PHYTOREMEDIATION OF MILITARY SOIL CONTAMINATED BY METALS AND ORGANOCHLORINE PESTICIDES USING MISCANTHUS

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SUMMARY

Peculiarities of applying the phytotechnologies with the second-generation biofuel crop miscanthus for soil restoration at the abandoned military site in Kazakhstan were explored. The research soil taken at Kyzyl Kairat village, Talgar district, Almaty region was contaminated by metals: Zn, As, Co, Ni, Cu, Cr, Ba, Sr, Fe, U and residues of organochlorine pesticides (OCPs): DDT and its metabolites. Concentrations of As exceeded maximum allowable concentration (MAC) in 6, Co in 3, Ni in 9, Cu in 26, Cr in 8, Ba in 1560, Sr in 21, Zn in 2 times, consequently. The average concentration of OCPs was 6181±8 μg/kg and exceeded MAC in 62 times. The impact of soil contamination to growth of two varieties of miscanthus plants: triploid hybrid *Miscanthus x giganteus* and diploid species *Miscanthus sinensis* was studied in greenhouse conditions. It was established that *M. giganteus* could grow in the soil with relatively low concentrations of pesticides till 2 MAC, while *M. sinensis* had the ability to grow in the soil with concentrations of OCPs till 62 MAC.

Key words: phytoremediation, residues of pesticides, metals, *Miscanthus x giganteus*, *Miscanthus sinensis*

INTRODUCTION

Kazakhstan was granted numerous military contaminated sites as an ecological heritage from the former USSR. One of the effective methods for cleaning these soils contaminated by metals, oils, organic pollutants including obsolete pesticides, is incineration at the special high-temperature stoke. However, that method requires high capital investments and is too costly for developing countries like Kazakhstan. Application of phytoremediation is promising because the approach can address cleanup requirements and may be managed at the acceptable cost. That technique uses vegetation to accumulate, degrade or stabilize environmental contaminants in soil, sediments, surface water, or groundwater (Kidd *et al.,* 2000; Linker 2000).
Selection of plants for that technology is determined by their ability to bring water to the surface soil by evapotranspiration, to break down contaminating substances through enzymes. Plants have to have high biomass productivity and growth rate, be adaptable to certain airborne contaminants and climate conditions as well as resistance to drought, cold, salinity, soil pH, etc. There are several advantages when using miscanthus for phytoremediation. That plant can be grown up to 30 years producing each season valuable biomass. The production process does not practically demand fertilizers and pesticides and, in addition, does not deplete the land. Growing miscanthus at the polluted land allows to restore the contaminated

sites and simultaneously obtaines biomass for production of solid biofuels (Cunningham *et al.,* 1996; Baker *et al.,* 2000; Pidlisnyuk *et al*, 2014).
The strategy for this study was to identify tolerant plant genotypes miscanthus that can be used for phytoremediation of contaminated military soil in Kazakhstan.

MATERIALS AND METHODS

M. x *giganteu*s is a highly productive triploid long-term grass, obtained by crossing diploid *Miscanthys sinensis* Anders with triploid *Miscanthys sacchariflorus Hack* (Jones and Walsh, 2001). Plant height reaches 3-4 meters; productivity is about 20-25 tons per hectare. *M.giganteus* reproduction is exclusively by rhizomes in *vivo* (rootstocks) which are sources of storing nutrients, vegetation development and breeding. Growth of rhizome is due to young shoots, supplying the aboveground part with nutrients and fixation of the plant organism in the soil.
M.sinensis is diploid long-term cereals reproduced by seeds. High degree *M.sinensis* genome affinity to the genome of *Sorghum bicolor* was shown (Rayburn *et al.*,2009*).* Currently *M. sinensis* and *M. giganteus* are considered as promising industrially cellulose-containing raw materials for production of cellulose due to their high productivity (Best practice guidelines, 2001; Pyter *et al.,* 2007; Heaton *et al.,* 2010; Budaeva *et al.,* 2013).

Method of research

The contaminated soil was taken from the polluted site at Kyzyl Kairat village, Talgar district, Almaty region. Standard (uncontaminated) soil was selected at the distance of 2 km from the source of contamination. These two soils were used for the experiment in green house conditions. Contaminated and uncontaminated soil was sieved through a sieve (3 mm) and then thoroughly mixed in different proportions (table 1). Each pot was initially filled with drainage (mass was 1000 g), sand (mass was 1000 g) and soil (mass was 3000 g), the total mass of it was 5000 g. Rhizomes of *M x giganteus* (2 rhizomes) and seedlings of *M. sinensis* were planted in each pot. The experiment was conducted in two replicates.
Before planting mixed soil samples were taken for analysis. Concentration of metals was determined by IC-MS using Agilent 7500 instrument. Gas chromatography (Agilent 7890A) was used for determination of the concentration of OCPs.

Table 1. Contamination and pH of research soil mixed in different proportions

Experiments	OCPs, μg kg^{-1}	MAC, 100 μg kg^{-1}	pH (water)
Contamin soil 200 g + Uncontam soil 2800 g	241±16	2	7.19±0.05
Contamin soil 500 g + Uncontam soil 2500 g	398±15	4	7.13±0.14
Contamin soil 750 g + Uncontam soil 2250 g	610±12	6	7.58±0.09
Contamin soil 1000 g + Uncontam soil 2000 g	1316±51	13	7.40±0.10
Contamin soil 1500 g + Uncontam soil 1500 g	3291±198	33	7.14±0.04
Contamin soil 2000 g + Uncontam soil 1000 g	4518±38	45	7.23±0.21
Contamin soil 3000 g	6181±8	62	7.36±0.13

Planting of *M x giganteus* rhizomes and seeding of *M. sinensis* were done same day, plants were watered every morning by running water.
After finishing the vegetation season plants were taken from the pots and washed 4 times by water. The length of the root systems and the height of the aerial part of the plants were measured; in addition, their biomass was determined. The above-ground part and root of the

plants were separately cut into small pieces. Then samples were collected, placed in sterile container and stored in refrigerator until being analyzed.
The concentration of metals and OCPs in plant's parts (root, stem, leaves) were detected. The determination of metals in the soils and plant tissues were done by the inductively coupled plasma mass spectrometer of Agilent 7500 series ICP-MS (Agilent, USA). For this purpose, a sample of the soil (1.00 ± 0.05 g) or plants (0.1360 g) was placed in a Teflon autoclave, after 5.0 ml of concentrated nitric acid (HNO_3) and hydrogen peroxide (H_2O_2) were added. The samples were taken out of the microwave and cooled to room temperature. The resulting solution was transferred to a 100.0 ml volumetric flask and brought up to the mark bi-distilled water. Finally, the sample was filtered and analyzed at the Mass spectrometer.
The concentrations of OCPs in soil and tissue plant samples were detected by gas chromatography with mass spectrometric detection 7890A/5973N (Agilent, USA), equipped with the Combi-PAL autosampler (CTC Analytics AG, Switzerland). Samples for analysis were prepared using the procedure detaily decribed by Sailaukhanuly *et al.,* 2013. Shortly, each sample was dried 1 day at room temperature, sieved using a 2-mm pore sieve until a homogeneous mixture. For extracting of OCPs 10 g of the sample was placed in a stopper 200 ml flask, 20 ml of n-hexane was added, and the mixture was shaken 1 hour. The volume of the obtained extract was measured and concentrated to 1 ml. The extract was placed in a 50-ml conical flask, 5-10 ml of n-hexane was added and the flask was stirred 30 minutes in a mixer. The resulting extract was filtered through filter paper, measured and concentrated to 1 ml. 1 µl volume of the finalizing extract was injected using an auto sampler for introducing a sample heated to 250 °C. For separation a capillary column DB-35MS (Agilent, USA) with length 30 m, internal diameter 0.25 mm and 0.25 mm thick film was used. Carrier gas (helium mark "A") was fed into the continuous-flow rate of 1.0 ml/min (the average linear flow velocity of 36 cm/s). The temperature of the thermostat column was programmed from 40 °C (hold time 1 min) to 160 °C (hold time 3 min) at a heating rate of 20 °C/min, followed by heating to 250 ° C (hold time 5 min) at 3 °C/min. Detection was carried out by electron capture detector. For quantitative determination of OCPs calibration plots were obtained over the concentration range of 1 – 500 ng/L. Six standard solutions of OCPs were analyzed using the GC-ECD method. The chromatography results were processed using MSD ChemStation software E.02.02 SP1.

Statistics

All experimental data is statistically processed by conventional methods, using "Microsoft Excel" computer program. As assessment criteria for plants' accumulation capacity the concentration of metals and the residual amount of OCPs in the tissue and soil; the extraction coefficient, the bioconcentration factor (BCF), and the translocation factor were calculated.
Translocation factor (TLF) was explored for estimation ability of plants to translocate metal from roots to aerial plant parts (Zu *et al.,* 2005) calculated using the following formula:

$$\mathrm{TLF} = \frac{\mathrm{C\ content\ in\ the\ aboveground}}{\mathrm{C_{content\ in\ the\ root}}} \qquad [1]$$

where
$C_{content\ in\ the\ aboveground}$ was the concentration of pollutant in the aboveground part;
$C_{content\ in\ the\ root}$ was the concentration of pollutant in the root of miscantus.

The coefficient of extraction of metals was calculated by the following formula:

$$C, \% = \frac{C_{HM} \text{ in plant}}{C_{HM} \text{ in soil}} * 100\ \% \qquad [2]$$

where
C % was a coefficient of extraction;
C_{HM} in plant was metal concentration in the plant;
C_{HM} in soil was metal concentration in the soil.

BCF was calculated as the ratio of metal concentration in the plant to the metal concentration in the soil (Zayed *et al.,* 1998). BCF is used as a measure of HM-accumulation efficiency in plants; in case BCF values are greater than 1 that indicates potential HM-hyper accumulator species (Zhang *et al.,* 2002). The value was calculated by the following formula:

$$BCF = \frac{C_{\text{ in the plant tissue}}}{C_{\text{in the soil}}} \qquad [3]$$

RESULTS AND DISCUSSION

Analysis of soil from Kyzyl Kairat village, Talgar district, Almaty region

It was revealed that research soil was contaminated by metals and metabolites of DDT (Table 2), and an average concentration of DDT metabolites was 6181 µg kg^{-1} which equal to 62 MAC, having MAC of DDT metabolites in soil 100 µg kg^{-1}. Measuring the concentration of DDT and its derivatives allows roughly estimate the time of appearance and dissolution of pesticide residual concentrations in soils. If the ratio (DDE + DDD) / DDT> 1 it means "old" use of DDT and its active transformation by microbiological activities. Genetic risk assessment showed (Sailaukhanuly *et al.,* 2016) that such pesticides concentrations can cause cancer. As mentioned, soil at the research site was additionally contaminated by metals and concentrations of As exceeded MAC in six times, Zn in 2, Co in 3, Ni in 9, Cu in 26, Cr in 8, Ba in 1560, Sr in 21 times, consequently. The results of analysis show that concentrations of metals in the control soil also exceeded the MAC and for some metals were about ten times higher than permitted MAC. It could be caused by intensive background agricultural activities in that region accompanied by keeping chemical plant protection products during a long time.

Table 2. Metals content in the soil from the research site at Kyzyl Kairat village, Talgar district, Almaty region, depth of soil 0-60 cm

Contaminants	MAC, µg kg^{-1}	Contaminated soil	Uncontaminated soil
		Heavy metals, µg kg^{-1}	
		1st Hazard class	
As	2	11.9±1/0	1.8±1.4
Cd	3-5	n/d	n/d
Pb	32	21.2±0.3	5.6±4.3
Zn	55	106.5±2.1	51.4±10.6
		2nd Hazard class	
Co	5	14.2±0.7	14.3±1.2
Ni	4	37.8±2.9	36.7±1.3
Cu	3	77.4±10.7	36.4±0.9
Cr	6	51.3±4.0	49.5±4.4
		3rd Hazard class	
Ba	0.1	156.0±5.6	143.0±12.7
V	150	47.6±3.9	45.0±4.4

Contaminants	MAC, µg kg^{-1}	Contaminated soil	Uncontaminated soil
Mn	1500	849.0±21.2	803.5±62.9
Sr	7	147.9±3.0	50.8±4.3
U		6.6±0.1	4.0±0.2
Fe		29020.0±1709.8	28605.0±2926.0
OCPs, µg kg^{-1}			
4,4'-DDE	100	2750±88	n/d
2,4'-DDD	< 1	933±48	n/d
4,4'-DDT	100	2498±45	n/d
Sum		6181	

Growth and development of miscanthus at the contaminated soils

The first year of experiment permitted to establish that *M. sinensis* species grew well at the metals and pesticides contaminated soil up to concentration of OCPs equal 62 MAC (6181 ± 8 µg kg^{-1}). *M. giganteus* grew satisfactory till concentration of 2 MAC (241 ± 16 µg kg^{-1}), at higher concentration the plant died.

It was shown that the growth and development of *M. giganteus* was determined by soil contamination: when the plant was grown in the soil contaminated by OCPs in the range 6 -45 MAC it died at the stage of seedlings. One of the reasons of death at the early stage of ontogenesis was the disturbance the meristematic zones of roots necessary for the growth and development of *M. giganteus*. The measuring of the plant's roots showed that the average height of the root system varied from 6.9±1.3 to 46.3 ±2.1 cm depending on the pesticides' concentration in the soil. When the concentration of pesticides was 62 MACs *M. x giganteus* did not grow at all. In case the concentration of pesticides in the soil was less than 2 MPC, the plant underwent a full cycle of development: the average height of the plant reached 137.6±6.1 cm (control 157.5±5.9 cm), and the length of the root was 57.2±4.2 cm (control 33.3±1.2 cm).

Another picture was observed for *M. sinensis* species. That plant grew well at the pesticide-contaminated soil up to 62 MAC. The average height of the plant reached 75.3±3.2 cm (control 67.6±1.1 cm), and the length of the root was 35.2±2.2 cm (control 65, 0±3.3 cm).

Table 3. The concentration of OCPs in the research soil and weight of miscanthus biomass

Samples	Vegetation	Mass. kg	Concentration. µg kg^{-1}			Phyto extraction. µg
			4,4'-DDE	2,4'-DDD	4,4'-DDT	
			M.giganteus			
2 MAC	Soil	3	146±22	3±1	92±21	723
	Aboveground	0.031	0.2±0.1	18.3±6.1	98.4±27.1	3.6
	Root	0.035	35.2±6.5	352.3±32.2	1810.4±126.3	76.9
	Soil	3	105±18	2±1	83±12	572
Dying sprouts of *M. giganteus*						
6MAC	Aboveground	0.015	50.2±14.2	15.3±6.2	36.3±12.5	1.5
	Root	0.026	158.1±87.5	134.3±25.1	139.8±23.6	11.2
13 MAC	Aboveground	0.010	41.4±21.2	17.3±11.5	36.7±15.7	0.9
	Root	0.022	246.3±123.3	135.5±27.1	191.1±55.1	8.8
33 MAC	Aboveground	0.008	22.2±9.2	36.1±18.2	32.0±16.0	7.2
	Root	0.020	267.4±42.1	60.4±19.1	212.6±27.1	10.8
45 MAC	Aboveground	0.007	36.1±27.2	112.3±20.5	27.9±9.9	1.2
	Root	0.021	453.3±30.1	113.4±15.2	52.1±11.1	13.0
62MAC	Aboveground	Died				

Samples	Vegetation	Mass. kg	Concentration. μg kg^{-1}			Phyto extraction. μg
			4,4'-DDE	2,4'-DDD	4,4'-DDT	
	Root					
			M. sinensis			
62MAC	Soil	3	2750±88	933±48	2498±45	18543
	Aboveground	0.014	151.2±45.3	78.4±29.3	12.4±7.0	3.4
	Root	0.009	570.5±53.3	45.1±22.0	247.2±76.4	7.8
	Soil	3	1230±49	888±79	1991±221	12327
MAC – Maximum Acceptable Concentrations of metabolites DDT in the soil 100 μr κg^{-1}						

It could be summarized that *M. sinensis* species was more tolerant to OCPs than *M. giganteus.* The species *M. giganteus* when produced at slightly contaminated soil accumulated up to 115 MAC of OCPs (2314 μg kg^{-1}) in vegetative organs. The species *M. sinensis* when produced at the highly polluted soil accumulated up to 55 MAC of OCPs (1105 μg kg^{-1}), having MAC of OCPs for plant 20 μg kg^{-1}. It should be noted that wet aboveground biomass of species *M. giganteus* was more than in two times greater in comparison with aboveground biomass of *M. sinensis*. For roots that ratio was in four times higher for *M. xgiganteus* in comparison with *M. sinensis* (Table 3).

Phytoextraction of DDT metabolites in vegetative organs of plants was calculated as concentration of pesticides in tissue multiplied by biomass. It was noted that species *M. giganteus* mainly extracted il 4,4'-DDT, 2,4'-DDD, and species *M. sinensis* mainly extracted metabolites 4,4'-DDE and 2,4'-DDD. The species *M. sinensis* had the ability to accumulate in the tissue up to 36 MAC the highly hydrophobic metabolite 4,4'-DDE and also had the high TLF (1.7) of the metabolite 2,4'-DDD from the root system to the aboveground part.

When evaluating the metal concentration in tissue of *M. giganteus* growing in slightly pesticide-contaminated soil (2 MACs), the plant accumulated metals along with DDT metabolites (Table 4).

Table 4. The total content of metals in the soil, tissue of plant, coefficient of extraction, coefficient of translocation

Heavy metals	MAC	The total content of metals in tissue. μr κg		C, %	TLF
		1st Hazard class			
As	2	0	0	0	0
Pb	32	2.0±0.1	0.9±0.1	14	0.4
Zn	55	33.0±2.1	26.2±1.5	56	0.8
		2nd Hazard class			
Co	5	0.3±	0.03±0.01	1	0.1
Ni	4	0.9±0.1	0.5±0.3	4	0.5
Cu	3	6.6±1.1	5.8±0.9	34	0.9
Cr	6	0.6±0.1	0	2	0
		3rd Hazard class			
Ba	0.1	47.7±2.1	30.0±1.7	50	0.6
V	150	1.1±0.2	0.1±0.1	3	0.1
Mn	1500	83.4±2.3	30.1±4.2	14	0.4
Sr	7	67.1±1.2	25.0±1.5	62	0.4
		Radioactive isotopes			
U		0.3±0.1	0.08±0	6	0.3
Other					
Fe		493.3±21.2	180.0±18.2	3	0.4

The metals were mainly accumulated in the roots, however, for some metals (Zn, Cu) TLFs were close to 1 (0.8-0.9). The consistency of metals accumulation in plant tissues had the following order: Sr> Zn> Ba> Cu> Mn> U> Ni> Fe> Cr> Co.

CONCLUSIONS

The research soil from contaminated site located in Kyzyl Kairat village, Talgar district, Almaty region was polluted by metals and organochlorine pesticides residues. Concentrations of As in the soil exceeded MAC in 6, Co in 3, Ni in 9, Cu in 26, Cr in 8, Ba in 1560, Sr in 21, Zn in 2 times, consequently. The average concentration of OCPs was 6181±8 μg/kg and exceeded MAC in 62 times.

The efficiency of using two species of *miscanthus* (second generation biofuel crop) for phytoremediation of these polluted soils was evaluated at green house conditions. It was established that *M. sinensis* had the ability to grow at the soil with high concentrations of OCPs up to 62 MAC. Another research species *M. giganteus* could grow only at the soil with relatively low concentrations of pesticides till 2 MAC. *M. giganteus* while growing at the slightly contaminated soil accumulated 115 MAC OCPs, while *M. sinensis* accumulated 55 MAC OCPs from the high contaminated soil. Translocation factor for metals in the system "soil – root –aboveground biomass" varied from 0.1-to 0,9 and was the highest for Zn and Cu. *M. giganteus* can be cultivated at the lands with relatively low concentration of OCPs: till 2 MAC. *M.sinensis* can be used for remediation of highly polluted soil, contaminated by OCPs and metals till 62 MAC of OCPs.

ACKNOWLEGEMENT

The research was supported by NATO SFP MYP G4687 and Projects of the Ministry of Education and Science of the Republic of Kazakhstan.

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