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AGRONOMSKI FAKULTET U ČAČKU

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FACULTY OF AGRONOMY
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17–18 March 2023

Faculty of Agronomy in Čačak, University of Kragujevac, Serbia

- PROCEEDINGS -

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PREFACE

"The scientific man does not aim at an immediate result. He does not expect that his advanced ideas will be readily taken up. His work is like that of the planter - for the future. His duty is to lay the foundation for those who are to come, and point the way."

Nikola Tesla

Agriculture is a primary and strategic activity that ensures food security and food market stability, and protects living standards for people. We have witnessed that, in crisis situations in the world, agriculture has responded to its task as the main support in supplying the market with food products. The production of sufficient quantities of safe food enables the development of the working-age population that actively participates in the economic development of society. The specific conditions in which agriculture develops require economic support measures and subsidies from the state to preserve domestic agriculture. Planned investments in agriculture through the introduction of modern technologies and efficient organization in both production and trade reduce economic and market risks, thus enabling stable business conditions. The development of agriculture must be based on a multifunctional connection with other activities (food industry, trade, tourism, etc.)

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, traditionally organizes the Symposium on Biotechnology every year. This year marks the 28th anniversary of the Symposium. The main goal of the Symposium 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.

At the 1st International Symposium on Biotechnology, a total of 71 papers were presented in the fields of Field, Vegetable and Forage Crops, Pomology and Viticulture, Livestock Production, Plant Protection, Food Safety and the Environment, Food Technology 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 financial support and patronage to this Symposium, which they provide every year. We thank companies, entrepreneurs, stakeholders and all

long-time friends of the Faculty of Agriculture for their material and organizational support.

Doing agriculture, in addition to economy and business, is also a noble social activity, considering that it satisfies people's basic daily needs of food. Agricultural producers deserve reputation and respect in society and should be enabled to make a decent living from their work, and society should recognize this.

In Čačak, March 2023

Programme and Organizing Committee
1st INTERNATIONAL SYMPOSIUM
ON BIOTECHNOLOGY
(28th SYMPOSIUM ON BIOTECHNOLOGY
with international participation)

Faculty of Agronomy in Čačak
University of Kragujevac

is organizing

1st International Symposium on Biotechnology

17–18 March 2023, Čačak, Republic of Serbia

in cooperation with

Univerza v Ljubljani
Biotehniška fakulteta

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University of Banja Luka

Faculty of Agriculture
University of Novi Sad

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Section: Field, Vegetable and Forage Crops

Section: Applied Chemistry

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THE INFLUENCE OF HEAVY METALS ON THE DEVELOPMENT OF THE SURFACE OF WHEAT LEAVES

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Abstract: Heavy metals represent a very significant group of environmental pollutants because they are potential metabolic inhibitors. Therefore, a study was conducted where the height of the wheat stem, as well as the surface of the leaves in the budding and leafing stages, were examined when contaminated with a mixture of heavy metals in concentrations of 250 and 500 ppm. The aim of the research was to determine the influence of heavy metals on the initial phenophase of wheat varieties *Pobeda* and *Ljiljana*. An experiment was carried out in volumetric flasks where two different concentrations of a mixture of heavy metals 250 ppm and 500 ppm were added under controlled conditions.

Keywords: atomic absorption spectrophotometry, heavy metals, leaf, *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 reach 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 are increasingly becoming 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 native rocks. Also, the irrational use of organic and mineral fertilizers can lead to soil contamination. Toxic metals are absorbed by the plant through its roots from the soil, and from the atmosphere through its leaves (Mickovski-Stefanovć et al., 2012).

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The constant increase of the human population, and the construction of large cities, industrial facilities, and roads significantly reduce agricultural areas, but also with various harmful agents.

As these areas are closer to the main polluters of the ecosystem, the effects on cultivated plants become more pronounced. The biggest polluters of agricultural land and crops are heavy metals. Several heavy metals (Fe, Mn, Cu, Zn, and Co) in small amounts are necessary for the growth and development of plants, but they are toxic to plants in high concentrations. Heavy metals come from contaminated air and soil, where they can be found if plant protection products and organic and mineral fertilizers are used irrationally (Abrahams, 2002; Rajković et al., 2012).

It is difficult to imagine agriculture today and its further progress without the application of various types of pesticides (Knežević et al., 2011). The consequences of their excessive and uncontrolled use are closely related to the withdrawal period and reach the point of creating a potential risk to human health. The withdrawal period is the shortest prescribed time that must elapse from the last application of pesticides to the time of harvest and is indicated on the product (http://pinova.hr/hr_HR/katalog-proizvoda/sredstva-za-zastitu-bilja, access date: 02.09 .2017). Although pesticides are generally expected in foods of plant origin, they simply enter the entire food chain and remain there due to slow decomposition and then accumulate in certain tissues. Aerosols enter the body in the form of vapors, solids, and liquids through the skin, ingestion, or respiratory system (Valić, 2001).

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).

Material and 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 plant growth was carried out through experiments in the greenhouse of the Faculty of Agriculture in Zemun, where the 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 flasks 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 - $Zn(CH_3COO)_2 \times 2H_2O$, lead in the form of lead- acetate $C_4H_6O_4Pb \times 3H_2O$, chromium in the form of chromium trioxide-CrO_3, copper in the form of copper sulfate-$CuSO_4$ and cadmium in the form of cadmium nitrate-$Cd(NO_3)_2 \times 4H_2O$.

The following concentrations of solutions were used:

0 ppm(control), 250 ppm, 500 ppm

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 - leaves that 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 80̊C. 1 g of sample was taken and poured with 20 ml of 60% HNO_3. The mixture was brought to a boil for 2 hours. After cooling, 3 ml of H_2O_2 was added and then boiled for 15 minutes. The procedure was repeated with peroxide. After cooling, 2 ml of $HClO_4$ was added and mild 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 50 ml volumetric flasks. The flasks 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.

Results and discussion

Stem height

The *Pobeda* variety had a slightly lower average stem height (42.40 cm), while the *Ljiljana* variety had a higher average height (39.67 cm). The average stem height per sample for the *Pobeda* variety varied from 34.00 to 50.60, and for the *Ljiljana* variety from 33.50 to 50.00 (Tables 1 and 2).

Table 1: Stem height, *Pobeda variety*, 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 height, *Ljiljana variety*, 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
500 ppm	33.50	3.3119	6.0795
Average	39.67		

When contaminated with a mixture of metals with a concentration of 250 ppm, the *Ljiljana* variety had a stem height of 35.50 cm, and the *Pobeda* variety was 34.00 cm. When the contamination was carried out with a concentration of 500 ppm, the stem height of the *Ljiljana* variety was 33.50 cm, while that of *Pobeda* was 50.60 cm.

Inhibition of chlorophyll biosynthesis occurs if the plant is exposed to the toxic effects of lead for a long time (Ernst et al., 2000; He et al., 2014). If the lead concentration is higher than 300 ppm, photosynthesis and stomatal conductivity are disturbed (Fu and Wang, 2015).

Leaf surface

Research shows that on average the surface area of the leaves was higher in the *Pobeda* variety (13.40) compared to the *Ljiljana* variety (14.71). The *Pobeda* variety had the smallest leaf area (3.19) in the sample variant where the soil was treated with 250 ppm, while the largest leaf area was in the control sample (31.80) (tables 3 and 4).

Table 3: Leaf surface, *Pobeda variety*, cm

Variant	Zn, Pb, Cr, Cu, Cd	LSD 5%	LSD 1%
Control	31.80	10.1436	18.6201
250 ppm	3.19	0.0183	0.0336
500 ppm	5.23	0.1606	0.2948
Average	13.40		

Table 4: Leaf surface, *Ljiljana variety*, cm

Variant	Zn, Pb, Cr, Cu, Cd	LSD 5%	LSD 1%
Control	13.18	0.6010	1.1032
250 ppm	10.41	4.1425	7.6041
500 ppm	20.54	1.2327	2.2629
Average	14.71		

The research was carried out by setting up vegetation trials in flasks. The uptake of heavy metals in different types of vegetables (lettuce, radish, and carrot) was studied. The soils were contaminated with different concentrations of a mixture of heavy metals. An enormous accumulation of heavy metals occurred in the leaves of examined plants, namely the following metals: iron, zinc, lead, and cadmium. The determined values are above the permitted (MDK) quantities. Somewhat higher contents of iron, zinc, lead, and nickel were found in the tested garden soil samples, but they did not have a negative impact on plant development (Stevanović et al., 2001).

Depending on the chemical properties of the soil, the proportion of adopted heavy metals is different. Thus (Youseff and Chino, 1991) point out that the intensity of uptake of heavy metals is significantly reduced on soils with a pH of 7 and higher. The intake of metals through the leaves is about 10 times higher than through the roots from contaminated soil, with the most efficient uptake of zinc ions through the leaves. The uptake of lead ions from the soil was negligible and the conclusion is that this metal primarily reaches the plants through the leaves from the air.

Conclusion

Vegetation experiments in *v*olumetric flasks were performed with different concentrations of heavy metal mixtures. Increasing the concentration of the metal mixture had a negative effect on the number of leaves and leafy area. Higher concentrations of heavy metals significantly reduced plant growth in both varieties, as well as leaf area in the *Pobeda* variety.

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