0.05$).

c (mg/kg dwt)		Year 1			Year 2		
	soil	roots	stems	leaves	roots	stems	leaves
As	**83±24c**	**7±7b**	0±0a	0±0a	**8±4b**	0±0a	0±0a
Cu	**152±35e**	**55±32d**	**4±1a**	10±11ab	**57±13d**	**8±3b**	**14±4c**
Fe	**136 550±10 641f**	**27 162±18 187e**	**316±146b**	**5 227±3 529d**	**20 238±3 034e**	**130±62a**	**1 107±251c**
Mn	**5 189±963e**	**953±552cd**	**128±32b**	**445±260cd**	**638±265d**	**46±23a**	**176±65bc**
Pb	**282±134c**	**60±60b**	1±1a	1±2a	**21±13b**	0±0a	0±0a
Sr	**788±128e**	**158±93d**	**7±3a**	**39±17c**	**95±4d**	**16±7b**	**29±12bc**
Ti	**19 327±1 668f**	**4 067±2 629e**	**67±24b**	**913±641d**	**2 800±360e**	**28±17a**	**158±34c**
Zn	**541±34e**	**138±75cd**	**18±8a**	**114±54cd**	**163±47d**	**49±15b**	**75±9c**
Zr	**1 355±364e**	**269±194d**	**1±1ab**	**19±13c**	**112±53d**	0±0a	**2±1b**

Table 4. Translocation ratios[1] of metals in *M. x giganteus* parts measured after first and second vegetation seasons (average ± std. deviation, n = 10). Letters indicate overlapping of intervals ($\alpha = 0.05$, see Table 3); bolded values indicate significantly non-zero values (t-test, $\alpha = 0.05$).

	Year 1			Year 2		
	stems/roots	leaves/roots	leaves/stems	stems/roots	leaves/roots	leaves/stems
As	0±0a	0.08±0.18a	0±0a	0.01±0.03a	0.07±0.14a	2.53±2.53a
Cu	**0.11±0.09b**	0.05±0.07a	**2.70±3.12bcd**	**0.12±0.03b**	**0.24±0.06c**	**1.76±0.34d**
Fe	**0.02±0.02**	**0.29±0.31ab**	**15.06±11.03c**	**0.01±0a**	**0.05±0.02b**	**7.94±2.73c**
Mn	**0.26±0.22ab**	**0.88±0.95ab**	**3.10±1.78bc**	**0.07±0.04a**	**0.29±0.16b**	**4.26±1.57c**
Pb	0.03±0.04a	0.01±0.01a	6.75±9.18a	0±0a	0±0a	0±0a
Sr	**0.10±0.10a**	**0.39±0.38a**	**5.97±3.25d**	**0.18±0.06ab**	**0.35±0.16b**	**1.93±0.75c**
Ti	**0.03±0.02bc**	0.37±0.46abc	**16.12±14.63d**	**0.01±0.01b**	**0.05±0.01c**	**5.66±1.73d**
Zn	**0.23±0.19a**	**1.04±0.86ab**	**5.55±2.47c**	**0.31±0.07a**	**0.42±0.07a**	**1.64±0.39b**
Zr	0±0a	0.13±0.16ab	22.15±24.16ab	0±0a	**0.02±0.02a**	2.67±1.38ab

[1] Generally the leaves/roots ratio divided by stems/roots ratio should be equal to leaves/stems ratio, which was predominantly observed. Nevertheless, due to high data variability and elimination of extremes concentration values by inner-fence test, sometimes the values differ. The leaves/stems ratio was calculated directly from the concentration values and not from two other ratios.

The metal accumulation data confirm the desired pattern required for simultaneous phytoremediation and biomass production. On the one hand, *M. x giganteus* extracted some metals from soil and thus it carried out slow soil decontamination. On the other hand, these metals are deposited predominantly in roots, which preserve upper plant parts relatively clean and thus enable their use as energetic biomass. Nevertheless, *M. x giganteus* is a perennial plant and supposed to be grown for far longer time than two years (PIDLISNYUK *et al.*, 2014a,b). Described promising results from two vegetation periods (2014 and 2015) need to be therefore verified during experiment, which is expected to be continued for 2016-2017. The results can illustrate the overall picture for the long-term growing of *M. x giganteus* at the former military contaminated site. The further research has also to be concentrated on interconnection between *M. x giganteus* biomass quality and quantity, nature and concentrations of contaminants at the military sites including those newly appeared at the east of Ukraine. The results indicate that production of energy biomass from *M. x giganteus* is attractive and might be developed into a profit making operation.

4. Conclusions

The greenhouse pot experiment with *Miscanthus x giganteus* during two vegetation seasons using soil taken from the abandoned military site located in Kamenetz-Podilsky, Ukraine and moderately contaminated by metals confirmed the ability of the research plant to grow on the marginal contaminated land. Accumulation of metals in *M. x giganteus* was observed predominantly in roots and order of magnitude less in stems and leaves preserving upper parts usable as energy biomass. While the experiment continues these preliminary results indicate applicability of this crop for simultaneous phytoremediation and energy biomass production.

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Supplementary materials: Additional illustrative materials are presented at supplementary material - Tables S1a-i nad Figures S1a and S1b.

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