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ACCUMULATION OF CADMIUM, LEAD AND MERCURY IN SEEDLINGS OF SELECTED SUGAR BEET VARIETIES AS A RESULT OF SIMULATED SOIL CONTAMINATION

Bogdan Saletnik, Grzegorz Zaguła, Marcin Bajcar, Czesław Puchalski*

Address(es): University of Rzeszow, Faculty of Biology and Agriculture, Department of Bioenergy Technologies, 4 Zelwerowicza St., 35-601 Rzeszow, Poland, (17) 785 50 46.

*Corresponding author: bogdan.saletnik@urz.pl

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ABSTRACT

The article presents the findings of a study designed to compare accumulation of cadmium, lead and mercury in seedlings of selected sugar beet varieties, as a result of simulated soil contamination. The analyses included seedlings of eight sugar beet varieties: Alegra C, Delano C, Milton C, Primadonna, Silvetta C, Finezja C, Agnieszka, Janosik, which in the course of growth were fortified with heavy metals. The acquired results were used to calculate Bioconcentration Factors (BCF) and to compare the contents of heavy metals in the examined plants. The greatest capacity to absorb the relevant heavy metals from the soil was found in seedlings of Milton C and Silvetta C varieties for cadmium and lead, and in Silvetta C and Finezja C varieties for mercury. It was determined that in the entire group of plants, the seedlings of Agnieszka and Janosik verities were least susceptible to bioaccumulation of cadmium and lead, while Primadonna variety had the lowest susceptibility to mercury.

Keywords: Heavy metals, soil contamination, sugar beet, bioconcentration factors

INTRODUCTION

The progress of civilization and the increasing pollution of the environment have resulted in numerous irreversible changes affecting natural areas **(Majtkowski *et al*., 2010)**. Human activity in recent years has led to an increase in quantities and distribution of heavy metals in the atmosphere, soil and aquatic ecosystems **(Gbaruko and Friday, 2007)**. The large-scale gas emissions, combustion of fossil fuels in heat and power generation plants, accumulation of industrial waste as well as utilization of artificial fertilizers and crop protection chemicals in agriculture have significantly contributed to the increased content of heavy metals in the soils and surface waters. Heavy metals taken up by plants from the soils may adversely affect their growth and development **(Majtkowski *et al*., 2010)**. Accumulation of heavy metals in soil causes an undesirable changes in the soil ecosystems and functioning of plants **(Khan *et al*., 2008a; Khan *et al*., 2008b)**. The consequences of exposing plants to heavy metals may include impairments in the processes of flowering, gametophyte development, sprouting and development of seedlings **(Siwek, 2008)**.

Considerable contamination of soils and plants with heavy metals is found in areas located along major roads. Carbon and nitrogen oxides, sulfur dioxide and heavy metals get into the atmosphere along with fumes produced by combustion engines. The use of car tyres and other parts of mechanical vehicles results in the fact that numerous substances, including cadmium and toxic lead compounds find their way into the environment **(Baran *et al*., 2007)**. Uptake of heavy metals through plants in agricultural areas could pose a serious threat to humans **(Liu, 2007; Yan, 2012)**. With regard to areas used for farming purposes it should be emphasized that the potential sources of heavy metals in the soils include mineral and organic fertilizers, lime as well as waste-based composts. Power plants and industrial facilities generate dust emissions which may spread over large areas, polluting soils at a significant distance from the industrial zones. Air pollution leads to accumulation of such elements as cadmium and lead in the soil. Regardless of the way heavy metals access the soils, once they exceed the acceptable quantities, they may pose significant hazard to plants, animals and people **(D'Amore *et al*., 2005; Gruca-Królikowska and Wacławek, 2006)**. Importantly, due to the low mobility of heavy metals in soil they are among the most persistent soil pollutants **(Potarzycki *et al*., 1999)**.

Heavy metals find their way into plants via their root systems and leaf blades. Metals, which in the soil are present in the form of free ions, are more available for plants than element occurring in the form of complexes **(Chaney *et al*., 1997)**. Compounds such as metal chelators and organic acids, released by plant roots, may increase availability of heavy metals occurring in insoluble soil complexes.

The degree of heavy metals accumulation in plants depends on the type of metals, their concentration in the soil substrate, the form in which they occur, as well as the plant variety. As for the contents of heavy metals in specific parts of the plant, it is possible to notice a decrease in metal concentration in the following order: root, leaves, stalk, flowers, and seeds **(Ociepa-Kubicka and Ociepa, 2012)**. Excessive quantities of heavy metals in the soil, resulting e.g. from their long-term accumulation, pose significant hazard for living organisms **(Grygierzec, 2013)**. High concentrations of heavy metals may impair physiological and biochemical processes occurring in organisms. The most harmful heavy metals include Pb, Hg, Cd, Sn, Cr, Zn, Cu **(Gosh, 2010)**. Metals which are considered to be most harmful for humans are cadmium and lead **(Sekara *et al*., 2005)**. Lead is believed to be responsible for many ailments affecting people, e.g. chronic neurological disorders **(Awofolu, 2005)**. In the case of children, lead poisoning may lead to neurological conditions whose symptoms include problems with coordination, loss of short-term memory or learning difficulties **(Padmavathiamma and Li, 2007)**. Toxic activity of cadmium, visible even with low concentrations of the element in the organism, contributes to neoplastic changes, and impairs the functions of liver and kidneys **(Czeczot and Majewska, 2010)**. In plants, cadmium can contribute to disturbances in the photosynthesis process and nutrients collecting **(Kuo *et al.* 2006, Alia *et al.* 2015)**. Cadmium can cause oxidative stress and decrease the rate of a new cells production **(Sandalio *et al*., 2001; Liu *et al*., 2004)**. In turn Pb affected to a decline of chlorophyll content and the length of roots and shoots **(Sharma and Dubey, 2005; Joseph *et al*., 2002)**. Hence, measurements of heavy metals contents in the air, soil and food seem to be a necessary element of monitoring and assessment of pollutants in the environment **(Gruca-Królikowska and Wacławek, 2006)**.

The purpose of the present study was to investigate the level of accumulation of three selected heavy metals (Cd, Pb, Hg) in seedlings of eight sugar beet varieties, with the use of simulated soil contamination. The study was also designed to compare the sugar beet varieties in terms of their ability to take up heavy metals from the soil.

MATERIALS AND METHODS

Experimental design

The materials used in the study included Pb, Cd and Hg (as $Pb(NO_3)_2$, $Cd(NO_3)_2$ and HgO) which were applied to contaminate the soil, as well as eight varieties of sugar beet coming from four companies (a - Alegra C, Silvetta C, b - Delano C, Milton C, c - Primadonna, Agnieszka, d - Finezja C, Janosik). The experiment was carried out in Petri dishes, with the use of MLR-352 phytotron. For this purpose 30g samples of soil were weighed and transferred to 16 dishes. In the study universal soil based on peat, pH = 6 and salinity not exceed 1.9 $g.dm^{-3}$ NaCl was used. The soil contained a starting dose of compound fertilizers NPK (14-16-18): 0.6 kg/m^3. The study using the optic method of inductively coupled plasma optical emission spectroscopy (ICP-OES) ruled out the presence of cadmium, lead and mercury in the soil. The dishes were divided into two groups: the controls (the substrate contained no heavy metals), and the experimental group, then identical number of seeds was placed in each of the dishes. During the experiment, the dishes were located in a climatic chamber, 5ml portions of demineralized water were added to the control dishes, and solution of cadmium, lead and mercury ions was introduced to the experimental sample. The total of 20mg of the metal ions per 1 kg of the soil was added to each dish. After 10 days of growth in constant conditions, i.e. temperature of 25°C, air humidity at the level of 90% and constant lighting, above-ground parts of the plants were collected for further analyses.

Metal extraction and analysis

The collected plant material was carefully rinsed with demineralized water to remove soil residues. Subsequently, the plant samples were subjected to microwave digestion in Teflon vessels using Milestone ETHOS ONE microwave digestion system. The experimental material was subjected to three digestion processes repeated in parallel. 5 g of fresh plants were accurately weighed in a 120 ml Teflon digestion vessel. Then 8 ml of nitric acid was added and dissolved using microwave mineralization. The acid clear solution were transformed to 50 ml volumetric flasks and diluted with deionized water. The contents of cadmium, lead and mercury were determined using the optic method of inductively coupled plasma optical emission spectroscopy (ICP-OES). All elements were blanked by blind sample with was clear nitric acid mineralized in the same time and conditions. In the calibration step, standard solutions for all elements were prepared from a spectroscopic grade reagent (Thermo) with 3 step curve. A curve fit factor for all elements were above 0,99.

Data and statistical analysis

The findings were used to determine the Bioconcentration Factor (BCF), which shows the capacity of plants to take up heavy metals from the soil and to accumulate them in their tissues. The factor shows the relation of the heavy metal concentration in the plant tissues to the concentration of the same metal in the soil. Bioconcentration Factor was calculated from the formula **(Zhuang *et al.*, 2007)**:

$$BCF = C_{harvested\ tissue} / C_{soil}$$

where $C_{harvested\ tissue}$ means the concentration of the heavy metal in plant tissues, and C_{soil} means the concentration of the metal in the soil from which it was absorbed.

The statistical analysis of the findings was performed using software Statistica, version 10.0. The mean values and standard deviations were computed, and the differences were statistically verified using Student's t-test, at the significance level of $p<0.05$, for $n = 3$.

RESULTS AND DISCUSSION

Figure 1 shows the mean contents of lead and the standard deviation in the examined plants.

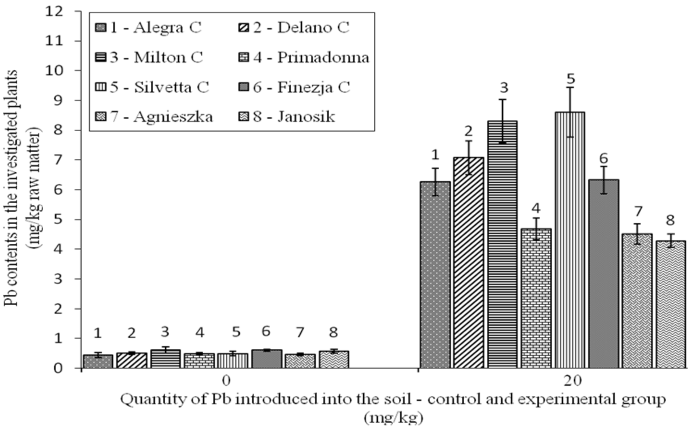

Figure 1 Contents of lead in the experimental and control group depending on sugar beet variety

Concentration of Pb in the investigated plants was significantly higher in all experimental samples, in comparison to the controls (the mean value for the experimental group differs significantly from the control - Student's t-test, p<0.05, n=3). The highest contents of lead were identified in Milton C and Silvetta C varieties, and the lowest contents of this metal were found in Agnieszka and Janosik varieties.

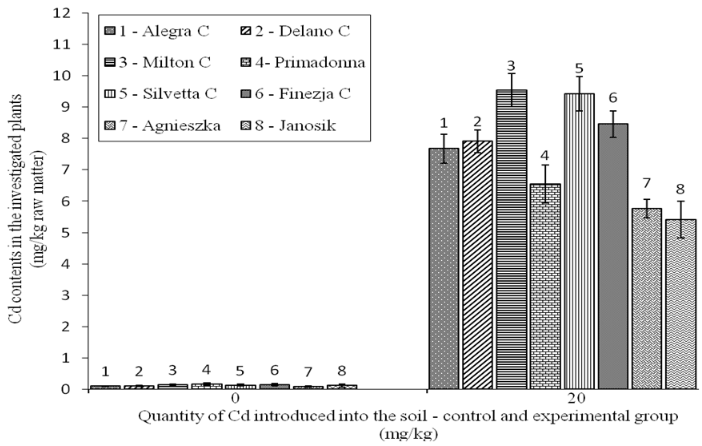

Figure 2 Contents of cadmium in the experimental and control group depending on sugar beet variety

Concentrations of cadmium in all studied varieties of beet were significantly higher, in comparison to the controls (the mean value for the experimental group differs significantly from the control - Student's t-test, p<0.05, n=3). The highest contents of cadmium in the experimental samples were found in the same plants which had the highest concentrations of lead (Milton C and Silvetta C). The lowest contents of cadmium were again identified in Agnieszka and Janosik varieties. Jankowska *et al.* **(2007)** measured the contents of lead in selected species of dicotyledonous plants growing in the proximity of an express way and they identified varied contents of lead in above-ground parts of the common dandelion, broadleaf plantain, and common sorrel **(Jankowska *et al.*, 2007)**. Differences in the capacity for accumulating lead and cadmium in several varieties of the same plant species were demonstrated by Tyksiński and Kurdubska (2004; 2005) who identified a diverse capacity for accumulating these metals in varieties of lettuce (*Lactuca Sativa* L.) and radish (*Raphanus Sativus* L.). In the case of radish these authors also identified the highest and the lowest concentrations of cadmium and lead in the same plant varieties (**Tyksiński and Kurdubska, 2004; 2005)**.

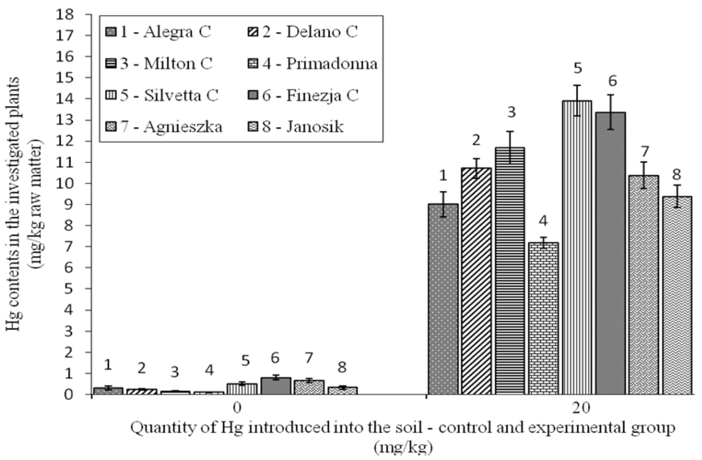

Figure 3 Contents of mercury in the experimental and control group depending on sugar beet variety

Concentrations of Hg in the examined sugar beet varieties were significantly higher in all the experimental groups in comparison to the controls (the mean value for the experimental group differs significantly from the control - Student's t-test, p<0.05, n=3). The findings showed the highest concentration of mercury in Silvetta C and Finezja C varieties. The lowest concentration of this element was identified in Primadonna variety. Just like cadmium and lead, mercury does not have any nutritional functions for plants, yet it finds its way into plant tissues. Due to the fact that in plant tissues mercury is bound by protein sulfhydryl groups, the element may induce adverse changes in cellular respiration processes, in particular related to enzymatic transformation **(Gworek and Reteńska, 2009)**. As reported by Kabata-Pendias **(1992)** higher plants are not excessively sensitive to the presence of mercury in the environment. In the case of plants which are vulnerable to toxic activity of mercury, e.g. beet, maize, and rose, increased concentration of the element may injure their roots, and lead to chlorosis **(Kabata-Pendias, 1992)**.

Table 1 shows the calculated values of correlation coefficients. High positive correlation (significant dependence) were identified between mercury and lead (a value of 0.69) as well as mercury and cadmium (a value of 0.69). Practically complete dependence were identified between cadmium and lead value of correlation coefficient 0.96.

Table 1 Correlation between the analyzed elements

	Mercury	Lead	Cadmium
Mercury	1	0.69	0.69
Lead		1	0.96
Cadmium			1

Bioconcentration Factor (BCF) was calculated in order to determine the degree of mobility of the selected heavy metals in the investigated plants. The value of BCF reflects the plants susceptibility to metals infiltrating from the soil and it provides information about the bioaccumulation properties **(Czech *et al.*, 2014)**.

Table 2 Values of Bioconcentration Factor (BCF) for lead, cadmium and mercury

Sugar beet variety	**Bioconcentration Factor (BCF) for the examined part of the plant**		
	Pb	Cd	Hg
Alegra C	0.313	0.384	0.450
Delano C	0.354	0.396	0.535
Milton C	0.415	0.477	0.584
Primadonna	0.234	0.328	0.360
Silvetta C	0.430	0.471	0.696
Finezja C	0.316	0.424	0.668
Agnieszka	0.226	0.289	0.519
Janosik	0.214	0.271	0.469

Table 2 shows the BCF values for lead, cadmium and mercury in the above-ground part of the plants. The present findings suggest that seedlings of Silvetta C variety are most susceptible to accumulation of the relevant heavy metals (Pb, Cd, Hg). The values of BCF for this variety were: 0.430 Pb, 0.471 Cd and 0.696 Hg. The findings also show that all the sugar beet varieties in question are characterized by higher bioaccumulation properties in relation to mercury than in the case of the remaining investigated elements (cadmium and lead).

CONCLUSIONS

It was demonstrated that all the investigated plants have the capacity to absorb heavy metals from the soil and to accumulate them in their tissues. The highest capacity to absorb cadmium and lead from the soil was found in seedlings of Milton C and Silvetta C varieties, and the highest concentrations of mercury were identified in seedlings of Silvetta C and Finezja C varieties. It was determined that in the group of the examined plants Agnieszka and Janosik varieties were least susceptible to cadmium and lead bioaccumulation, while Primadonna variety was least susceptible to mercury. This study show that the seedlings of the specific varieties of sugar beet differed in terms of their capacity to absorb heavy metals from the soil.

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