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14–15 March 2024

Faculty of Agronomy in Čačak, University of Kragujevac, Serbia

- PROCEEDINGS -

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XXIX Savetovanje o biotehnologiji sa međunarodnim učešćem

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PREFACE

"From the mouth of my immortal teacher Pasteur, I heard these words:
It is true that science is international, but every scientist must be a man who in his scientific work is warmed by love for the people from which he sprang and to whom he owes all his strength".

Prof. Dr. Milan Jovanović Batut (1847-1940)"
The first dean of the Faculty of Medicine in Belgrade

Agricultural science and agriculture as a profession monitor and study changes occurring in this area, point out problems in agricultural practice, and find solutions. The Faculty of Agronomy in Čačak, in addition to educating students, 29y traditionally organizes the Symposium on Biotechnology (SYMBIOTECH) every year. The main goal is to acquaint the wider scientific and professional public with the results of the latest scientific research, and bring together domestic and foreign scientists in the fields of primary agricultural production, food processing, and environmental protection. We work tirelessly in pursuit of excellence.

At the 2nd International Symposium on Biotechnology, a total of 80 papers were presented in the 7 sections: Field, Vegetable and Forage Crops, Pomology and Viticulture, Livestock Production, Plant Protection, Food Safety and the Environment, Food Technology, Nutritionism, and Applied Chemistry.

We owe great gratitude to the **Ministry of Science, Technological Development and Innovation of the Republic of Serbia** and the **City of Čačak** for their traditional financial support and patronage of SYMBIOTECH24. We thank companies, entrepreneurs, stakeholders and all long-time friends of the Faculty of Agriculture for their material and organizational support.

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is organizing

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29th Symposium on Biotechnology with International Participation

14-15 March 2024

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in cooperation with

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DEVELOPMENT OF WHEAT STEAM UNDER CONTAMINATION WITH HEAVY METALS

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Abstract: Heavy metals are significant environmental pollutants because they represent potential metabolic inhibitors. A study was conducted where the content of heavy metals in the wheat steam was examined, as well as the height of the plants from the soil level to the top of the highest coot. The aim of the research was to determine the content and influence of heavy metals on steam development in the Pobeda and Ljiljana varieties. An experiment was set up in pots where two different concentrations of a mixture of heavy metals 250ppm and 500ppm were added under controlled conditions.

Key words: atomic absorption spectrophotometry, heavy metals, wheat steam, *Triticum sp*

Introduction

Interest in the use of bioindicators as a means of monitoring and assessing environmental pollution with toxic metals is constantly increasing. Heavy metals enter the environment from natural sources, but also through anthropogenic activities, and once they reach the environment, they do not disappear, but accumulate in the soil, sediment and biota and increasingly become a growing global problem (Stanković, 2015).

Toxic metals come from contaminated air and soil. Toxic metals can be found in the soil as a result of their presence in the parent rocks. Also, irrational use of organic and mineral fertilizers can lead to soil contamination. Toxic metals are absorbed by the plant through the roots from the soil, and from the atmosphere through the leaves (Mickovski-Stefanović et al., 2012).

Knowing the factors that influence the behavior and bioavailability of heavy metals in soil is essential. In the past period, the concentration of zinc in some soils has increased, especially in industrialized countries as a result of inadequate environmental protection from factory pollution. The most

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important factors for the dynamics of Zn accumulation are pH-value, $CaCO_3$ content and mechanical composition of the soil. Plants grown on contaminated soil absorb and accumulate heavy metals in their aerial organs (Jakovljević et al., 1997).

Due to the rapid increase in the population of people on earth, the requirements for the quantity of food are increasing. However, increasing anthropogenic accumulation of heavy metals can reduce yield and product quality (Popović-Vukeljić, 2002).

The concentration of heavy metals in plants indicates the degree of contamination, but also the ability of different plants to accumulate metals from soil treated with sewage sludge. The study showed the mobility and transfer of heavy metals from sewage sludge soils to different wheat cultivars. The experiment was carried out to study the transfer of heavy metals to wheat grain, namely wheat varieties grown on soil without waste sludge (control) and soil with waste sludge. A very high concentration of all heavy metals was found in the wheat grain from the land with waste sludge. And that only with the two varieties TJ-83 and Mehran-89, while with the varieties Anmol and Abadgar this was not the case as pointed out by Jamali et al. (2008).

Uncertainty of elevated cadmium concentration causes a number of negative effects in plants, such as inhibition of growth, enzyme activity, photosynthesis and change in stomatal activity (Prasad, 1995). The toxic effect of cadmium is reflected through its affinity to the thiol group, as a result of which cadmium can block the functional groups of biomolecules, the result of which is the inhibition of the enzymatic activities of the plant's metabolic processes, such as photosynthesis, respiration, assimilation of nutrient elements and transport (Ferreira et al., 2002).

Inhibition of any component of the photosynthetic apparatus will have a negative effect on physiological activities, and therefore most likely on plant growth. Elevated concentrations of toxic metals also affect parameters that show the capacity for photosynthetic gas exchange, such as stomatal conductance (Li et al., 2013). In one study, the growth and concentration of elements in wheat that grew on 5 different soils treated with heavy metals was monitored. Each soil was mixed with $CdCl_2$ or $ZnCl_2$. The decrease in the concentration of elements in the soil varied depending on the type of soil. Wheat yield was reduced in all soil types and varied by soil type (Hattori and Chino, 2001).

The accumulation of heavy metals in a wheat crop exposed to different pollutants was studied. These experiments lasted 18 years on two types of soil.

The subject of research was spring wheat. The increased degree of soil contamination affected the activation of enzymes in the leaves and roots of plants and increased the degree of antioxidant protection against heavy metal ions. The concentration of heavy metals cadmium, zinc, lead and chromium in wheat grains was significantly lower than in straw, while nickel and copper were more in the grain. The roots contained the most cadmium, nickel, zinc and copper. The pH values had a slight influence on the content of copper, lead and chromium in plants (Murzaeva, 2004).

In some parts of China, research has been conducted on how heavy metal contamination affects plants and how plants provide resistance. Plant growth was inhibited, plant structure was damaged, physiological and biochemical activities fell. The bioavailability of heavy metals depends on numerous factors, such as environmental conditions, pH, types of elements and types of plants. There are also studies on plant defense mechanisms against the toxic effects of heavy metals, such as combining heavy metals with proteins and enzyme detoxification. These are mechanisms for protecting plants from damage by heavy metals. There are two aspects to the interaction of plants and heavy metals here. On the one hand, heavy metals have negative effects on plants, but plants also have their own defense mechanisms against the harmful effects of heavy metals (Cheng, 2003).

The effect of chlorimuron oxide and cadmium on common wheat was investigated. The combined effect on wheat contributed to weaken the possibility of chlorophyll formation. Wheat was able to protect itself by increasing the activity of the antioxidant peroxidase enzyme. Thus, the content of degradable proteins and peroxidase activity represent biomarkers for the negative effect of chemicals on the plant (Wang and Zhou, 2006).

Material and working methods

Research on the influence of the concentration of a mixture of heavy metals on the dynamics of the accumulation of heavy metals in the wheat stem and the growth of the plant was carried out through experiments in the greenhouse of the Faculty of Agriculture in Zemun, where the conditions of heat and humidity were controlled. The vegetation experiment was set up in three repetitions, with a total of 36 pots in which two varieties of wheat, Pobeda and Ljiljana, were sown. Before sowing, the pots were filled with 2 kg of Novobalt dry extract, which was subsequently contaminated with a mixture of chemical compounds of heavy metals in the form of a solution of the following

compounds, namely: zinc in the form of zinc-acetate-$Zn(CH_3COO)_2$ x $2H_2O$, lead in the form of lead acetate $C_4H_6O_4Pb$ x $3H_2O$, chromium in the form of chromium trioxide-CrO3, copper in the form of copper sulfate-$CuSO_4$ and cadmium in the form of cadmium nitrate-$Cd(NO3)_2$ x $4H_2O$.
The following concentrations of solutions were used:
0 ppm (control), 100 ppm, 250 ppm

In addition to the content of heavy metals in the wheat stem, the height of the stem from the soil level was first measured. After that, the content of heavy metals in wheat stems was determined.

Wheat varieties Pobeda and Ljiljana were chosen because they are mostly grown in the area of southern Banat. 12 seeds were sown in each pot at a depth of 5 cm. Samples from the experiment in pots were analyzed in the budding and leafing stages, when four plants were taken from each pot. After sampling the plant, the root was manually separated from the wheat stem. After that, the plant mass-steams were previously washed with distilled water and kept for several hours in 0.1 M HCl, in order to remove soil and mineral oxides from the surface. Then the plant mass was ground and dried in an oven at 80C. 1 g of sample was taken and poured with 20 ml of 60% HNO_3. A gentle boiling was performed for 2 hours. After cooling, 3 ml of H_2O_2 was added, and then boiling was performed for 15 minutes. The procedure with peroxide was repeated. After cooling, 2 ml of HClO4 was added and gentle evaporation was carried out until thick white fumes of perchloric acid appeared (Jones and Case, 1990). After cooling, 5 ml of 5M HCl was added, and then the samples were quantitatively transferred into normal 50 ml vessels. The vessels were filled to the final volume with distilled water. The solution was filtered through quantitative filter paper. The reading was performed by atomic absorption spectrophotometry (Varian Spectr AA 220FS apparatus), in an acetylene/air flame. The analysis of the obtained data was done with the statistical package STATISTICA 8 for Windows and SPSS Statistics 17.0.

Research results and discussions

Steam height

The Pobeda variety had an average height (42.40 cm), while the Ljiljana variety had a lower average height (39.66 cm). The average height of the steam per sample for the variety Pobeda varied from 34.00 to 50.60, and for the variety Ljiljana from 33.50 to 35.50 (tables 1 and 2).

Table 1: Stem height, variety Pobeda, cm

Variant	*Zn, Pb, Cr, Cu, Cd*	*LSD 5%*	*LSD 1%*
Control	*42,60*	*7,9889*	*14,6647*
250 ppm	*34,00*	*1,8371*	*3,3723*
500 ppm	*50,60*	*1,0625*	*1,9503*
Average	*42,40*	-	-

Table 2: Stem hight, variety Ljiljana, cm

Variant	*Zn, Pb, Cr, Cu, Cd*	*LSD 5%*	*LSD 1%*
Control	*50,00*	*1,8371*	*3,3723*
250 ppm	*35,50*	*5,1143*	*9,3881*
500ppm	*33,50*	*3,3119*	*6,0795*
Average	*39,66*	-	-

At a mixture concentration of 250 ppm, the height of the steam in the variety Ljiljana was 35.50 cm, while in Pobeda it was lower and amounted to 34.00 cm. When contaminated with a mixture of metals with a concentration of 500 ppm, the variety Ljiljana had a stem height of 33.50 cm, and the variety Pobeda was slightly taller at 50.60 cm.
Differences in stress-induced effects of elevated concentrations of toxic metals indicate different tolerance of plants to metals (Zhao et al., 2017).

The content of heavy metals in the stem of wheat

The content of heavy metals in the varieties Pobeda and Ljiljana varies greatly (tables 3 and 4).
According to their average content in the wheat stem of the Pobeda variety, the investigated heavy metals can be arranged in the following order:
• zinc > cadmium > copper > lead > chromium
The lead content of the Pobeda variety in the wheat stem is on average 4.13 mg kg -1 and varies from 2.46 mg kg -1 (control) to 5.45 mg kg -1.
According to their average content in the wheat stem of the Ljiljana variety, the examined heavy metals can be arranged in the following order:
• zinc > cadmium > copper > lead > chromium
The lead content of the Ljiljana variety in the wheat stem is on average 4.25 mg kg $^{-1}$ and varies from 2.14 mg kg $^{-1}$ (control) to 6.28 mg kg $^{-1}$.

The results of statistical processing (analysis of variance) show that the experimental factor-concentration of the mixture of heavy metals for contaminating the soil had a very significant influence on the content of heavy metals in parts of wheat, which can be seen on the basis of the calculated F-values.

Table 3: Content of heavy metals in the stem of wheat, variety Pobeda, mg kg-1

Variant	*Zn*	*Pb*	*Cr*	*Cu*	*Cd*	*Ftest*	*LSD 5%*	*LSD 1%*
Control	*19,39*	*2,46*	*1,23*	*4,49*	*0,65*	*38832,27***	*0,1197*	*0,1655*
250 ppm	*49,10*	*5,45*	*1,75*	*5,54*	*9,12*	*49105,52***	*0,2678*	*0,3703*
500 ppm	*122,77*	*4,49*	*0,64*	*8,14*	*27,00*	*56428,15***	*0,6525*	*0,9023*
Prosek	*109,41*	*4,13*	*1,21*	*6,05*	*12,26*	-	-	-

Table 4: Content of heavy metals in the stem of wheat, variety Ljiljana, mg kg-1

Variant	*Zn*	*Pb*	*Cr*	*Cu*	*Cd*	*Ftest*	*LSD 5%*	*LSD 1%*
Control	*29,99*	*2,14*	*0,58*	*4,65*	*0,39*	*38832,27***	*0,1197*	*0,1655*
250 ppm	*79,13*	*4,33*	*1,23*	*5,41*	*17,31*	*58501,43***	*0,4087*	*0,5652*
500ppm	*203,47*	*6,28*	*1,15*	*6,39*	*34,87*	*87931,24***	*0,8808*	*1,2181*
Prosek	*104,20*	*4,25*	*0,99*	*5,48*	*17,52*	-	-	-

Conclusion

Vegetation experiments in pots were performed with different concentrations of heavy metal mixtures. Increasing the concentration of the metal mixture had a negative impact on steam height. Higher concentrations of heavy metals significantly reduced plant growth in both cultivars.

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