UNIVERZITA J. E. PURKYNĚ V ÚSTÍ NAD LABEM

DEVELOPING PERENNIAL PHYTOTECHNOLOGY FOR CONTAMINATED MILITARY SITE: CASE OF KAMENETZ-PODILSKY, UKRAINE

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Projects involved:

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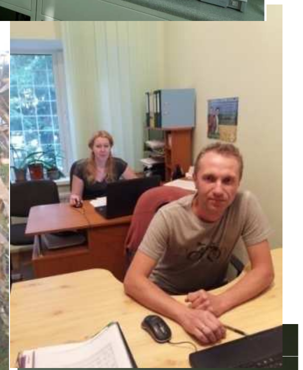
Finished:

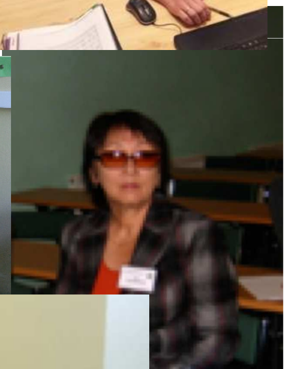
- NATO SPS Planning Grant #984687 " New technology for phytoremediation of military contaminated sites" (2014-2015)
- **On-going:**
- NATO SPS MYR G4687 " Military Site Cleaning" (2016-2018)
- Scientific Project from the Ministry of Education and Science, Republic of Kazakhstan (2015-2017) –bilateral IPBB and UJEP
- FEMS from European Federation of Microbiology Society, bilateral UJEP-TUBAF FU

Submitted for funding:

- Sustainable management of post-mining areas - institutional cooperation for strengthening of Czech-Saxony cross-border region (INSTANT)/ Bergbaufolgeflächen nachhaltig bewirtschaften – Institutionelle Zusammenarbeit zur Stärkung des Grenzgebietes Sachsen-Tschechien (INSTANT)- under reviewing

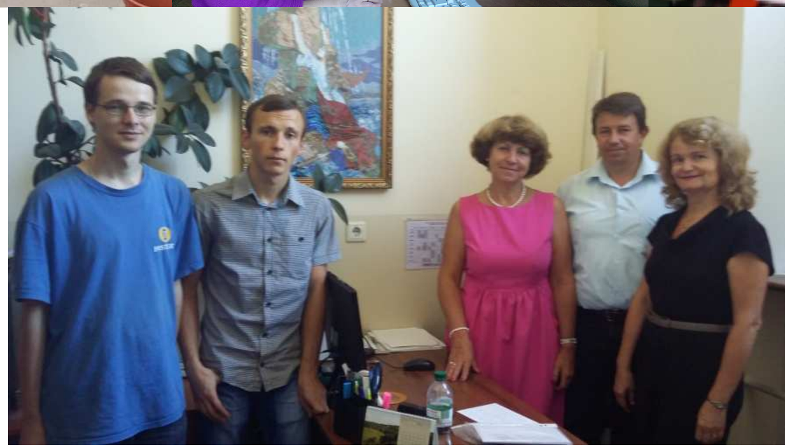
UJEP, Czech Republic
NULES and NULP, Ukraine
KSU, USA
KAES, USA
IPBB, Kazakhstan
Liasoing institutions:
UZ, Croatia
WULEC, Poland
WMU, Poland





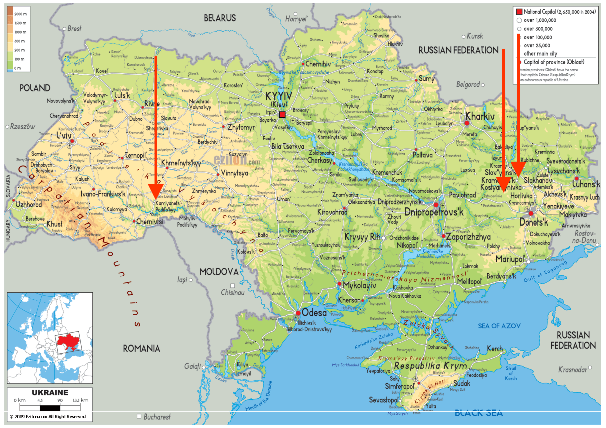
The primary goals

- to improve environmental security by developing methods to produce biomass in large quantities on contaminated military lands
- to improve soil effectively and efficiently



Research sites for developing biotechnology

Ukraine



Kamenetz-Podilsky, since 2014
ATO zone: Mariinka and Kurakhovo, since 2016

Czech Republic

Mimon, since 2016

USA

USA- Fort Riley, since 2015

Directions of current research:

UJEP, Czech Republic

- Using microbiology indicators: phospholipid fatty acids and enzymes for assessment changing in military contaminated soil's ecosystem during application of phytotechnology
- First year semi field experiment on growing *M.xgiganteus* at the soil from Mimon
- Biomass production-impact of soil

KSU and KAES, USA

- Testing appropriate soil amendments or amendment mix to optimize production of miscanthus, improve soil quality, and/or reduce bioaccessibility of soil contaminants
- Establish research plantation on contaminated lands located at Fort Riley Army installation

NULES and NULP, Ukraine

- Exploring nematodes as indicators of process effectiveness for semi-field research in Kamenetz
- Biomass production: impact of soil properties
- Semi-field research on soil from Mariinka, Eastern Ukraine
- Establish research plantation at Kurachovo, Eastern Ukraine
- Working out curricula for the new graduate course in Ecology including phytotechnology

IPBB, Kazakhstan

- Establish plantation of *M.xgiganteus* and exploring adaptation of *M.xgigateus* to Kazakhstan conditions
- Possibilities of growing *M.xgiganteus* at pesticide's contaminated soil

Advantages of the phytotechnology

- The union of phytoremediation and production of biofuel crops is perspective approach (delivering additional benefits – phytoproducts)
- That method permits to restore marginal contaminated land to agricultural use or urban land bank and simultaneously meet the demand for biomass production as alternative energy sources.
- The additional request is to stabilize the soil and to decrease maximally extraction of contaminants to the above surface part of the plants to be used for energy production

Biofuel crops for biotechnology

- Second generation biofuel crops represent not-food crops and are less directly in conflict with food crops

- 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)

Advantages and disadvantages of *Miscanthus* for phytotechnology with biomass production *

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 phytotechnology with biomass production*

In 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*

Military sites in Ukraine

- In 1991, military sites included territory of 4500 garrisons, testing areas and military individual sites occupying about 600,000 hectares.
- In the period between 1991 and 2003, approximately 140,000 hectares of territory, 147 military bases and 507 separate defense objects were withdrawn from Ministry of Defense jurisdiction
- Currently numerous new military contaminated sites appeared at the Eastern part of the country as result of anti-terroristic operation mainly polluted by metals, oils and products of their decompositions.

- The contaminated research site was located in city Kamenetz-Podilsky, Western Ukraine and had the following coordinates: Latitude-48.680910; Longitude-26.58025. The land was used as a military storage of former Soviet UnionArmy.

- The control soil was taken from nearby agricultural field and had the following coordinate: Latitude-48.715954; Longitude-26.577356

Agronomic characteristic of the soil from the research site , Kamenetz-Podilsky, Ukraine

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

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

Research conditions

✓There were 7 kg of mixture soil in each pot, and two experiments were done in parallel.

✓In each pot the contaminated soil was mixed with control soil using the next combinations: 4:0; 3:1;1:1;1:3; 0:4.

✓In each pot two rhizomes of *M. x giganteus* were planted.

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

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

Concentration of the selected metals in the soil samples in pots

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

✓Soil due to former intensive military activities was contaminated by metals, in particular by Fe, Mn,Sr,Ti and Zr.

✓Concentrations of As, Cu, Pb,Zn were elevated compare to inherent soil in the area

Correlation between metal concentration in different plant parts and two sampling period .*

a) As

	1.	2.	3.	4.	5.	6.	7.
1. Soil	1,00						
2. Roots – year 1	-0,45	1,00					
3. Stems – year 1	-0,18	0,10	1,00				
4. Leaves – year 1	-0,18	-0,04	-0,11	1,00			
5. Roots – year 2	-0,20	0,00	-0,17	**0,68**	1,00		
6. Stems – year 2	-0,22	-0,02	0,52	-0,16	0,06	1,00	
7. Leaves – year 2	-0,27	0,18	**0,59**	-0,17	-0,07	0,22	1,00

c) Fe

	1.	2.	3.	4.	5.	6.	7.
1. Soil	1,00						
2. Roots – year 1	0,34	1,00					
3. Stems – year 1	-0,44	-0,17	1,00				
4. Leaves – year 1	-0,32	-0,66	-0,14	1,00			
5. Roots – year 2	-0,68	-0,27	0,47	0,18	1,00		
6. Stems – year 2	-0,38	-0,33	**0,67**	-0,09	0,02	1,00	
7. Leaves – year 2	0,01	0,19	0,31	-0,48	-0,26	**0,62**	1,00

b) Cu

	1.	2.	3.	4.	5.	6.	7.
1. Soil	1,00						
2. Roots – year 1	-0,31	1,00					
3. Stems – year 1	-0,13	-0,18	1,00				
4. Leaves – year 1	0,16	0,05	-0,29	1,00			
5. Roots – year 2	0,10	**0,56**	0,25	0,20	1,00		
6. Stems – year 2	**0,60**	-0,23	0,40	-0,03	0,33	1,00	
7. Leaves – year 2	0,47	-0,42	0,32	-0,35	-0,20	**0,71**	1,00

d) Mn

	1.	2.	3.	4.	5.	6.	7.
1. Soil	1,00						
2. Roots – year 1	-0,37	1,00					
3. Stems – year 1	-0,15	-0,46	1,00				
4. Leaves – year 1	0,54	-0,57	0,42	1,00			
5. Roots – year 2	-0,11	-0,34	0,10	-0,01	1,00		
6. Stems – year 2	-0,14	-0,19	**0,78**	0,02	-0,16	1,00	
7. Leaves – year 2	-0,22	0,24	0,46	-0,16	-0,57	**0,76**	1,00

****Significant correlation (P<0.05) are in bold red***

Correlation between metal concentration in different plant parts and two sampling period .*

e) Pb

	1.	2.	3.	4.	5.	6.	7.
1. Soil	1,00						
2. Roots – year 1	-0,30	1,00					
3. Stems – year 1	-0,04	-0,34	1,00				
4. Leaves – year 1	**0,58**	-0,30	-0,07	1,00			
5. Roots – year 2	0,33	-0,32	-0,06	0,03	1,00		
6. Stems – year 2	0,21	-0,35	-0,24	-0,22	0,39	1,00	
7. Leaves – year 2	---	---	---	---	---	---	1,00

f) Sr

	1.	2.	3.	4.	5.	6.	7.
1. Soil	1,00						
2. Roots – year 1	-0,36	1,00					
3. Stems – year 1	0,08	-0,40	1,00				
4. Leaves – year 1	0,44	-0,66	0,26	1,00			
5. Roots – year 2	-0,15	-0,33	0,26	0,38	1,00		
6. Stems – year 2	0,14	-0,51	0,27	0,28	0,49	1,00	
7. Leaves – year 2	0,49	-0,17	0,10	0,54	0,03	0,46	1,00

g) Ti

	1.	2.	3.	4.	5.	6.	7.
1. Soil	1,00						
2. Roots – year 1	-0,19	1,00					
3. Stems – year 1	0,38	0,10	1,00				
4. Leaves – year 1	0,19	-0,59	-0,20	1,00			
5. Roots – year 2	-0,17	-0,24	0,30	0,23	1,00		
6. Stems – year 2	0,41	-0,67	0,49	0,18	0,08	1,00	
7. Leaves – year 2	-0,19	0,10	0,19	-0,56	-0,58	0,28	1,00

h) Zn

	1.	2.	3.	4.	5.	6.	7.
1. Soil	1,00						
2. Roots – year 1	-0,12	1,00					
3. Stems – year 1	-0,06	-0,21	1,00				
4. Leaves – year 1	-0,25	-0,56	0,33	1,00			
5. Roots – year 2	0,52	-0,40	0,17	0,03	1,00		
6. Stems – year 2	**0,58**	-0,59	**0,55**	0,37	**0,61**	1,00	
7. Leaves – year 2	**0,83**	0,05	-0,02	-0,36	0,21	0,53	1,00

****Significant correlation (P<0.05) are in bold red***

✓The variability of metal concentration in the soils was not high, max relative deviation was ±33% around average

✓The correlation between metal concentrations in soil and aboveground parts were insignificant (As,Fe,Mn,SR,Ti, Zr) or occasional (Cu,Pb,Zn)

✓That permitted to consider all variants 1-5 equal and to compare them together in order to increase significance of statistical comparisons

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 (α = 0.05), i.e. values with the same letters can be considered comparable; bolded values indicate values significantly higher than zero (t-test, α = 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**

- ✓**Accumulation of metals took place predominantly in the roots, translocation to above surface parts was order of magnitude lower.**
- ✓**Fe,Mn,Ti were accumulated more intensive in the first year and less tangible in the second**
- ✓**Cu,Pb,Zn were insignificant accumulative in both seasons**
- ✓ **As and Pb were accumulated a little**

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 (α = 0.05, see Table 3); bolded values indicate significantly non-zero values (t-test, α = 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.

Shoot/roots coefficients were significantly lower than 1 (with exception of Zn in 1 year) That indicates absence of hyper accumulation of metals by *M.giganteus* growing at the soil from the military contaminated site in Kamenetz-Podislky

Summary

✓Despite high metals' concentrations in the research soil no evident growth inhibition was observed and concentrations of metals in the over surface parts were minor. The translocation ratio was calculated for roots, stems and leaves; coefficient was significantly lower than 1 and indicated absence of hyper accumulation

✓***The metal accumulation data confirmed the desired pattern requested for the phytotechnology with biomass production.***

✓ The research shows that utilization of the biomass obtained is attractive and can turn the process into a profit making operation.

✓The further research has also to be concentrated on interconnection between *M. x giganteus* biomass quality and quantity grown at the military sites including those newly appeared at the East of Ukraine (Mariinka and Kurakhovo)

Acknowledgements

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