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MACROELEMENTS AND HEAVY METALS CONTENT IN *PANICUM VIRGATUM* CULTIVATED ON CONTAMINATED SOIL UNDER DIFFERENT FERTILIZATION

SUMMARY

Heavy metal contamination of soils is a major problem worldwide. As a result, arable land polluted with heavy metals is unsuitable for food production. Utility of energy crops which allow the commercial exploitation of these soils by establishing biofuel feedstock production systems can offer a solution. Additionally, plant cultivation offers opportunities for site remediation.

Field experiments have been performed on heavy metal contaminated arable soil located in southern Poland, in the vicinity of a former smelting factory. Although heavy metal concentration exceeded standards, the area has been used for agricultural purposes. Experiments involved testing Switchgrass (*Panicum virgatum*) cultivated with standard NPK fertilizers and commercially available microbial inoculum. Biomass water, macronutrients (N, P, K, Mg, Ca) and heavy metal (Cd, Pb, Zn) content in aboveground plant organs were assessed at the end of two growing seasons.

Switchgrass biomass water content was higher after the second year for nearly 40%. Additionally, after the first as well as the second year fertilizers increased it. Magnesium content, essential in chlorophyll biosynthesis, was higher in the first year and additionally more evident in fertilized variants after every year. Heavy metals accumulation in aboveground organs was lower after the second year compare with the first year. Similar trend was observed for Ca and N plant accumulation. However P and K accumulations were higher after the second year of experiment.

In conclusion, due to acclimatization, switchgrass reduce heavy metal uptake, what could result in increase of two biogenic elements (P, K) essential in plant growth.

Keywords: *Switchgrass, Heavy metals, Macronutrients, Inoculum, Biomass composition.*

INTRODUCTION

Heavy industry (*i.e.*, smelters, coal mine) is main emitter of heavy metals (HMs) to the environment. The most dangerous directly for human health is spreading HMs to vicinity agricultural areas where it can be introduce to food

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chain by accumulation in consumed plant organs (Järup, 2003). Such contaminated arable lands should be excluded from agricultural production. The alternative for such production can be establishing biofuel feedstock production systems on those areas. Except biomass production for energy purposes there is additional benefit in those land management what protect soil from erosion (Meers*et al.,* 2010). Mineral macro- and micronutrients are essential in plant development and proper growth of plants is undeniable depends on its content in soil. Mineral macronutrients are need for plants in higher amount in comparison to micronutrients. Mineral macronutrients can be divided in to two groups: primary mineral macronutrients (N, P, K) and secondary mineral macronutrients (Ca, Mg, Fe, S) (Tripathi *et al.*, 2014). There are reports indicates negative impact of HM on macronutrients accumulation in above ground organs in agricultural plant as well as in plant cultivated for medical purposes (Bello *et al.*, 2004, Siedlecka, 2014). There is a dearth of papers concerning on macronutrients status in energy crop cultivated HMs contaminated soils. *Panicum virgatum* (Switchgrass) is C4 photosynthesis perennial grass belongs to second generation energy crops, however it can be also cultivated for feed purposes. It is originated from prairie of Northern America where it is dominant species. Switchgrass due to those features is plant very resistant to drought conditions (Parrish and Fike, 2005).

The aim of the study was to describe relationships in HMs and mineral macronutrients accumulation in fertilized Switchgrass during two growing season (2014 and 2015) after plantation establishment. Plants were treated with standard NPK fertilizer and commercial available microbial inoculum (EmFarma, Probiotics Magdalena Górska, Poland).

MATERIALS AND METHODS

Site description

The trial was established in Bytom (Upper Silesia Region, 50°20'43.0"N 18°57'19.6"E, Poland) on arable land contaminated with HMs. Soil was contaminated over the last century due to dust fall with HMs deposition (Zn, Cd and Pb) emitted by already non-existent Zn/Pb smelter. The climate conditions at the site are moderate, with monthly average temperature and precipitation presented in Figure 1 (based on Institute of Meteorology and Water Management data).

Experiment design

Switchgrass plots were established at the beginning of May 2014 from the seedlings obtained from seeds, pre-cultivated in growing room. On each of the three plots 49 plants were planted with density of 3plants per 1 m^2. Due to high soil homogeneity (Tab. 1) and apprehension of uncontrolled fertilizer application pseudo-replication were performed. On each plot four section were distinguish (Fig. 2): edge plants excluded from further analysis(i) and three section (pseudo-replication) from which samples were taken(ii). Each plot was treated in a different way and 4 meter buffer zone between each experimental plot was set:

•**P - C** - control (no treatment);
•**P - NPK** - NPK standard fertilization, applied directly to the soil once before planting (nitrogen 70 kg ha^{-1}, phosphorus 30 kg ha^{-1} as P_2O_5 and potassium 45 kg ha^{-1} as K_2O);
•**P - INC** - commercial microbial inoculum (EmFarma, ProBioticsPolska Magdalena Górska, Poland) applied on seedlings roots before plantation and on the leaves as aerosol in the middle of every month during the growing seasons (from May to September 2014 and 2015).
Initial soil samples were collected before planting, one from each plot sections. The plant samples for HMs and mineral macronutrients analysis were collected in the middle of October each year: one sample were collected from the middle plot section and two samples were collected from upper and down plot section.

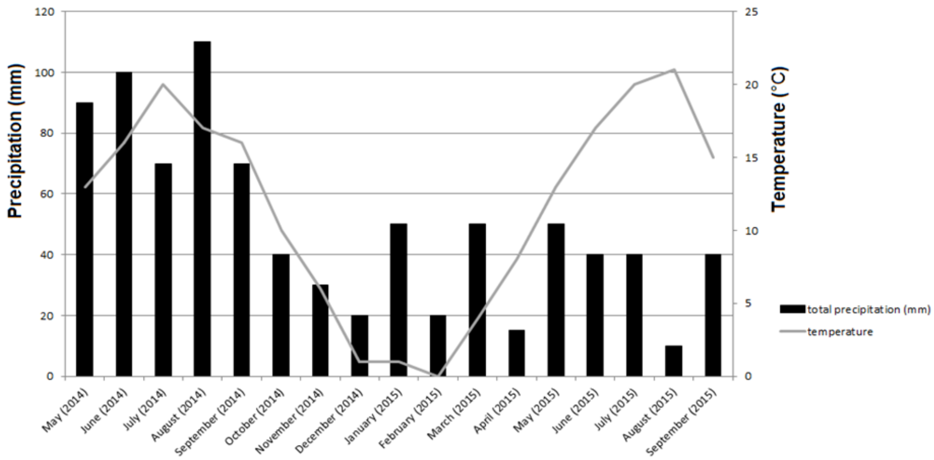

Figure 1. Meteorological data for growing seasons 2014-2015

Soil and plant samples analysis

Soil pH was measured in H_2O (1:2.5 m/v) with a combination glass/calomel electrode (OSH 10-10, METRON, Poland) and a pH-meter (CPC-551, Elmetron, Poland) at 20°C.
The conductivity was determined by an ESP 2ZM electrode (EUROSENSOR, Poland) according to the Polish norm PN-ISO 11265:1997.

Soil texture was evaluated by the hydrometric method according to the Polish norm PN-R-04032:1998.

The total content of metals in the soil and plant tissues was obtained using hot plate digestion (HNO_3 and $HClO_3$, ratio 4:1) and flame atomic absorption spectrometry (SpektrAA 300, Varian INC., USA).
Total nitrogen content (N) in plant (PB.06 edition 1 from 2011.09.01) and soil (ISO 13878:1998), available phosphorus (P) in plant (PB.03 edition 1 from 2011.09.01) and soil (PN-R-04023:1996) as well as potassium (K) content in

plant (PB.01 edition 1 from 2011.09.01) and soil (PN-R-04022:1996) samples was assessed in Institute for Ecology of Industrial Areas laboratory.

Soil organic matter (OM) was measured by loss on ignition as follows: air dry soil was sifted to pass a 2 mm sieve, dried at 105°C for 24 h and then (5 g) treated with 550°C for 4 h. Soil organic carbon (C-org) was assessed according to PN-ISO 14235:2003.

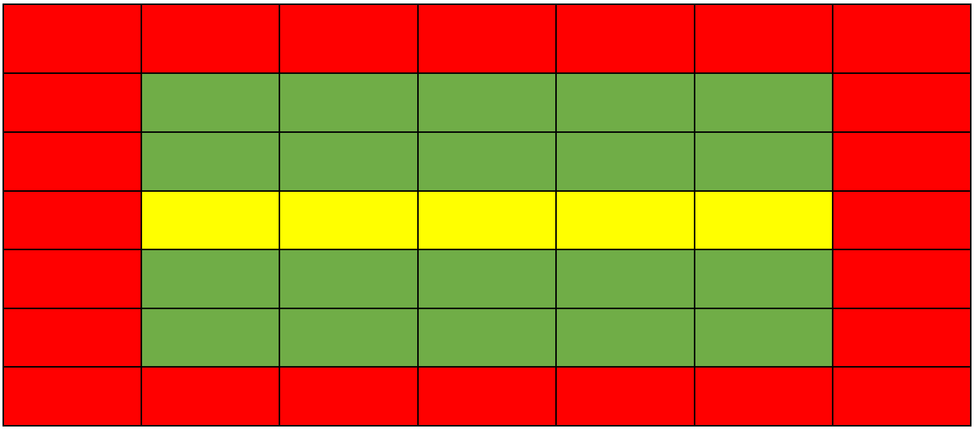

Figure 2. Section on plot distinguished for sample collection. Red - section excluded from sampling, green – section where two samples were collected, yellow – section where one sample was collected

Statistical analysis

Data were analyzed using a one-way ANOVA, followed by a *post hoc* comparison using the Fisher LSD test ($P < 0.05$). Statistical analyses were performed using Statistica 10 (Statsoft, USA). Spider charts were constructed using Excel MS Office (Microsoft, USA) and standardization of data for charts construction were performed using Statistica 10 Software (Statsoft, USA).

RESULTS AND DISCUSSION

Results of soil analyses indicate that among experimental plots there were no significant differences between measured parameter, except Zn (Tab. 1). Soil Zn content was slightly higher on P – INC plot when compare to control. There were no significant differences between P – NPK plot and P – INC plot. Soil texture on the experimental field was classified as silty loam. According to obtained results and regulation of Polish law (D.2002. r.165 poz.1369) soil HMs content exceed the limits defined by government regulation. In this case experimental area should be classified as marginal, moreover whole agriculture production should be abandoned (Gopalakrishnan *et al.*, 2011). Spider charts (Fig. 3) were used as a tool for assess values pattern changes of measured parameters in above ground organs of Switchgrass among different treatments, as well as annual changes for two growing seasons (2014 and 2015). Spider charts can be divided in to three representative sections: water with secondary macronutrient content (Ca, Mg) (1), primary macronutrients content (N, P, K) (2) and HMs content (Zn, Cd, Pb) (3). Overall, the highest HMs accumulation in above ground organs were found for control plants and it was conditioned by Cd accumulation in the first growing season. The lowest values of Mg and Ca for control plants could be associated with that highest HMs uptake. Hermans *et al.* (2011) reported that in *Arabidopsis thaliana* old leaves the content of Cd was lower forplants treated with Cd and Mg, when compare to plant treated only with Cd. It can be assumed with agreement to presented results that higher Mg content can reduce Cd accumulation in elder plants. It is well known that magnesium plays significant role in chlorophyll biosynthesis due to this is essential for photosynthesis process (Cakmak, 2014). Other measured parameters were equal among each treatment in the first growing season. In the second growing season it was found, that there is similar trend among treatments for Mg and Ca accumulation, however it was found that those parameter values are lower when compare to the second growing season.

Table 1. Soil physico-chemical characteristic, heavy metal content and macronutrients content in initial soil samples

	Soil physico-chemical characteristic				
	pH (H_2O)	pH (KCl)	EC (μS cm^{-3})	OM (%)	C-org (%)
P - C	6.49 ± 0.03a	5.97 ± 0.05a	89.92 ± 1.41a	5.39 ± 0.02a	2.18 ± 0.03a
P - NPK	6.57 ± 0.04a	6.06 ± 0.07a	82.28 ± 2.80a	5.52 ± 0.06a	2.11 ± 0.03a
P - INC	6.57 ± 0.03a	6.12 ± 0.02a	89.33 ± 2.89a	5.44 ± 0.06a	2.10 ± 0.01a

	Soil heavy metals content		
	Pb (mg kg^{-1})	Cd (mg kg^{-1})	Zn (mg kg^{-1})
P - C	514.77 ± 14.26a	17.94 ± 0.05a	1659.50 ± 7.22b
P - NPK	487.30 ± 3.18a	18.06 ± 0.05a	1700.00 ± 6.35ab
P - INC	496.50 ± 4.33a	18.02 ± 0.11a	1750.33 ± 34.04a
	Soil primary mineral macronutrients content		
	N_{total} (%)	P (mg kg^{-1})	K (mg kg^{-1})
P - C	0.15 ± 0.00a	834.30 ± 6.64a	950.30 ± 17.32a
P - NPK	0.15 ± 0.00a	833.20 ± 8.66a	970.60 ± 1.15a
P - INC	0.15 ± 0.00a	835.87 ± 15.90a	1014.27 ± 24.62a
	Soil secondary mineral macronutrients content		
	Fe (mg kg^{-1})	Mg (mg kg^{-1})	Ca (mg kg^{-1})
P - C	9854.50 ± 44.74a	1557.00 ± 30.60a	3015.00 ± 49.07a
P - NPK	10135.00 ± 13.28a	1592.00 ± 4.04a	2998.50 ± 3.17a
P - INC	10219.00 ± 164.93a	1669.33 ± 51.49a	3185.17 ± 98.48a

P – *Panicum virgatum*, C – control plot; NPK – NPK fertilized plot, INC – microbial inoculated plot. Values are means ± SE (n=3). A lower case letters (a, b, c, d) denotes significant differences among soils samples taken from different plots at P ≤ 0.05 according to Fisher LSD test.

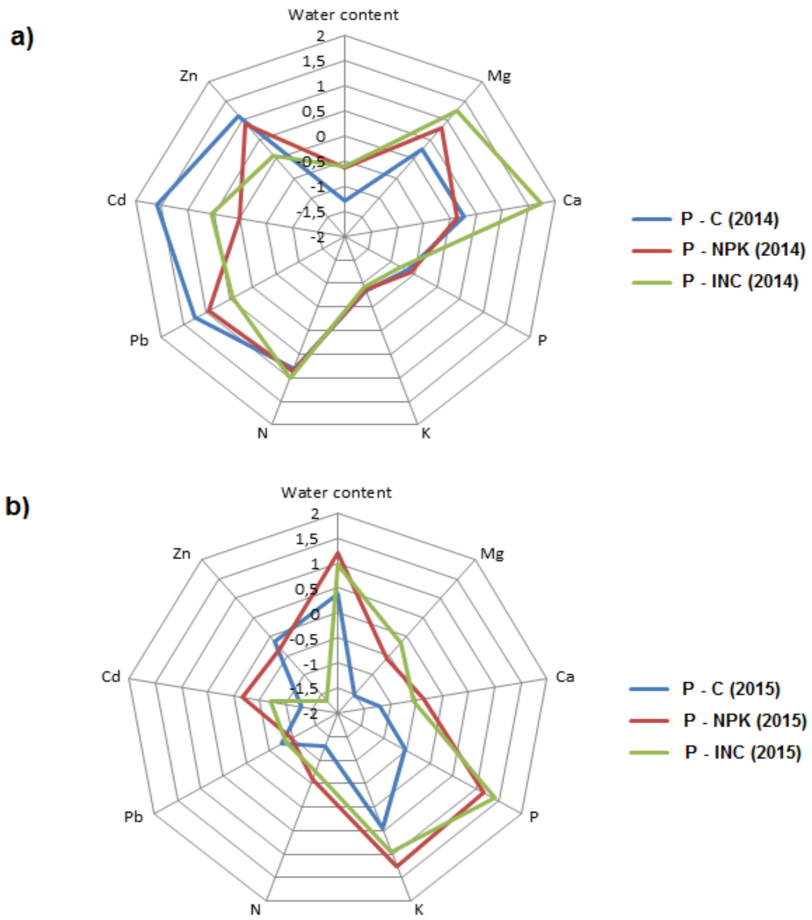

Figure 3. Spider charts constructed on macronutrients, heavy metals and water content show patterns indicated changes of those parameters; among treatment (**C** – control, **NPK** – NPK fertilizer treated plant, **INC** – microbial inoculum treated plant) and growing season (a-**2014** and b-**2015**) for *Panicum virgatum*(**P**). **Mg, Ca, P, K, N, Pb, Cd, Zn** – elements accumulation in above ground plants organs. For better data visualization all presented values were standardized. Each measurement was performed in 5 replicate (n = 5)

It also has been found that there is overall decrease in accumulation of HMs and N for plants from each plot, except Cd accumulation in NPK treated plants when compare to the first growing season. Increased K concentration in plant samples was found for each variant and P was found to be increased on each plot treated with fertilizer, when compare to the first growing season. Gonçalves *et al.* (2009) reported, that higher Cd concentration in hydroponic solution can decrease K uptake to above ground organs. This paper is with agreement with obtained results, however the tendency is only contrary to NPK treated plants. Water content in plant stems was 40% higher for plant in the growing season 2015 when compare to the growing season 2014 and treatments slightly increased it. This phenomena can be associated with ability to water capture, which is correlated to root distribution. It is known that perennial grasses root system become fully developed after 4th growing season (Ferchaud *et al.*, 2015). In this case water content could be increased due to annual increase of roots density. Spider charts with description corresponds to overall annual and treatment changes in biomass HMs, water and mineral macronutrient content. Its allows to track changes in tendencies while detailed statistical analysis for measured parameters is presented in Table 2.

Table 2. Matrix of statistical significant differences among analyzed parameters presented on spider charts (Fig. 2)

	P-C (2014)	P-NPK (2014)	P-INC (2014)	P-C (2015)	P-NPK (2015)	P-INC (2015)
Water content	b	b	b	a	a	a
Mg	bc	ab	a	e	d	cd
Ca	ab	ab	a	b	ab	ab
P	b	b	b	b	a	a
K	b	b	b	a	a	a
N	a	a	a	b	b	b
Pb	a	a	b	c	c	c
Cd	a	bc	ab	c	bc	bc
Zn	a	a	a	ab	ab	b

P – *Panicum virgatum*, **C** – control; **NPK** – NPK fertilized plants, **INC** – microbial inoculated plants.
A lower case letters (a, b, c, d, e, f – where “a” corresponds to the highest value and “f” to the lowest) denotes significant differences among plants elements and water content taken from different plots at $P \leq 0.05$ according to Fisher LSD test. Each measurement was performed in 5 replicate (n = 5)

CONCLUSION

It could be concluded that HMs in soil affect mineral macronutrients status in *Panicum virgatum*, especially Mg and K. Additionally there are differences mostly in each measured parameters when compare growing seasons. It can be associated with acclimatization of plant to contaminated site. There is a dearth of papers concerning mineral nutrients as well as HMs status in the first two years after establishment, because most of them are focused on fully establishment trials, where perennial energy crops achieves its maturity after 3rd -4th year after establishment.

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REFERENCES

Bello, M. O., Ibrahim, A. O., Ogunwande, I. A., Olawore, N. O. 2004. Heavy trace metals and macronutrients status in herbal plants of Nigeria. Food Chemistry 85, 67-71.

Cakmak, I. 2014. Major functions of calcium and magnesium in crop plants. In World Fertilizer Congress , 16, 30-32.

D.2002. nr.165 poz.1369 – Regulation of the Polish Ministry of Environment on Soil and Ground Standards (in Polish).

Ferchaud, F., Vitte, G., Bornet, F., Strullu, L., Mary, B. 2015. Soil water uptake and root distribution of different perennial and annual bioenergy crops. Plant and Soil, 388, 307-322.

Gonçalves, J. F., Antes, F. G., Maldaner, J., Pereira, L. B., Tabaldi, L. A., Rauber, R., Rossato, L. V., Bisognin, D. A., Dressler, V. L., de Moraes Flores, E. M., Nicoloso, F. T., 2009. Cadmium and mineral nutrient accumulation in potato plantlets grown under cadmium stress in two different experimental culture conditions.Plant Physiologyand Biochemistry. 47, 814-821.

Gopalakrishnan, G., Cristina Negri, M., and& Snyder, S. W. 2011. A novel framework to classify marginal land for sustainable biomass feedstock production.Journal of environmental quality, 40, 1593-1600.

Hermans, C., Chen, J., Coppens, F., Inzé, D., Verbruggen, N. 2011. Low magnesium status in plants enhances tolerance to cadmium exposure. New phytologist, 192, 428-436.

ISO 13878:1998 - Soil quality - Determination of total nitrogen content by dry combustion ("elemental analysis")

Järup, L. 2003. Hazards of heavy metal contamination.British medical bulletin, 68, 167-182.

Meers, E., Van Slycken, S., Adriaensen, K., Ruttens, A., Vangronsveld, J., Du Laing, G., Witters, N., Thewys, T., Tack, F. M. G. 2010. The use of bio-energy crops (*Zea mays*) for 'phytoattenuation'of heavy metals on moderately contaminated soils: a field experiment. Chemosphere, *78*, 35-41.

Parrish, D. J.and Fike, J. H. 2005.The biology and agronomy of switchgrass for biofuels. BPTS, 24, 423-459.

PB.01 edition 1 from 2011.09.01 – Potassium content, Flame photometry method (in Polish)

PB.03 edition 1 from 2011.09.01 – Phosphorus content, Spectrophotometric method (in Polish)

PB.06 edition 1 from 2011.09.01 - Total nitrogen content (Kjeldahl), Titration method (in Polish)

PN ISO 14235:2003 Soil Quality - Determination Of Organic Carbon By Sulfochromic Oxidation

PN-ISO 11265:1997 -Soil quality - Determination Of Electrical Conductivity

PN-R-04022:1996 - Assessment of assimilable potassium content in mineral soils (in Polish)

PN-R-04023:1996 - Assessment of available phosphorus content in mineral soils (in Polish)

PN-R-04032:1998 – Soil and mineral pieces – Soil sampling and texture assessment (in Polish)

Siedlecka, A., 2014. Some aspects of interactions between heavy metals and plant mineral nutrients.ActaSocietatisBotanicorumPoloniae 64, 265-272.

Tripathi, D. K., Singh, V. P., Chauhan, D. K., Prasad, S. M., Dubey, N. K., 2014. Role of macronutrients in plant growth and acclimation: recent advances and future prospective, in: Ahmad, P., Wani, M. R., Azooz, M.M., Tran, L.-S. P. (Eds.), Improvement of Crops in the Era of Climatic Changes. Springer, New York, pp. 197-216.