

Phytotechnology with Biomass production (for sustainable management of military contaminated sites)

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***CHE650 Seminar,
K-State, USA, January 9, 2018***



Overview

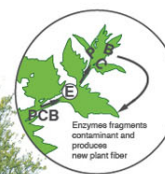
- ▶ Introduction to Phytoremediation
- ▶ Introduction to Biomass production
- ▶ Synergy: Phytotechnology with Biomass Production
- ▶ Miscanthus as perspective crop for Phytotechnology with Biomass production
- ▶ Military contaminated sites and place of Phytotechnology with Biomass production
- ▶ Results of NATO SPS MYP G4687 “New Phytotechnology for cleaning contaminated military sites”
- ▶ Conclusion

Phytoremediation*

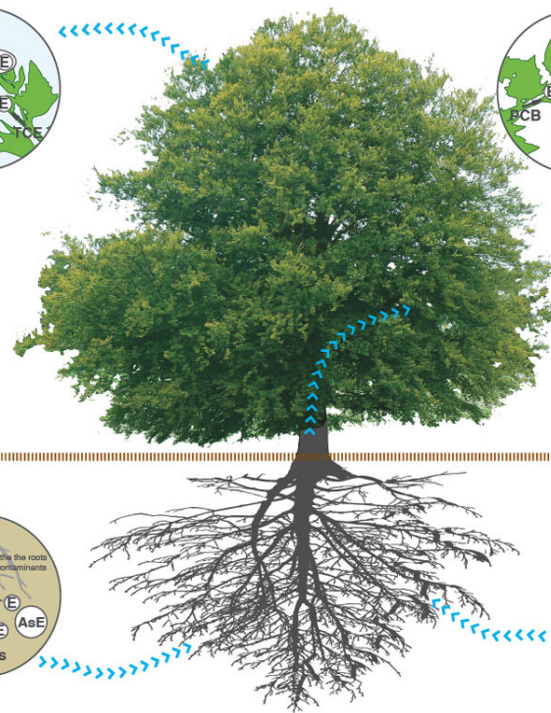
- ▶ Phytoremediation is the use of vegetation to remediate, contain or prevent contaminants in soils, sediments and groundwater, and/or add nutrients, porosity and organic matter
- ▶ It is also a set of planning, engineering and design tools and practices that can assist landscape architects, site designers, engineers and environmental planners in working on individual sites, the urban fabric and regional landscapes
- ▶ It is a cost effective, green-clean technology with long-term applicability for the cleaning up of contaminated sites. It involves the cleaning up of contaminated soils and water by either root colonizing microbes or by the plants them selves and it best applied at sites with shallow contamination of organic and/or inorganic pollutants

** Definition of Kirkwood and Kennen (PHYTO, 2015) as expansion of previous definition (Rock S., 2000)*

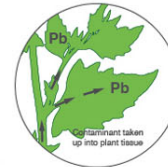
PHYTO VOLATILIZATION:
Some plants take up volatile contaminants and release them into the atmosphere through transpiration. The contaminant is transformed or degraded within the plant to create a less toxic substance before and then released into the air.



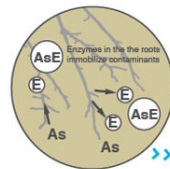
PHYTO DEGRADATION:
Plants take up and break down contaminants through the release of enzymes and metabolic processes such as photosynthetic oxidation/reduction. In this process organic pollutants are degraded and incorporated into the plant or broken down in the soil.



PHYTO EXTRACTION:
Plants take up contaminants - mostly metals, metalloids and radionuclides- with their roots and accumulate them in large quantities within their stems and leaves. These plants have to be harvested and disposed as special waste.



PHYTO STABILIZATION:
Some plants can sequester or immobilize contaminants by absorbing them into their roots and releasing a chemical that converts the contaminant to a less toxic state. This mechanism limits the migration of contaminants through water erosion, leaching, wind, and soil dispersion.



The following fundamental processes can be identified when plants are used for remediation of contaminated sites:

- **Phyto-immobilization:** plants prevent transport of dissolved contaminants in the soil
- **Phyto-stabilization:** plants mechanically stabilize polluted soils, and prevent bulk erosion and air born transport to other environment
- **Phyto-extraction:** plants extract metallic and organic compounds from soil to plant tissue
- **Phyto-volatilization:** plants volatilize contaminants in soil or water to air
- **Phyto-degradation:** plants mineralize or assimilate contaminants in soil or water

✓ For most inorganic contaminants and crops the phyto-immobilization and phyto-stabilization are the major processes that prove useful

Biomass for energy

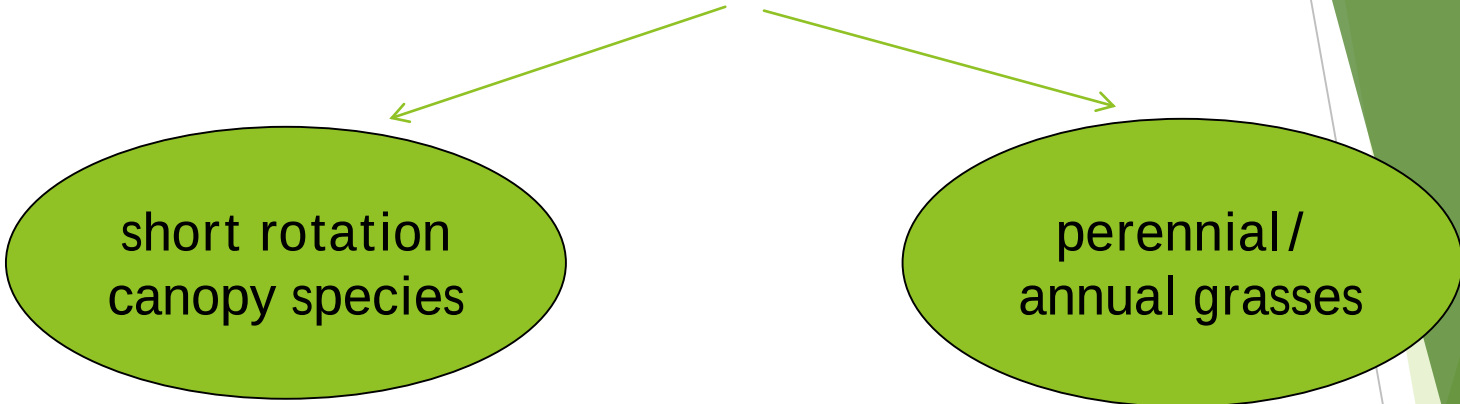
- ▶ Biomass is a storable form of renewable energy (most other renewable energies are not storable) that can be used as a direct replacement for fossil fuels in many applications. Biomass is the only renewable energy source capable of replacing products such as platform chemicals, plastics and aviation fuel, all currently derived from fossil fuels
- ▶ Biomass for energy production can be categorized as:
 - Residues from agriculture and forestry
 - Organic waste
 - Surplus forestry
 - Energy crops. It is predicted that *Mxgiganteus* may supply up to 12% of the EU energy need by 2050**
- ▶ Calculation of available energy contained in biomass show that it ranged from 27 to 130 EJ/year around the world*, and 5,4 EJ/year in EU*** (Exajoule (EJ): $1 \text{ EJ} = 10^{18} \text{ J}$)
- ▶ *Beringer et al, 2011

**Fruhworth and Liebhard, 2004

***Stampfl et al, 2007

➤ 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

▶ That crops can be divided into two main categories:



Poplar
(*Populus*
spp.)



Willow
(*Salix*
spp.)



Locust
(*Robinia*
spp.)



Switchgrass
(*Panicum*
virgatum L.)



**Reed canary
grass**
Phalaris
arundinacea L.



***Miscanthus x giganteus*, a
sterile triploid hybrid of:
Miscanthus sinensis,
diploid called Chinese
silvergrass,) and
Miscanthus sacchariflorus,
tetraploid often called
Amur silvergrass,**

Miscanthusxgiganteus Greef et Deu

- ▶ Discovered in Japan in 1935, and for many years was treated as an exotic ornamental plant
- ▶ Beginning of 1980s- first plantations were established in Denmark and Germany, than – in other EU countries, including Czech Republic, Poland and Slovakia
- ▶ the estimated area of land under miscanthus cultivation in the EU is currently about 20,000 ha. The above-ground biomass yield in EU may reach 20-35 t.ha-1.yr-1**. In the USA the yield is reported as 24-35 t.ha-1.yr-1***

Over a decade of moving Miscanthus forward...



from Asia...



to Aberystwyth...



and beyond

* Illustration from Aberystwyth University web

**Venendaal et al, 1997

***USDA, 2011



St.Helena, Croatia, 20.12.2017

Miscanthusxgiganteus Greef et Deu

- ▶ *Mxgiganteus* was 59% more productive than maize in USA (Illinois)*
- ▶ Full establishment of *Mxgiganteus* stand takes from 2 to 5 years, depending of climate conditions, productive life span is estimated between 20 to 30 years **
- ▶ *M.xgiganteus* can produce much higher biomass yield after applying a fertilizer, including municipal sewage sludge***

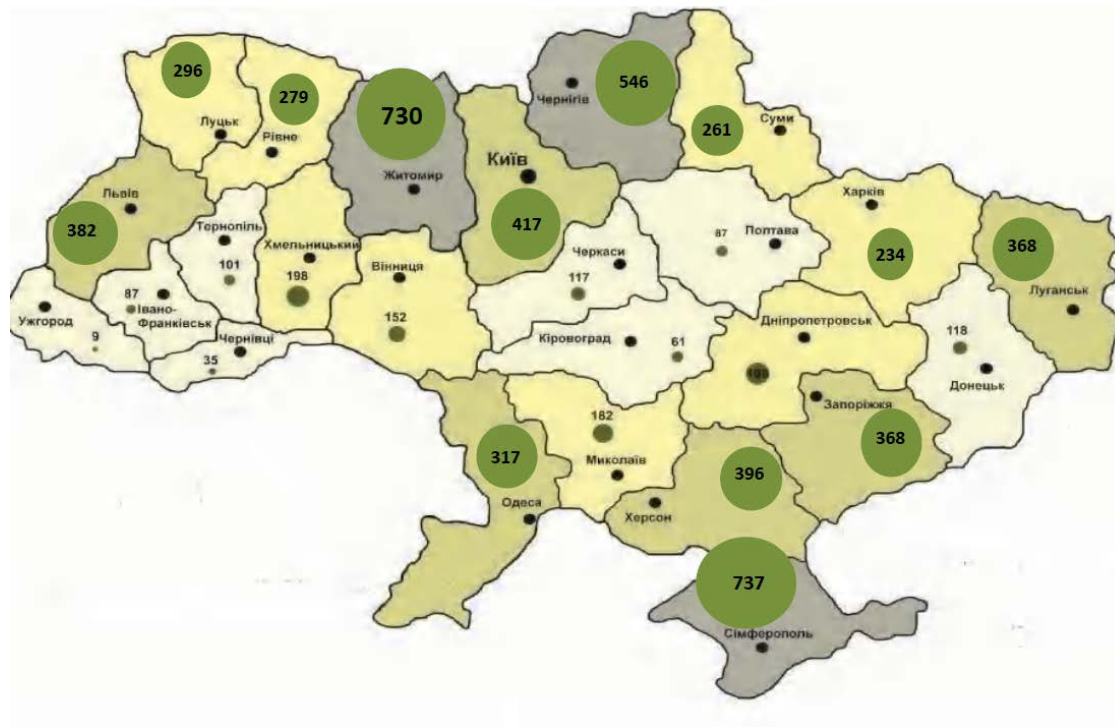
*Dohleman F.G. and Long S.P. 2009. More productive than maize in the Midwest: how does miscanthus do it? *Plant Physiol*, 150, 2104 -2115

**Heaton et al, 2004; Larsen et al, 2014

***Kolodziej B., Antonkiewicz j. Sugier D. 2016. *Miscanthusxgiganteus* as a biomass feedstock grown on municipal sewage sludge. *Industrial Crops and Products*, 81, 72-82



Prognosis of request for energy crops in Ukraine (thousands tons)*

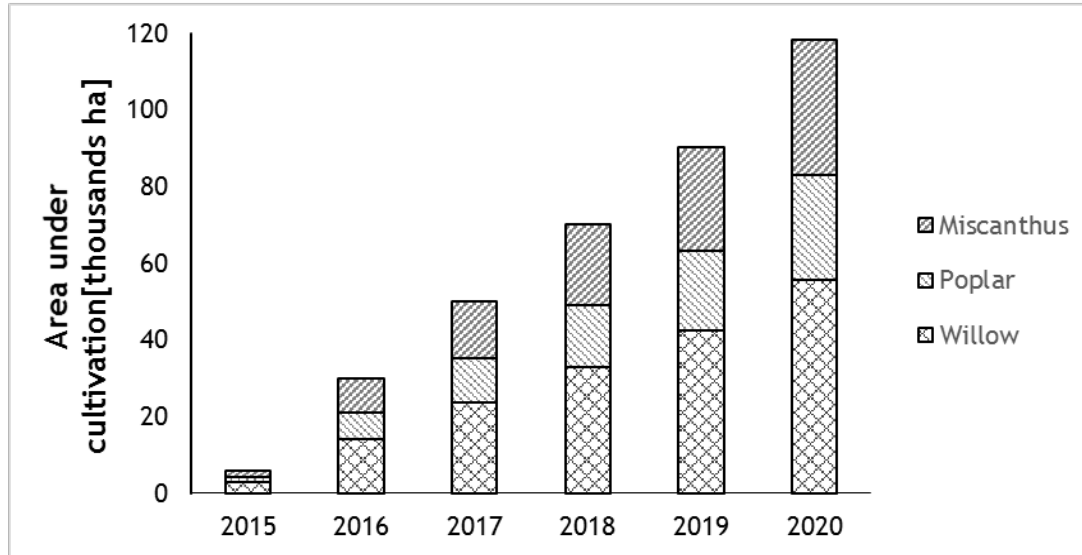


Growing energy crops in Ukraine

	2014	2020	2035
Land under energy crops, thousands ha, from those	3	100	500
Willow	2	25	150
Miscanthus	0,5	15	90
poplar	0,5	10	60
Sharing of the surface, %			

**Savchuk S., Head of State Energy Committee, Report at the Information Day "Use of underutilized land for sustainable bioenergy feedstock production – additional income to farmers", December 12, 2017, Kyiv, Ukraine*

Ukraine*



Pellets from *Mxgiganteus*

- 2007- first plantation was established in Ukraine
- 2017- the estimated area of land under *Mxgiganteus* was about 7,500 ha



Miscanthus planting in Ternopil region, 2016



Bioenergy heating station using pellets (Kyiv oblast, firm "Kameliya")

*Geletukha et al, 2017. Report at the Information Day "Use of underutilized land for sustainable bioenergy feedstock production – additional income to farmers", December 12, 2017, Ukraine

Miscanthus and Carbon Sequestration

- ▶ Growing miscanthus has the potential to mitigate atmospheric carbon dioxide emissions by storing carbon in the soil
- ▶ It also reduces emissions of carbon and NO_x (nitrogen oxides) into the atmosphere and uses less energy than conventional crops, due to limited inputs and limited tillage, as its planted once and is harvested annually for 20 + years
- ▶ The magnitude of increase of soil C by growing miscanthus depends on total production and time of harvest because a significant fraction of foliage drops slowly during autumn and winter
- ▶ This is significant carbon sequestration for marginal land*: it was found that during 15 years monitoring of miscanthus yield in Ireland with using C isotope dilution an average 0.6 tons.ha⁻¹ carbon was recorded*

*Clifton-Brown et al, 2007

Breeding efforts in Europe:

FP7 project Optimisc* was a consortium headquartered at University of Hohenheim, Germany

Partner organizations were from across Europe, Ukraine, Russia, China, Turkey
Seven trial locations for multiple germplasms. Varietal trial locations: U.K.,
Netherland, Ukraine, Russia, Turkey, establishing of plots in 2012; observation
2012-2016



Potasch, Poltava region, Ukraine



Blankney, England, U.K.

*Lewandowski I. et al, 2016

Using of *Mxgiganteus* biomass

- ▶ For energy (direct burning; pellets are a particularly convenient form of biomass and can be used in a wide range of boilers)
- ▶ Biorefining: Advances in biorefinery techniques are allowing more products to be created from biomass. These range from transport fuel to plastics and papers.
- ▶ Animal bedding (range of livestock: horses, sheep, poultry)
- ▶ Supporting hare population: according to research, once established, the crop can act as a valuable habitat for the brown hare
- ▶ Game cover: Miscanthus provides thick cover which will work well as a wind break and stand all season. It can easily be planted with other game crops allowing both warmth and food with easy flushing
- ▶ Paper industry, cellulose production

Miscanthus for Phytotechnology with Biomass Production

- ▶ Due to this shortcoming the utilization of the approach involving high biomass fast growing crops for remediation purposes combined with biofuel production has gained momentum in recent years
- ▶ The goals are two-fold*: firstly to avoid environmental damage and to protect the population from negative effects caused by contamination of such sites, and secondly to render contaminated sites fit for subsequent commercial, recreation and other civilian uses with simultaneous production of biomass
- ▶ *Mxgiganteus*- one of the main crop at that technology while it is applied to metal-contaminated soils

**Forman et al, 1998*

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*

Poland

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

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

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

Confirmation possibility to using miscanthus for phytoremediation of metal-contaminated soils*

- ▶ Researching the behavior of selected metals (Co and Cu) in the soil artificially contaminated by metals
- ▶ Exploring the dynamic of the process (32 days and 86 days) and evaluation the differences between behavior of Cu and Co**



**Pidlisnyuk V., Erickson L., Kharchenko S., Stefanovska T., 2014. Journal of Environmental Protection, Special Issue in Environmental Remediation 5, 723-730*

***Methodology of research: Claim for the Invention #12471, Ukraine. Pidlisnyuk V., Stefanovska T. Method for growing plants in heavy metals contaminated soils, issued on January, 2014.*

Concentration of Co in miscanthus after 32 and 86 days of soils' treatment by $\text{CoCl}_2 \cdot x\text{H}_2\text{O}$

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

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

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

Concentration of Cu in Miscanthus after 32 and 86 days of soils' treatment by $\text{CuSO}_4 \times 5 \text{H}_2\text{O}$

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

Military sites contamination/damaging

Various military installations: training areas, airfields and air bases, rocket fuel and chemical storage centers, tank regiments, military towns and naval bases have caused extensive damage to the environment

Soil and groundwater deterioration is the most common problems and also the most expensive to rehabilitate. The military sector has only recently become seriously engaged in environmental site investigation and remediation and often is technologically behind the civilian sector

Hydrocarbons (petrol, diesel, kerosene) are the dominant pollutants found at military bases. Other common contaminants are chlorinated hydrocarbons and heavy metals. In some cases PCB and other chlorinated hydrocarbons are special problems



Categories of militart polluted sites



Oil tanks



Open oil ponds



Hidden pollution



Carelessness



Oil on rails



Chemical accidents

The Soviet troops operated in 73 locations on the territory of the **Czech Republic** and sixty of them were left considerably contaminated. The main problem was the contamination of ground water and soil by fuels like petrol or diesel and other toxins like oil-based hydrocarbons, chlorinated hydrocarbons or polychlorinated biphenyls as well as heavy metals

In 1991 there were around 420 military sites in **Ukraine** left after the Soviet troops. In 90th the inventory was done and 2/3 of the former military land was passed to the local/state authority management; some of that localities were considered as abandoned land. In 2013 there were about 170 localities contaminated, mainly by oil-based carbons and heavy metals

After beginning of the Russian-Ukraine (February 2014- currently) a new military contaminated/damaged land appeared at the East of Ukraine

Methods used for military sites' treatment

- ▶ **Excavation** and further treatment (incineration)
- ▶ **Soil composting** was used for remediation of former military bases in Baltic countries, EU. It is a biological remediation process that can be adapted to treat a wide variety of organic contaminants, including rocket fuel. Composting works in cold climate too, duration about 2 - 4 years
- ▶ In case of little contamination/damaging of former military bases in Germany, Czech Republic, Slovakia **transformation to the natural zones** was applied
- ▶ Phytotechnology with biomass production: for slightly contaminated/ damaged military sites-more profitable



Estonia

NATO SPS MYP G4687: Research Goals

- ▶ to enhance environmental security at the former and recently appeared military sites by developing phytotechnology to produce biomass in large quantities on contaminated lands
- ▶ to conduct laboratory and field research in order to investigate biomass production as affected by concentrations and nature of contaminants, soil moisture, and nutrients
- ▶ to study the effect of miscanthus biomass production on soil quality and microbiology, insect and nematode biodiversity
- ▶ to test biomass for the using as:
 - direct burning
 - pellets

G4687 Team

Main partners:

Jan Evangelista Purkyně University, Czech Republic

National University of Life and the Environment, Ukraine

KSU, USA

KAES, USA

Institute of Plant Biology and Biotechnology, PBB, Kazakhstan

National University "Lvivska Polytechnika", Ukraine



End-users:

Kamenetz-Podilsky city council, Ukraine

Dolyna city authority, Ukraine

State Committee of Energy Saving, Ukraine

Slovakian Environmental Agency, Slovakia

Environmental Division, Fort-Riley, USA

Private firm "Ecology Engineering", Kazakhstan



Liasoning institutions:

University of Zagreb, Croatia

Warminsko-Mazursky University, Poland

Wroclaw University of Life Science

and the Environment, Poland

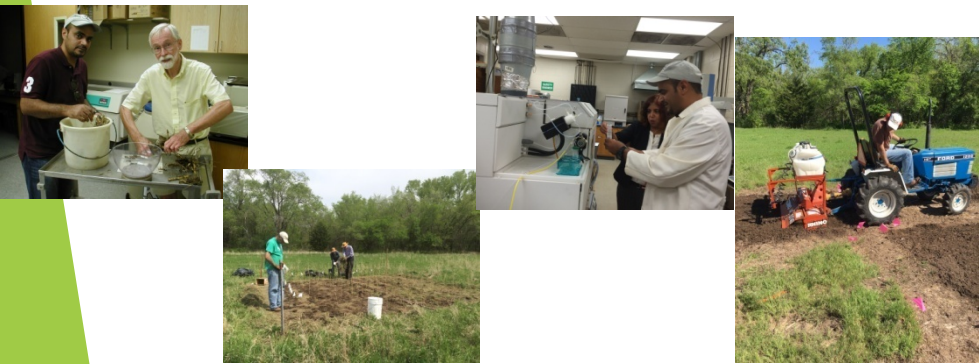
Directions of the research:

UJEP, Czech Republic

- ▶ Lab experiment on growing *M.xgiganteus* at the soil from Mimon
- ▶ Using microbiology indicators: phospholipid fatty acids and enzymes for assessment changing in military contaminated soil's ecosystem during application of phytotechnology
- ▶ Biomass production-impact of PGRs
- ▶ Lab experiment with soils from Bakar, Croatia
- ▶ Erasmus course in Phytotechnology

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
- ▶ Research plantation established at the contaminated lands located at Fort Riley Army installation



NULES and NULP, Ukraine

- ▶ Exploring nematodes as indicators of process effectiveness for semi-field research
- ▶ Biomass production: semi-field research on soil from Dolyna and Kurakhove, impact of soil properties
- ▶ Impact of PGRs – field experiment in Tokarivka
- ▶ Establishing and monitoring of field plots in Kurakhove, Eastern Ukraine and Dolyna, Western Ukraine



IPBB, Kazakhstan

- ▶ Establish plantations of *M.xgiganteus* and exploring adaptation of *M.xgigateus* to Kazakhstan conditions
- ▶ Lab experiment on growing *M.xgiganteus* at metals and pesticide's contaminated soils



Ukraine: Dolyna, Ivano-Frankivsk region

Former military training polygon. GPS of the contaminated plot: 48°58'05.1"N 23°59'41.6"E. 48.968094, 23.994881.

GPS of the control plot: 48°58'01.4"N 23°59'33.3"E. 48.967066, 23.992574.

Size of the plot: 0.4 ha contaminated and 0,2 ha –control. Main contaminants: metals, unidentified organic substances



MAP OF UKRAINE



Tokarivka, Mid-Ukraine, Kyiv region

Unused agricultural land, since May 15, 2017. (GPS 49°40'14"; 29°39'03"). 100 rhizomes were planted from which half was treated by PGR Stimp, plantation has a size 100 m².



MAP OF UKRAINE



Mimon, Czech Republic

- Since 2016. Former SU air-space base. The source of the contamination was caused by previous release of oil-products into the soil by soviet troops while using airport from 1968 to 1990.
- Long-term remediation was carried out in the locality between 1996-2006 during implementation of projects supported by Czech Ministry of the Environment and other governmental agencies based at oxidation of oil-products inside the soil depth. However, the locality found is still remained contaminated
- GPS coordinates: Mimon-2 (contaminated) 50.6239286N, 14.7467006E.
Mímon-1 (control) 50.6255317N, 14.7228317E
- Main contaminated substances: oil products; nitrificated organic substances; metals (Pb, Fe, Cu, Zn)
- Both localities were prepared by ploughing and 92 rhizomes were planted in each in spring 2017, half of them was treated by PGR Stimpo. The rhizomes planted were the same as planted in Kurakhove and Dolyna.
- On November 6, 2017 the plots were enriched by 200 rhizomes each (without treatment of PGRs)

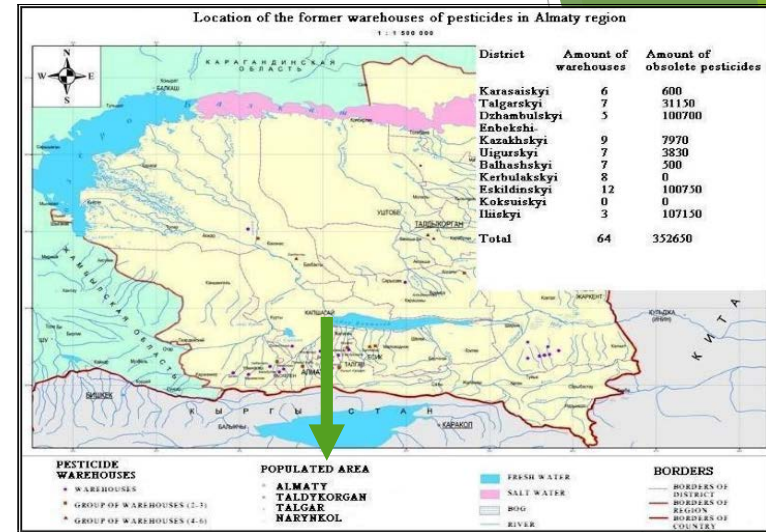


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- On November 6, 2017 the plots were enriched by 200 rhizomes each (without treatment of PGRs)



Research sites: Maili, Kazakhstan, since 2016.

Former military polygon and storage of pesticides



Village is in about 280 km from Almaty. Former polygon was closed in 1970 and further used as storage for pesticides. Currently the locality is in private farming property and former military storages are used as stokes for animal feed. Main contaminants: metals and pesticides residues

Research site- Ft.Riley, Kansas, USA.

Former shooting range, main contamination - Pb



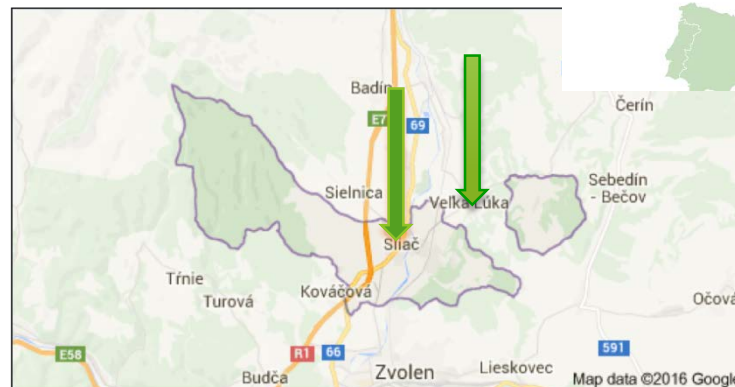
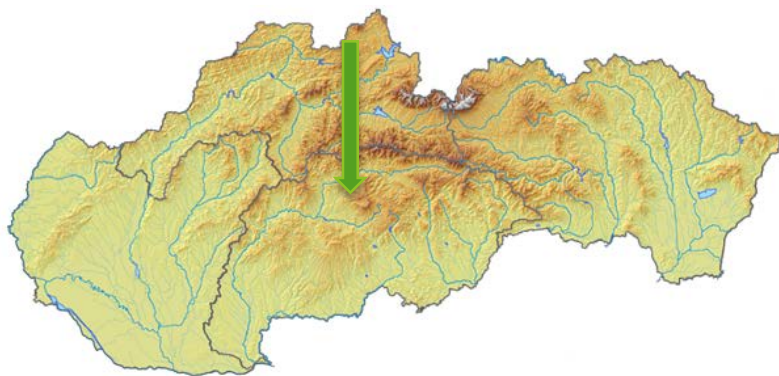
FORT RILEY, JUNCTION CITY, KS

The 100,656-acre Fort Riley site is located in Clay, Geary and Riley counties in Kansas.

Sources of contamination, including landfills, dry cleaners, furniture shops, pesticide storage facilities, incinerator, OB/OD range, and MMRP sites. Contaminated groundwater and soils with volatile organic compounds (VOCs) and solvents are present at this site. Some of the five subsites are cleaned up. However, environmental risks remain and long-term cleanup is ongoing. Operation and maintenance activities and monitoring are ongoing.



Sliac, Slovakia, 2014-2015*



- ▶ The contaminated research site was located in village Sliac, Middle Slovakia and was used as an airport of the former Soviet Union Air Force. It had the following coordinates: Latitude: 48°38'38"; Longitude: 19°08'26".
- ▶ The control soil was collected from cultivated arable land at village Velka Luka, it had the following coordinates: Latitude: 48°62'92", Longitude: 19°16'12".
- ▶ The time of experiment: April, 30th, 2014- October, 21st, 2015.

* *Pidlisnyuk V. et al, Polish Journal of Chemical Technology, 2018- in press.*



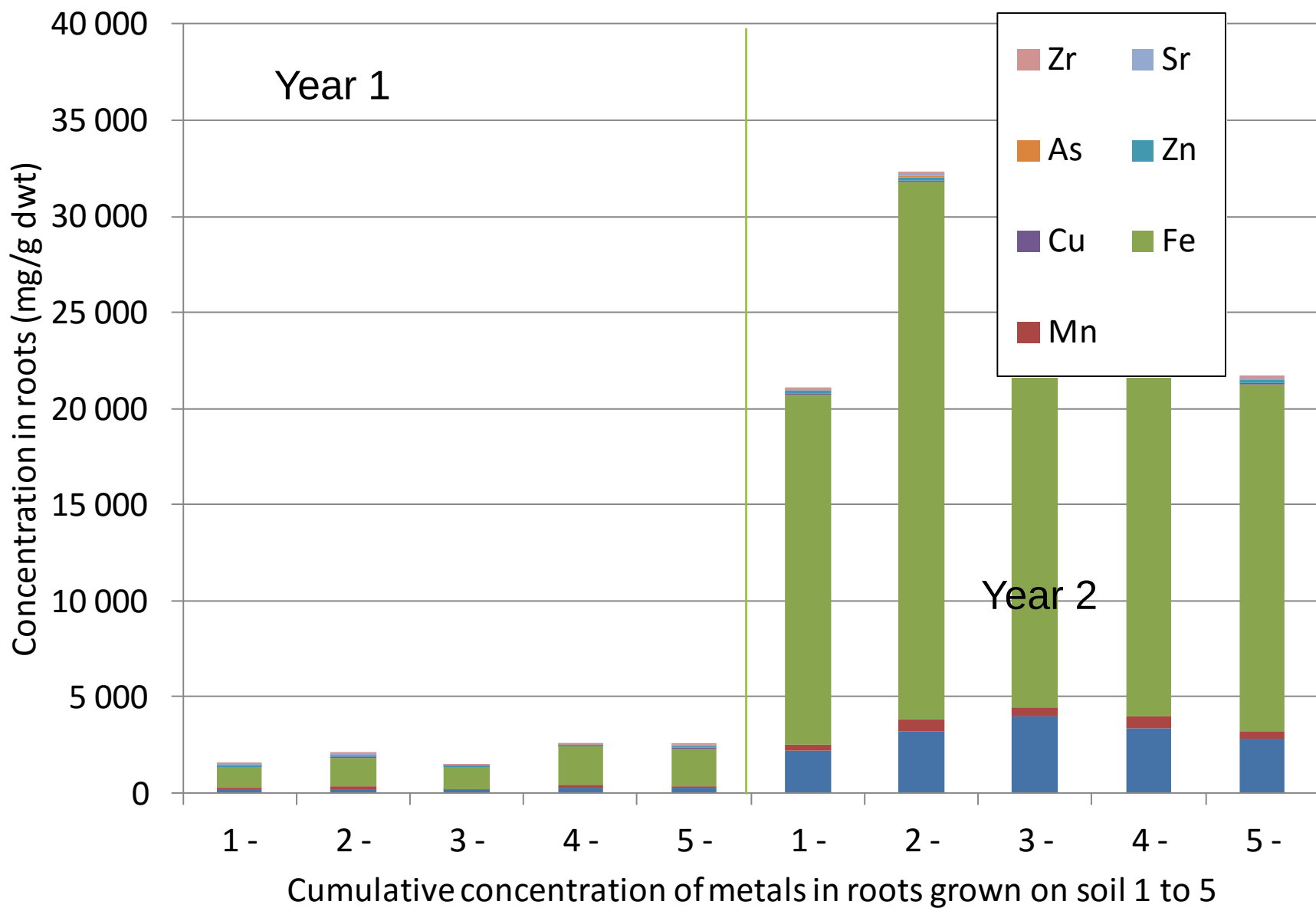
Concentration of metals in the soil, Sliac

soil label	1	2	3	4	5
control soil	100%	75%	50%	25%	0%
contaminated s.	0%	25%	50%	75%	100%
As [mg/kg]	290±40	515±15	430±79	465±15	425±45
Cu [mg/kg]	310±0	380±60	395±5	440±100	565±125
Fe [mg/kg]	174555±5395	194485±8645	205640±7540	209480±6830	215210±5720
Mn [mg/kg]	2995±185	3605±485	4110±340	4495±555	4660±500
Sr [mg/kg]	685±65	695±15	925±15	1185±75	1200±40
Ti [mg/kg]	20620±0	24410±260	25935±615	27940±2420	28170±530
Zn [mg/kg]	960±90	1025±45	1115±115	1205±335	1015±235
Zr [mg/kg]	1275±275	1455±75	1345±205	1500±60	1625±225

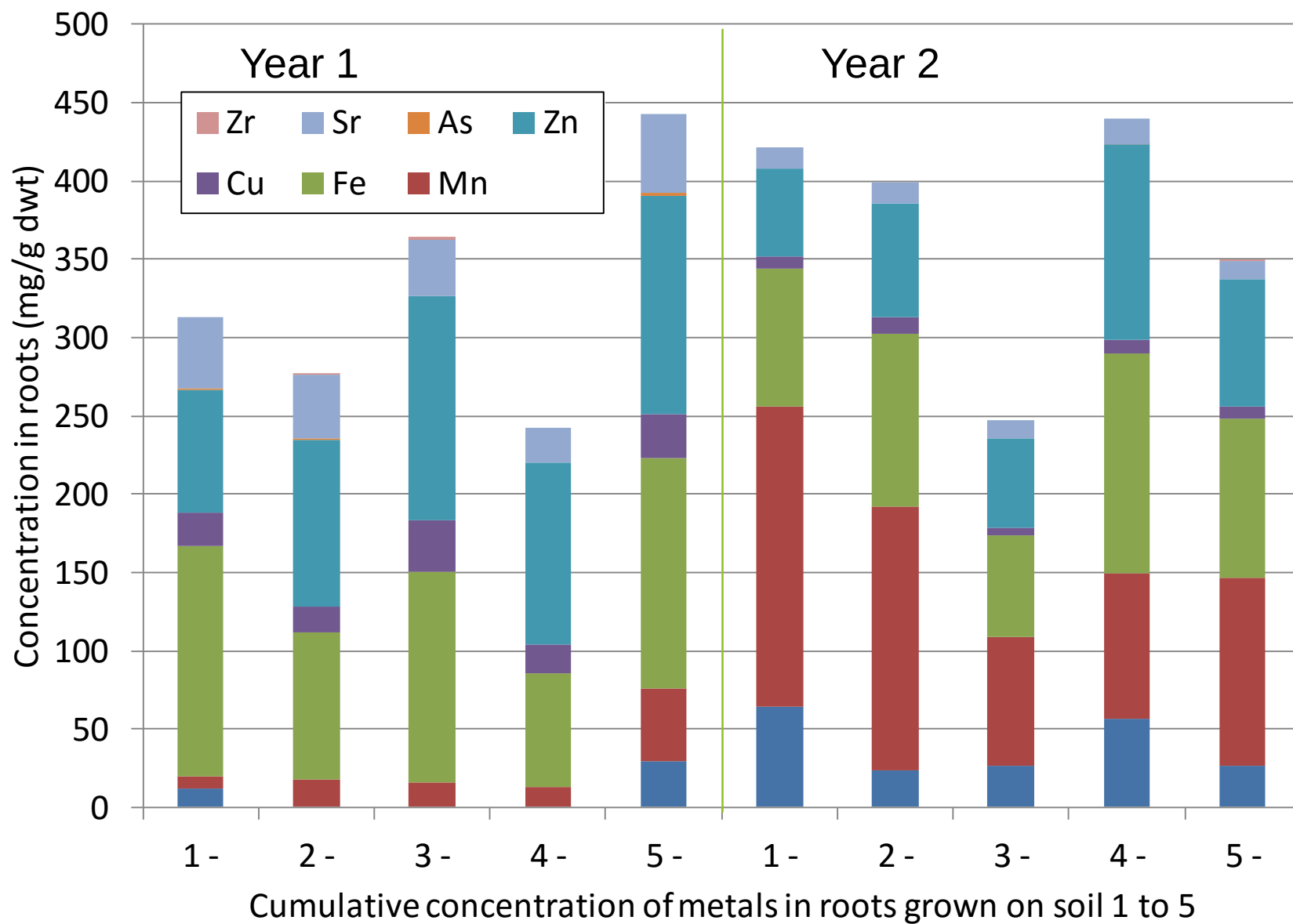
Concentration of metals in the plants' tissues

	[mg/kg dwt]	Year 1			Year 2		
	soil	roots	stems	leaves	roots	stems	leaves
As	425±86d	6±6bc	1±1ab	1±1a	25±10c	0±0a	0±0a
Cu	418±114e	58±24cd	23±11bc	26±4c	76±48d	8±3ab	8±3a
Fe	199874±15943e	1514±455c	119±34ab	213±33b	23779±10167d	101±36a	252±41b
Mn	3973±748d	85±52b	20±20a	181±54bc	488±266c	131±55bc	131±43b
Sr	938±230c	37±17ab	39±21ab	51±19b	135±78b	13±2a	24±10a
Ti	25415±2994d	225±72b	8±19a	2±3a	3114±1180c	40±30a	33±24a
Zn	1064±214e	63±19ac	117±33bd	40±4a	179±74cd	78±34ab	61±13a
Zr	1140±224d	13±7b	1±1a	0±0a	125±61c	0±1a	0±1a

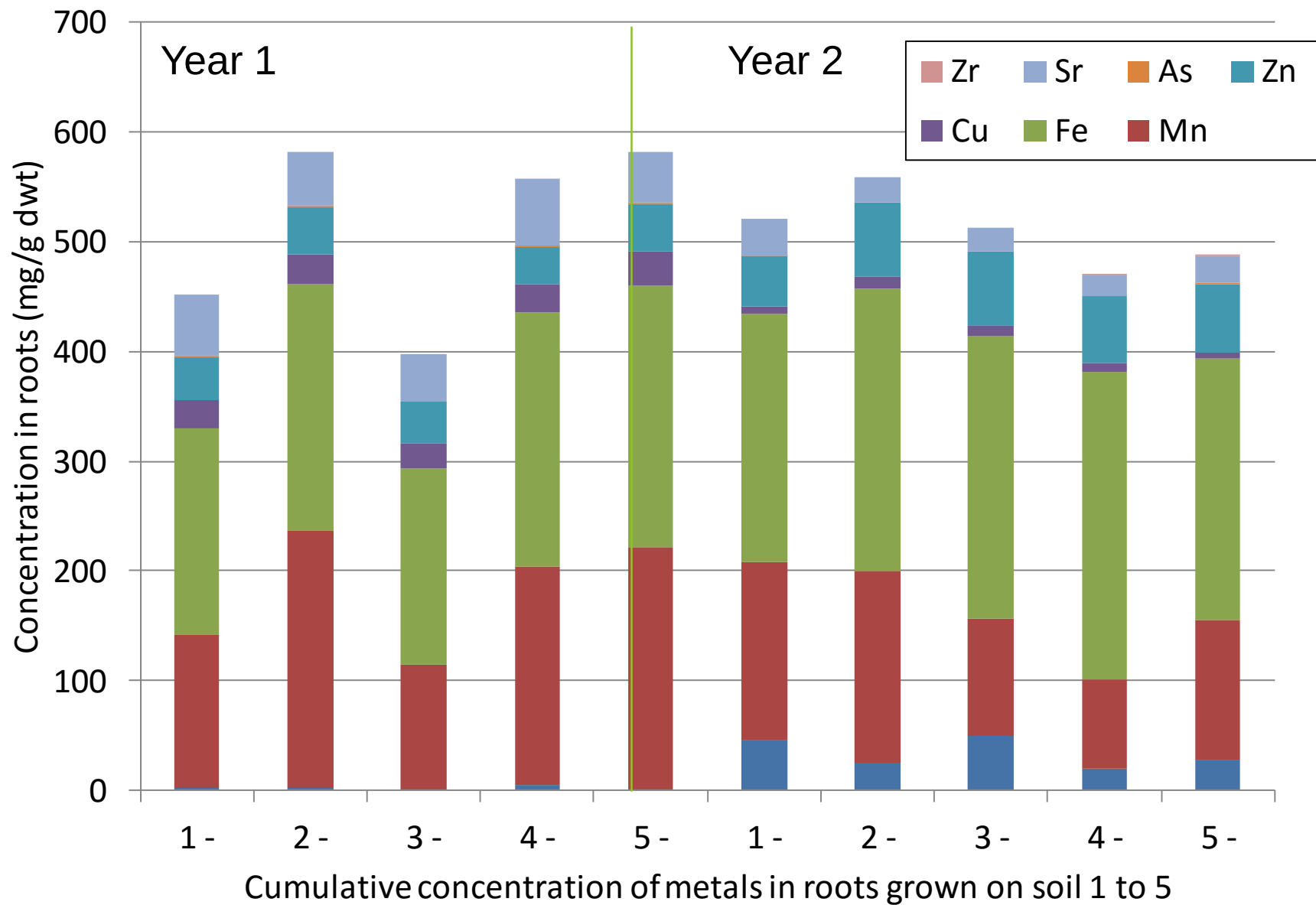
Cumulative concentration - roots



Cumulative concentration - stems



Cumulative concentration - leaves



Conclusions

- ▶ The data illustrated the differences in metals' uptake between first and second growing seasons
- ▶ The results show that metals' up taken by above part of the plant is very limited during both seasons referring to the concentration of metals in the soils.
Consequently, As and Zr are almost not accumulated by stems and leaves during both vegetation seasons and accumulation of Cu, Mn, Zn and Sr is not essential which confirmed that biomass may be processed for the energy.
- ▶ Data illustrates the interesting fact: for some metals accumulation by the above part of *M.xgiganteus* decreases at the second year of vegetation in comparison with the first year: that effect is observed for Cu and Sr (stems and leaves); Fe (stems); Mn (leaves) and Zn (stems).

Conclusion

- ✓ 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 with limited concentration of metals is attractive and can turn the process into a profit making operation

Impact of *Mxgiganteus* rhizomes' treatment by PGRs to the biomass produced (Lab experiment, year 2016)

Table 3. First year experiment of *M.x giganteus* biomass production at the soils from Mimon, Kurakhove and Maili

Index	Mimon		Kurakhove			Maili		
	Site soil	Control soil	Site soil	1:1 site: control	Control soil	Site soil	1:1 site: control	Control soil
Without treatment of PGRs								
Plant height (at harvest), m	2.2 ±0.02	2.4±0.01	1.3±0.04	1.4±0.01	1.4±0.05	1.2±0.05	1.4±0.04	1.6±0.03
Dynamic of plant growing (from seedling to harvesting), day	180	180	195	195	195	210	210	210
Plant weight (wet), kg	0.06±0.01	0.08±0.02	0.07±0.01	0.07±0.01	0.08±0.01	0.05±0.01	0.07±0.01	0.08±0.02
With treatment by PGR								
Plant height (at harvest), m	-	-	1.4 ±0.03	1.5±0.02	1.7±0.05	0.9±0.045	1.3±0.07	1.6±0.07
Dynamic of plant growing (from seedling to harvesting), day	-	-	200	200	200	210	210	210
Plant weight (wet), kg	-	-	0.08±0.01	0.08±0.01	0.09±0.02	0.05±0.02	0.08±0.003	0.09±0.02

Impact of treatment by PGRs *Mxgiganteus* rhizomes to biomass produced, field experiment, Tokarivka, Ukraine, 2017 (unused agricultural land)



Research site:

- field length – 13,50 м
 - field width – 4,20 м
 - distance between rows – 1,40 м
 - distance between plants in the row – 0,60 м
- 4 replications; 27 rhizomes planted in one row.
Rhizomes before planting were treated by PGR Stimp 25 ml/t; control rhizomes were treated by distilled water



PGRs- bio stimulator for plant growth. It is composition of biologically active substances: phytohormons, microelements, bio protective compounds

First vegetation season 2017, Rhizomes treated by PGRs (1) and control (2)

	Height, m	Number of stems, unit	Mass of fresh biomass, g	Dry content, %	Mass of dry biomass, g	% of surviving	Harvest of dry biomass, t/ha	Yield of solid biofuels, t/ha	Exit of energy J/ha
1	127.0	6.4	69.3	41.2	28.6	90.0	0.4	0.5	8.2
2	96.0	1.4	17.6	42.3	7.4	90.0	0.1	0.1	2.1

Soil contamination by heavy metals, Maili, Kazakhstan*

Metal	Limited levels,, mg/kg	Content of the metals in the soils, mg/kg	
		Clean soil	Contaminated soil
1 class of danger			
As	2	2,6±0,5	9,8±1,6
Cd	3-5	0	1,2±0,2
Pb	32	14,1±0,7	772,7±82,9
Zn	55	32,6±3,4	615±7
2 class of danger			
Co	5	5,4±0,5	9,8±0,1
Ni	4	17,0±0,7	24,0±1,4
Cu	3	24,5±2,1	36,5±2,1
Cr	6	16,4±0,9	28,5±3,5
3 class of danger			
Ba	0,1	56,6±6,6	115±7
V	150	17,0±1,2	32±0,7
Mn	1500	335,8±31,1	500±0
Sr	7	133,9±19.1	150±0
Other metals			
Fe		21 450±2757	15100±3535
radioactive elements			
U		1,1±0,1	1,3±0,3

Clean soil- 1 km from storage at the agricultural land; contaminated soil- at the location near the storage (1m).Soil pH 8,27-9.89.

*Nurzhanova A., Pidlisnyuk V., Sailaukhanuly Y., Kenessov Y., Trogl J., Aligulova R., Kalugin S., Nurmagambetova A., Abit K., Stefanovska T., Erickson L.,2017 Phytoremediation of military soil contaminated by metals and organochlorine pesticides using miscanthus. Comm. Agricul Appl Biological Science,82/3, 61-68.

Physiological characteristics of *Miscanthus x giganteus* growing at the soils, artificially contaminated by heavy metals

Concent ration, mg/kg	T, month							
	29.03.16		23.05.16.		19.07.16		01.10.16.	
	High of the plants, cm							
	m±n	K _B %	m±n	K _B %	m±n	K _B %	m±n	K _B %
control	33,2±5, 8	100	116,5±4 ,5	100	158,5±5 ,3	100	157,5±5,9	100
ZnSO ₄ x 7H ₂ O (Zn limited level LL 55 mg/kg)								
3 LL	41,8±2, 3	125	103,1±1 ,3	87	148,7±2 ,6	94	154,2±2,8	98
9 LL	33,7±2, 1	101	101,7±7 ,4	87	138,0±9 ,2	87	144,1±5,8	91
15 LL	21,2±1, 2*	64	92,2±4, 5**	79	133,5±8 ,7*	84	135,2±9,2 *	85
Pb(NO ₃) ₂ (Pb limited level LL - 32 mg/kg)								
3 LL	37,5±4, 6	113	107,7±2 ,6	92	151,5±7 ,5	96	153,25±8, 0	97
9 LL	32,3±2, 1	97	103,7±2 ,9	88	142,4±5 ,6	89	149,4±5,8	92
15 LL	17,2±2, 9*	52	84,2±2, 4***	72	114,5±1 ,4**	72	119,2±1,4 ***	76

Dynamic of growing showed that high of the plant depended of the level of contamination: at 15 LL high decreased for about 24% which may be cause by inhibition of the growth

U.S.A. Site:

- ▶ Study site: Fort Riley County, KS ($39^{\circ}11'30''\text{N}$ $96^{\circ}35'30''\text{W}$)
- ▶ Plot size will be 6ft x 6ft with a plant spacing of 2ft
- ▶ 4 replications



Soil property	
pH (1:10 soil: water)	6.85
CEC, $\text{cmol}^+ \text{kg}^{-1}$	19.5
Sand, silt, and clay, %	11.3, 59.8, 28.9
Mehlich III-P, mg kg^{-1}	40.8
Ext. K, mg kg^{-1}	589.4
Total Pb, mg kg^{-1}	1231

Future plans

- Creation network of West-East demonstration sites of *Mxgiganteus* growing at the contaminated lands
- Further research on impact of PGRs to the *Mxgiganteus* biomass production
- Further research on impact of soil amendments to the biomass production
- Model for Phytotechnology with biomass production
- Testing *M.xgiganteus* and hemp for revitalization of marginal lands (former mining)
- Testing energy value of *Mxgiganteus* biomass produced at the military contaminated sites
- Indicators of the process (soil and plant health)

Thanks to the following co-authors

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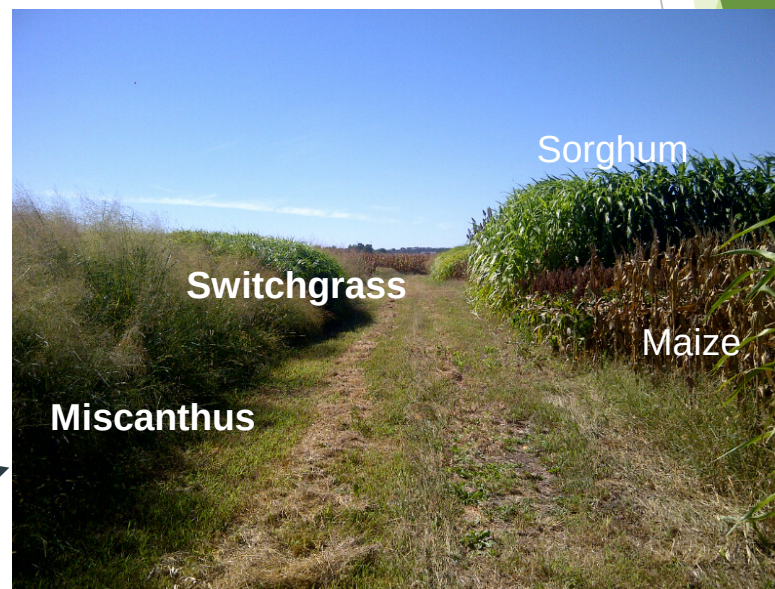
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