# New Phytotechnology for cleaning contaminated military sites in Slovakia and Ukraine

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***Contaminate Sites***
***Bratislava, Slovakia***
***May 29th, 2015***

# Overview

- Advantages and disadvantages of using biofuel crops for phytoremediation
- Miscanthus as prospective crop for soil remediation/biofuel production
- Confirmation the possibility to use miscanthus for phytoremediation
- Semi-field research while growing miscanthusxgigantheus at the contaminated soils in Ukraine and Slovakia
- Summary

# Biofuel crops

- Second generation biofuel crops which represented by not-food crops are less directly in conflict with food crops and would not effect the price of food
- Crops for second generation biofuels can be divided into two main categories:

short rotation canopy species

perennial/ annual grasses

***Poplar ( Populus spp.)***

***Willow ( Salix spp.)***

***Locust ( Robinia spp.)***

***Switchgrass (Panicum virgatum L.)***


***Reed canary grass Phalaris arundinacea L.***

***Miscanthus (Miscanthus sinensis A.., Miscanthus sacchariflorus M., Misccanthus x giganteus)***

# Miscanthus composition, value, and processing

- Miscanthus is of interest as an energy crop because of its perennial growth habit and relatively high yield of biomass with minimum inputs of fertilizers
- Plant grows well in mildly contaminated soil and where soil quality is poor
- As a biofuel crop *M. x giganteus* may supply up to 12% of the European Union's energy need by 2050 (Fruhwirth and Liebhard, 2004)
- The hybrid *M. x giganteus* is a large, sterile triploid perennial grass derived from a cross between M. sinensis and M.sacchariflorus native to southern and eastern Asia, is adaptable to areas not experiencing deep freezing of the soil and neither excessively wet or dry. There are 20 different species of miscanthus and under some conditions, higher production may be obtained with species other than those in common use (Liu et al. 2013)
- The annual harvestable energy production of it is favorable at > 17 MJ/kg dry matter (Collura et al., 2006) and > 10,000 kg.ha-1 yield (total 170,000 MJ/ha/year).
- Its total above-ground biomass yield in European conditions may reach 20 to 35 t.ha-1.yr-1 ( van der Werf et al, 1993; Venendaal et al, 1997)
- Similar total production is reported for the U.S. - 24-35 t.ha-1.yr-1 (Lewandowski, 2003; USDA, 2011).

## Advantages and disadvantages of *Miscanthus* for production and use in phytoremediation*

| Advantages | Disadvantages |
|---|---|
| In production | |
| Perennial, established stands last ~20 years | Takes 2-3 years to fully establish |
| Effectively suppresses weeds once established | Tall, dense growing perennial grass monoculture with limited wildlife friendly uses |
| High productivity of biomass compared to other energy crops (20 up to 35 tons.$ha^{-1}$.$yr^{-1}$) | Bioenergy processing immature technology; expensive pre-processing needed |
| Uses water efficiently by C-4 photosynthesis; total usage ~ 1 m.$yr^{-1}$ | Yields are influenced by water availability; under low-rainfall conditions may be poor |
| Grows at lower temperatures than other warm season (C-4) grasses; hence longer season | Limited tolerance of low winter temperatures so not suited to severe continental climates |
| Does not require as much N as sorghum, maize, oil palm, or sugar beets | Off-take of K ~3 x more than coppice willow |
| Mineral content of biomass relatively low compared to common biomass crops | Mineral nutrient content per unit energy high compared to coal |
| The winter harvested crop is relatively dry, so drying costs are low | Field drying and mineral leaching results in significant biomass loss as leaf fall |

* *Pidlisnyuk et al, Critical Review in Plant Science, 2014 ,N1, p.1-19*

Advantages and disadvantages of *Miscanthus* for production and use in phytoremediation*

| In use for phytoremediation | |
|---|---|
| Economic return can be obtained from contaminated land with employment and market value of biomass fuels (possibility of developing a more economical approach to remediation of soils with heavy metals such as mine land) | Dedicated energy crops can result in displacement of other crops with significant changes in land use, food crop prices |
| Easier to clear than trees for the site to be transformed for future use | Sterile hybrid so propagation for initial establishment is labor intensive |
| **In both processes** | |
| Potential for income generation through carbon credits through $CO_2$ sequestration | Less C storage than forest wood crops over next 50 years |
| Reduction of soil erosion due to rainfall, or wind. Reduces dust | Can serve as reservoir for insect pests of other species |

** Pidlisnyuk et al, Critical Review in Plant Science, 2014, 1, p.1-19*

Annual yields over three years (g/plot) of aerial part of *Miscanthus giganteus* and *Sida hermaphrodita* (Virginia Mallow) for soil previously contaminated by Zn and Pb **

| Plant species | Soil type | pH | 2008 | 2009 | 2010 |
|---|---|---|---|---|---|
| *Miscanthus giganteus* | Loam | 5.7<br>6.3 | 194<br>375 | 1216<br>1390 | 1518<br>2014 |
| | Sand | 5.2<br>6.1 | 379<br>546 | 2067<br>2087 | 3084<br>3454 |
| *Sida hermaphrodita* | Loam | 5.7<br>6.3 | 49<br>130 | 255<br>429 | 854<br>1199 |
| | Sand | 5.2<br>6.1 | 248<br>499 | 720<br>1531 | 1171<br>2128 |

Plot size was 1m x 1m. Each plot was filled with loamy or sandy soil, at two different pH levels. More than 20 years previously, the soil in each plot was artificially contaminated by metals. The loam was contaminated with 700 $mg.kg^{-1}$ of soil by Pb and with 1100 $mg.kg^{-1}$ of soil by Zn. The sand was contaminated with 600 $mg.kg^{-1}$ of soil by Pb and 900 $mg.kg^{-1}$ of soil by Zn. In 2008, the year of establishment, two plants were set per plot. Above ground biomass yield was determined for biomass dried several days at $60^oC$.

*****Kocon and Matyka, J.Food Agric.Environ., 2012***

# Research in Slovakia (conformation of using Miscanthus for phytoremediation of metal-contaminated soils)

- To research the behavior of selected metals (cobalt and copper) in the soil artificially contaminated by metals
- To explore the dynamic of the process (32 days and 86 days) and to evaluate the differences between behavior of copper and cobalt


❑**Evaluation of Cu/Co in the plants' parts were done by using Spectrometer AAS AVANTA ∑ by** GBC Scientific with the electrothermal atomization. Autosampler PAL 3000 was used for electrothermal analysis. Analysis and results' evaluation were supported by software GBC Avanta ver.2.0

❑Methodology of research is summarized in <u>Claim for the Invention</u> #a2013 12471 (Ukraine), Pidlisnyuk V., Stefanovska T. Method for growing plants in heavy metals contaminated soils, issues on January 29, 2014

| Concentration of Co in soil, ppm | Parallel tests, concentration in roots, ppm | | Average | Coefficient K | Parallel tests, concentration in stems, ppm | | Average | Coefficient K | Parallel tests, concentration in leaves, ppm | | Average | Coefficient K |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | | | 1 | 2 | | | 1 | 2 | | |
| 12.58 | ND* | ND | ND | - | ND | ND | ND | - | ND | ND | ND | - |
| 25.16 | ND | ND | ND | - | ND | ND | ND | - | ND | ND | ND | - |
| 50.32 | 0.43 | 0.62 | 0.525 | 1.04 | ND | ND | ND | - | 0.03 | ND | 0.03 | 0,05 |

| Concentration of Co in soil, ppm | Parallel tests , concentration in roots, ppm | | Average | Coefficient K | Parallel tests, concentration in stems , ppm | | Average | Coefficient K | Parallel tests, concentration in leaves, ppm | | Average | Coefficient K |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | | | 1 | 2 | | | 1 | 2 | | |
| 12.58 | ND | ND | ND | - | ND | ND | ND | - | ND | ND | ND | - |
| 25.16 | 0.44 | 0.62 | 0.53 | 2.1 | ND | ND | ND | - | ND | ND | ND | - |
| 50.32 | 0.84 | 0.81 | 0.82 | 1.64 | 0.05 | ND | 0.05 | 0.09 | 0.02 | ND | 0.02 | 0.04 |

$$\text{Coefficient K} = \frac{\text{Concentration of metal in plant's part x 100\%}}{\text{Concentration of metal in soil}}$$

(Li G.-Y. et al, 2011)

# Concentration of Cu in Miscanthus after 32 and 86 days of soils' treatment by $CuSO_4 \times 5 H_2O$

| Calculated concentration of Cu in soil, ppm | Parallel tests, concentration in roots, ppm | | Average | Coefficient K | Parallel tests, concentration in stems, ppm | | Average | Coefficient K | Parallel tests, concentration in leaves, ppm | | Average | Coefficient K |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | | | 1 | 2 | | | 1 | 2 | | |
| 22.10 | 2.40 | 3.60 | 3.00 | 13.57 | 1,20 | 2.20 | 1,70 | 7.69 | 2.10 | 2.00 | 2.05 | 9.28 |
| 44.20 | 7.20 | 4.60 | 5.90 | 13.35 | 1.00 | 2.00 | 1.50 | 3.39 | 3.20 | 7.20 | 5.20 | 11.76 |

| Calculated concentration of Cu in soil, ppm | Parallel tests, concentration in roots, ppm | | Average | Coefficient K | Parallel tests, concentration in stems, ppm | | Average | Coefficient K | Parallel tests, concentration in leaves, ppm | | Average | Coefficient K |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | | | 1 | 2 | | | 1 | 2 | | |
| 22.10 | 7.40 | No data | 7.40 | 33.4 | 1.00 | 2.40 | 1.70 | 7.69 | 2.60 | 2.00 | 2.30 | 10.40 |
| 44.20 | 6.30 | 10.20 | 8.25 | 18.66 | 5.00 | 7.20 | 6.10 | 13.8 | 6.80 | 7.40 | 7.10 | 16.06 |

# Research sites

Slovakia

Ukraine

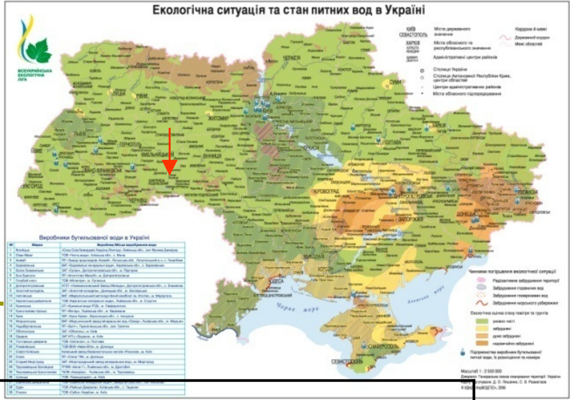


2014 →

2013-2014 →

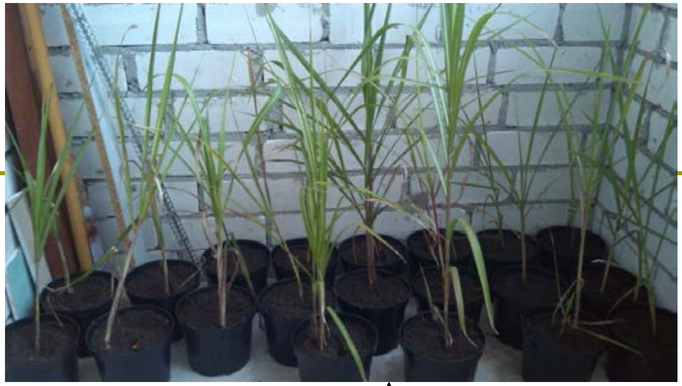
Place in Slovakia:48$^{0}$38’38.6’’N 19$^{0}$08’25.9’’E
Place in Ukraine:Latitude-48,680910;
Longitude-26,58025

# Research Kamenetz-Podilsky, Ukraine




| Number of test | Depth (cm) | Weight of example for test , (g) | Volume of acetate extract, (ml) | Concentration of heavy metals (mg/kg) | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | Cu | Zn | Co | Mn | Cd | Pb |
| 1 | 0-30 | 10 | 50 | 1.31 | 9.26 | 2.43 | **151.6** | 0.37 | **9.74** |
| | 31-60 | 10 | 50 | 1.63 | 10.5 | 4.38 | **337.0** | 0.36 | **20.60** |
| | 61-90 | 10 | 50 | 1.11 | 5.29 | 1.64 | **141.7** | 0.41 | **7.38** |
| 2 | 0-30 | 10 | 50 | 1.09 | 4.25 | 3.47 | **268.8** | 0.20 | **10.50** |
| | 31-60 | 10 | 50 | 1.49 | 5.24 | 3.53 | **351.0** | 0.50 | **10.70** |
| | 61-90 | 10 | 50 | 1.22 | 5.24 | 3.94 | **517.7** | 0.28 | **8.58** |
| 3 | 0-30 | 10 | 50 | 0.88 | 2.70 | 1.32 | 139.3 | 0.32 | **6.87** |
| | 31-60 | 10 | 50 | 0.73 | 0.85 | 1.09 | 26.9 | 0.30 | 3,73 |
| | 61-90 | 10 | 50 | 1.11 | 1.18 | 2.10 | 115.6 | 0.44 | **6.28** |
| Limited concentration of metals in the soil (mg/kg) | | | | 3.0 | 23.0 | 5.00 | 140.0 | 0.60 | 6,00 |

Kamenetz-Podilsky,Ukraine



Miscanthus plantation, 2013

| | Concentration of heavy metals (mg/kg) | | | | | |
|---|---|---|---|---|---|---|
| | Cu | Zn | Co | Mn | Cd | Pb |
| Soil | 1,63 | 10,50 | 4,38 | 337,0 | 0,36 | 20,68 |
| Roots* | ND | 0,07 | 0,03 | 3,64 | ND | 0,18 |
| K,% | - | 0,67 | 0,68 | 1,08 | - | 0,87 |
| Stems* | ND | 0,02 | ND | 0,96 | ND | 0,04 |
| K,% | - | 0,19 | - | 0,28 | - | 0,19 |

* Average from three plants

# Research ,2014

Zagreb University , Croatia

June,6th July,31st

Plantation, planted on May,3rd

Soil from Sybenik, planted on July,31st

Matej Bel University, Slovakia
Plantation planted on April,29th

May,14th

August,2nd

Soil from Sliac, planted on April 30th

May,14th

May,25th,2015

August,13th

November,19th

Plantation at NULES research station, established in 2011

NULES, Ukraine, soil from KP, planted on June, 6th

July,5th

October, 30th

# Semi-field research in Slovakia, 2014 ➔

- The soils sampling were tken in May,2014 from Sliac in accordance with the standard approach presented at GOST,1984
- In each of the pots the contaminated soil was mixed with the relatively clean soils from the territory located near the appointed research places, and different combinations of mixtering were applied. There were 14 kg of mixtured soil in each of the pots
- Slovakian experiment: started on April,$30^{th}$, finished on December, $10^{th}$
- The analysis of heavy metals content in the soil, and plants' parts: roots, stems and leaves was provided by Rentgen-fluorescent analysis using analizator Expert-3L, produced in Ukraine. The preparation of soil and plant samples to the analysis was done in accordance with ISO 11464-2001 and three parallel measurements of each testing example was done

# Content of contaminated elements in soils and rhizomes

Soil ( experiment A)

| Elements (mg/kg) | 1a | 2a | 3a | 4a | 5a |
|---|---|---|---|---|---|
| Ti | 27640 | 20620 | 24670 | 30360 | 26550 |
| Mn | 4160 | 3940 | 3120 | 3770 | 2810 |
| Fe | 209490 | 202650 | 198100 | 185840 | 179950 |
| Cu | 690 | 540 | 390 | 320 | 310 |
| Zn | 910 | 880 | 1230 | 1540 | 1050 |
| As | 470 | 390 | 500 | 500 | 330 |
| Sr | 1240 | 1110 | 910 | 680 | 750 |
| Zr | 1400 | 1560 | 1950 | 1530 | 1550 |

Soil ( experiment B)

| Elements (mg/kg) | 1b | 2b | 3b | 4b | 5b |
|---|---|---|---|---|---|
| Ti | 25200 | 24150 | 25520 | 25320 | 28700 |
| Mn | 4000 | 5160 | 4450 | 6090 | 3180 |
| Fe | 216310 | 220930 | 203130 | 213180 | 169160 |
| Cu | 310 | 340 | 400 | 440 | 400 |
| Zn | 780 | 870 | 1000 | 1070 | 870 |
| As | 380 | 450 | 360 | 530 | 250 |
| Sr | 1160 | 1260 | 710 | 940 | 420 |
| Zr | 1440 | 1850 | 1140 | 1380 | 1000 |

Rhizomes ( experiment A)

| Elements (mg/kg) | 1a | 2a | 3a | 4a | 5a |
|---|---|---|---|---|---|
| Ti | 441,36 | 1370,71 | 177,59 | 228,03 | 143,02 |
| Mn | 296,44 | 221,55 | 36,55 | 71,94 | 34,27 |
| Fe | 4158,01 | 11538,50 | 1359,36 | 1862,22 | 926,64 |
| Cu | 48,10 | 93,66 | 34,68 | 14,25 | 30,46 |
| Zn | 49,31 | 228,75 | 56,23 | 19,00 | 34,27 |
| As | 2,41 | 16,21 | 1,87 | 3,39 | 121,86 |
| Sr | 49,31 | 64,84 | 22,49 | 26,47 | 20,31 |
| Zr | 11,41 | 32,42 | 3,75 | 12,22 | 4,65 |

Rhizomes ( experiment B)

| Elements (mg/kg) | 1b | 2b | 3b | 4b | 5b |
|---|---|---|---|---|---|
| Ti | 545,68 | 1269,05 | 141,31 | 371,57 | 292,12 |
| Mn | 270,70 | 287,65 | 41,11 | 110,10 | 84,13 |
| Fe | 5744,56 | 10489,82 | 865,83 | 3166,12 | 1916,32 |
| Cu | 32,45 | 81.62 | 61,15 | 43,01 | 74,78 |
| Zn | 62,34 | 76,64 | 52,41 | 83,43 | 102,83 |
| As | 5,12 | 7,96 | 1,03 | 4,30 | 2,34 |
| Sr | 62,34 | 63,70 | 11,82 | 30,10 | 22,20 |
| Zr | 19,64 | 60,72 | 2,57 | 23,22 | 4,67 |

# Content of contaminated elements in stems and leaves of miscanthusxgiganteus

Stems( experiment A)

| Elements (mg/kg) | 1a | 2a | 3a | 4a | 5a |
|---|---|---|---|---|---|
| Ti | 24,59 | 38,21 | n/d | n/d | n/d |
| Mn | 15,37 | 19,10 | 31,05 | 13,49 | 19,06 |
| Fe | 124,82 | 91,54 | 129,18 | 75,72 | 121,51 |
| Cu | 18,45 | 18,31 | 42,23 | 10,50 | 11,91 |
| Zn | 80,55 | 117,81 | 144,09 | 118,45 | 88,15 |
| As | 0,61 | 2,39 | n/d | 0,75 | n/d |
| Sr | 19,68 | 35,03 | 53,41 | 14,99 | 22,24 |
| Zr | n/d | n/d | n/d | n/d | n/d |

Leaves ( experiment A)

| Elements (mg/kg) | 1a | 2a | 3a | 4a | 5a |
|---|---|---|---|---|---|
| Ti | 47,79 | n/d | n/d | 89,18 | n/d |
| Mn | 130,42 | 220,50 | 62,04 | 202,06 | 194,04 |
| Fe | 192,15 | 243,65 | 150,87 | 260,58 | 220,20 |
| Cu | 23,89 | 26,80 | 22,56 | 30,66 | 28,34 |
| Zn | 40,82 | 42,64 | 38,07 | 39,02 | 42,51 |
| As | 1,99 | 2,44 | n/d | 2,79 | 1,09 |
| Sr | 66,70 | 71,88 | 50,76 | 89,18 | 33,79 |
| Zr | n/d | n/d | n/d | n/d | n/d |

Stems ( experiment B)

| Elements (mg/kg) | 1b | 2b | 3b | 4b | 5b |
|---|---|---|---|---|---|
| Ti | n/d | n/d | n/d | n/d | 59,74 |
| Mn | n/d | 16,40 | n/d | 12,36 | 73,12 |
| Fe | 269,85 | 96,68 | 141,02 | 69,77 | 173,03 |
| Cu | 24,23 | 13,81 | 23,67 | 26,49 | 44,29 |
| Zn | 76,00 | 94,95 | 142,99 | 113,92 | 190,54 |
| As | n/d | n/d | n/d | n/d | 4,12 |
| Sr | 71,59 | 46,61 | 47,34 | 30,03 | 77,24 |
| Zr | n/d | 2,59 | 3,94 | n/d | n/d |

| Elements (mg/kg) | 1b | 2b | 3b | 4b | 5b |
|---|---|---|---|---|---|
| Ti | n/d | 42,05 | n/d | n/d | n/d |
| Mn | 147,50 | 247,35 | 166,97 | 195,59 | 248,32 |
| Fe | 184,37 | 307,95 | 205,41 | 203,57 | 258,14 |
| Cu | 27,94 | 27,21 | 24,02 | 19,96 | 32,27 |
| Zn | 39,11 | 43,29 | 39,64 | 30,60 | 46,30 |
| As | n/d | 1,24 | n/d | n/d | n/d |
| Sr | 44,70 | 24,73 | 34,84 | 33,26 | 57,72 |
| Zr | n/d | n/d | n/d | n/d | n/d |

# First year of long-term semi-field research in Slovakia

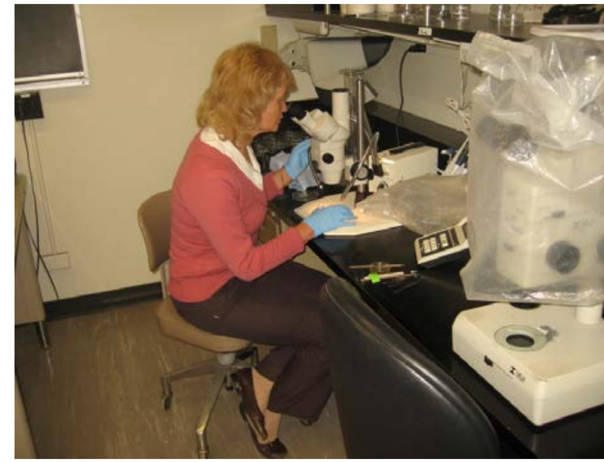
- Results shown that there is only slight correlation between increasing concentrations of contaminants at the diluted soil and their up taken to the above part of the plants.
- The main part of heavy metals was uptaken to the leaves at the beginning of the vegetation season (mainly during first two month) and remains relatively stable till the end of vegetation season. The uptaken amount of heavy metals was not essential by above surface plants and preliminary was under the limited levels.
- The heavy metals were in the following order in terms of soils : Fe>> Ti>>Mn. For rhizomes the similar order was observed. Much smaller content of elements was detected in stems and leaves, in both Fe was in the similar concentrations and less concentrations of Zn and Sr was detected in leaves in comparisons to stems. Only a limited amount of others was observed and Ti and Zr did not detected at all.

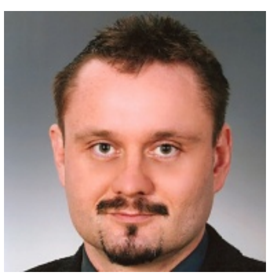
# Conclusion

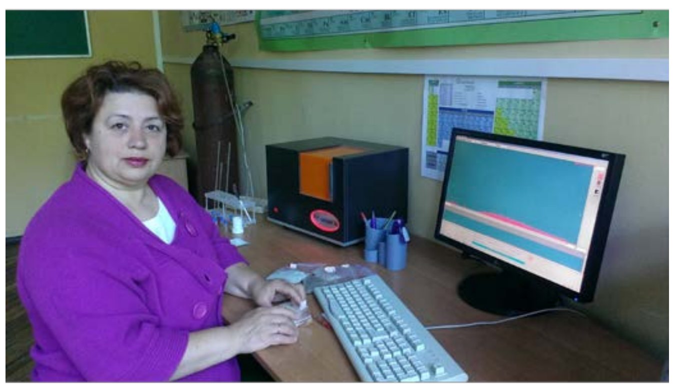
- The obtained results confirmed the ability of Miscanthusxgigantheus to grow at the slightly contaminated soils The highest concentrations of metals were detected in the roots and smaller concentrations were in stems and leaves during all monitored time
- Miscanthus biomass received at cobalt contaminated soil may be used for energy production because the above surface part accumulated only limited traces of the metal and fit the requests
- Miscanthus showed good growing at the contaminated military soils in KP . During first year of growing Zn,Co,Mn and Pb were detected in small concentrations in the plants and preliminary at roots, coefficient of taken K was rather low (around 1% and below)

Acknowledgements

NATO SfP Planning Grant #98468
Kansas State University Alumni association, USA







Thank you for the attention!

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