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THE INFLUENCE OF MICROBIAL PREPARATION ON THE MIGRATION OF WATER SOLUBLE FORMS OF COPPER IN SOIL–PLANT SYSTEM AND GRAIN QUALITY OF WINTER WHEAT

Lyudmila Chaikovska[1,3], Olga Ovsienko[1], Marina Baranskaya[1], Valentina Klyuchenko[2], Nina Klimenko[2]

Address(es): Lyudmila Chaikovska,
[1]Research Institute of Agriculture of Crimea, Department of Agricultural Microbiology, str. Kyevskaya 150, 95000 Simferopol, Crimea, +7(978)852 18 63.
[2]Agroindustrial College of V.I.Vernadsky Crimean University, Studencheskaya str. 1, 97517 Simferopol district, v. Malenkoe, Crimea.
[3]Academy of Life and Environmental Science of V.I.Vernadsky Crimean University , 95000 v. Agrarnoe.

*Corresponding author: ludachaika@mail.ru

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ABSTRACT

One of the most dangerous and most widespread chemical pollutions of the biosphere are heavy metals (HM). Therefore, the assessment of their biogenic migration in the "soil–plant" system and the search for ways to reduce toxic effects on the environment are priority and relevant. The task of our researches consisted of the determination of influence the microbial preparation Phosphoenterin on the migration of water soluble forms of copper in the soil–plant system and grain quality (protein and gluten content) at growing winter wheat under the conditions of model field experiments. The quantitative content of water soluble forms of copper in soil samples (chernozem southern calcareous) and plants (roots, straw, grain) by atomic absorption analysis has been obtained. The content of gluten and protein in the grain of winter wheat was determined by infrared spectroscopy. The positive effect of the microbial preparation Phosphoenterin on the migration of water soluble forms of copper in the soil–plant system was established. In the rhizosphere of inoculated plants their accumulation decreased by 29-39 %, roots – 7-28 %, straw – 7-39 %, grain –- 6-40 % in comparison to the control variant. Correlation analysis showed the presence of direct, close, reliable relationships between the content of water soluble Cu compounds in the rhizosphere, roots, straw and winter wheat grain (r=0.89-0.98). The positive effect of presowing inoculation of seeds on the quality (protein and gluten content increased to 15% and 28% against 13 % and 24% on the control variant, respectively) of grain winter wheat, growing on a natural background has been showed.

Keywords: Phosphoenterin, winter wheat, water soluble copper compounds, rhizosphere

INTRODUCTION

Heavy metals (HM) play a priority role in modern environmental pollution processes. The ways of their arrival in natural and agroecosystems are different: emissions of factories, plants, vehicles, the use of plant protection chemicals, etc. As a rule, HM have high biochemical activity. Some of them play a significant role in metabolism, being important trace elements for animals and plants (Fe, Cu, Co, Mo, etc.). However, the organisms need for most of them is small and is expressed by micrograms, and in large quantity they become toxicants. The danger of HM is also compounded by their ability to accumulate in organisms and concentrate in food chains. As a result, HM, in particular copper, accumulate in the soil and hydrosphere, and also migrate to plants grown in contaminated areas. To reduce the toxic effect of HM in agrophytocenosis is possible in the transition to biological farming, one of which methods is the use of environmentally friendly microbial preparations (**Tikhonovich *et al.*, 2011; Zavalin *et al.*, 2010**). It is known, that the economically useful microorganisms, included in their composition, improve the growth and nutrition of crops, and also have protective properties against pests and stress (**Belimov, 2008; Belimov *et al.*, 2011**). Thus, a number of researchers have shown the ability of microbial agents to convert plant-toxic forms of HM into less toxic insoluble complexes (**Arkchipova *et al.*, 2004; Belogolova *et al.*, 2011, 2013**). It is known that some associative bacteria have the ability to significantly improve the condition of plants on soils contaminated with TM. This is due to their excretion of growth substances that accelerate the development of plants and enhance their absorption of nutrients compounds (**Dodd *et al.*, 2010**). In addition, bacteria can mobilize of HM ions and prevent their accumulation in plant cells (**Safronova *et al.*, 2006**).
The task of our researches consisted to determination of the effect of microbial preparations (for example Phosphoenterin) on the migration of water soluble forms of copper in the soil-plant system and qualitative indices of grain (protein and gluten content) at the cultivation of winter wheat in the conditions of model field experiments.

MATERIALS OF RESEARCHES

Field experiments were conducted at the experimental plot of the Crimean Agroindustrial College (Simferopol district) in 2012-2014. The Soil – Chernozem southern calcareous. Agrochemical characteristics of soil: humus content – 2.5%; mobile forms of nitrogen and phosphorus – 5.3 and 2.6 mg/100 g of soil, respectively; pH of water extraction – 7.0-7.2. The area of the cultivated plots was 10 m^2, accounting – 5 m^2, placed randomly; repetition of experiment fourfold. In early spring, $CuSO_4$ solution was introduced into the soil from calculations (according to Cu content) corresponding to the pollution levels: 5, 10 and 20 MPC (for soils 6 mg/kg). In the control variants, no $CuSO_4$ solution was introduced. Microbial preparation Phosphoenterin, created on the basis of phosphate mobilizing bacterium *Enterobacter nimipressuralis* 32-3 (**Chaikovska, 2011; Chaikovska *et al.*, 2006, Chaikovskaya *et al.*, 2017**), was used for presowing inoculation of wheat seeds; the seeds were moistened with water in the control.

METHODS OF RESEARCHES

Determination of the quantitative content of water-soluble forms of copper in soil and plants (grain) was carried out by atomic absorption analysis according to the guidelines of GOST (**GOST, 1998, 2010; RD, 1991**). The content of gluten and protein in grain is determined by infrared spectroscopy (**DSTU, 2007**). Field experiments and statistical processing of the obtained data were carried out in accordance with the generally accepted methods (**Dospekhov, 1985**) and the program Statistica 7.0 (**Bureeva, 2007; Meshalkina 2008**).

RESULTS AND DISCUSSION

The analysis of the results showed a small background content of water-soluble forms of copper in the soil of experimental plots: it did not exceed the permissible values of maximum permissible concentration (MPC) for soils and

constituted 0.5-0.6 mg/kg. It is necessary to note, that these data are in accordance with the results established by other authors for arable soils of the Crimea (**Sychevskiy *et al*., 2012**).

Our results is evidence, that depending on the level of contamination of soil, in the rhizosphere of wheat accumulated a significant amount of water-soluble compounds of copper: in the range of 18.6-97.6 mg/kg. Thus, by contamination of the soil at 5, 10 and 20 MPC, their content in the rhizosphere amounted to 18.6, 45.4 and 97.6 mg/kg, respectively. Results are shown in the figure 1.

It was found, that the use of Phosphoenterin for presowing inoculation of wheat seeds allowed to reduce the concentration of water-soluble forms of Cu in the rhizosphere of inoculated plants in comparison to the control variant. Thus, in according to the results, their contents decreased by 29-39% against control at every level of soil pollution and amounted to 13.1, 30.2 and 64.2 mg/kg at 5, 10 and 20 MPC, respectively.

It is known that for migration of HM in the soil–plant system is influenced by many factors: physical and chemical properties of soils, their biological activity, as well as physiological mechanisms of different plant species. These factors may determine the general form and ability of HM transfer in the soil–plant system (**Zheng *et al*., 2015**). In the literature there is information, that the primary source of HM in wheat plants are heavy metals contained in the soil (**Wu *et al*., 2013; Xue *et al*., 2014**).

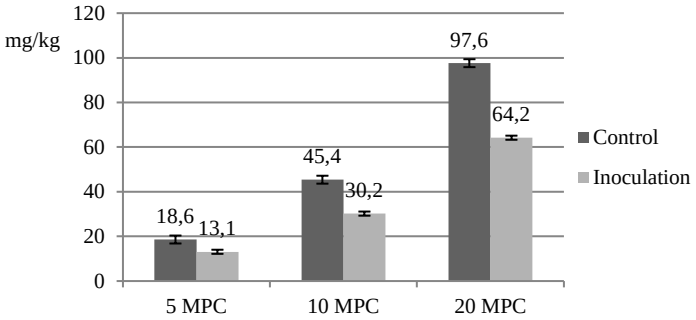

Figure 1 Content of water soluble copper compounds in the rhizosphere of winter wheat (mg/kg)

The results of our studies showed, that in the roots of plants of the control variant growing in the background area, the content of water-soluble compounds Cu was 6.0 mg/kg, and in the roots of plants grown on plots contaminated TM – varied within 7.3-31.9 mg / kg (Tab. 1). It was found that the accumulation of water-soluble forms of copper in wheat straw was significantly less than in the roots (3.0-3.8 mg/kg): twice – in the background areas and with pollution at the level of 5 MPC. At high levels of soil contamination (10 MPC and 20 MPC), the content of water-soluble Cu compounds in straw reached 4.4-8.9 mg/kg: 3-4 times less than in wheat roots.

Table 1 Content of water-soluble copper compounds in winter wheat phytomass (mg / kg)

Variants	Roots	Straw
Background (less HM)		
Control	6.0	3.0
Inoculation	5.1	2.7
LSD_{05}	2,60	2,35
5 MPC		
Control	7.3	3.8
Inoculation	6.2	3.1
LSD_{05}	1,96	1,69
10 MPC		
Control	20.1	4.4
Inoculation	18.5	4.1
LSD_{05}	4,97	1,72
20 MPC		
Control	31.9	8.9
Inoculation	23.0	5.5
LSD_{05}	8,98	1,35

Legend: LSD_{05} –Least Significant Difference at 5% significance level

The use of Phosphoenterin for seed-presowing inoculation of wheat contributed to the reduction of water-soluble copper compounds not only in the rhizosphere, but also their migration to the roots and straw. Thus, the accumulation of Cu compounds in the roots of inoculated plants decreased by 7-28%, in straw – by 7-39% in comparison to the control variant (plants grown from seeds without inoculation).

According to the results of our research, the content of water-soluble copper compounds in the rhizosphere of winter wheat on natural background and plots with different levels of soil pollution significant fluctuations has been reached. However, despite this, their accumulation in the grain was within 1.0-5.2 mg/kg, which did not exceed the MPC (for grain 10 mg/kg). It should be noted the positive influence of Phosphoenterin on the content of water-soluble compounds of Cu in the grain of inoculated plants: it was lower than in the control (by 6-40%, depending on the variant) and varied within 0.6 - 4.9 mg/kg (Figure 2).

The results of our research confirmed the data obtained by other authors. Thus, in the study of TM accumulation in various parts of winter wheat was founded the largest content of them to accumulated in the roots and the least – in the grain (**Wang *et al*., 2016**).

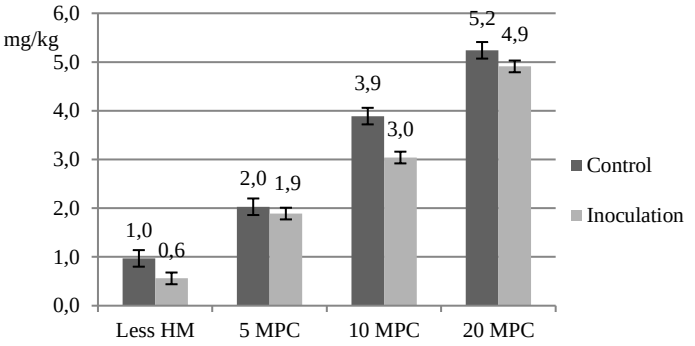

Figure 2 Content of water-soluble copper compounds in winter wheat grain (mg / kg)

The correlation analysis to determine the dependence of the content of water-soluble forms of copper in the rhizosphere and various parts of winter wheat plant has been carried. The obtained data showed (Tab. 2), that in variants without using Phosphoenterin a direct, close and reliable relationship between Cu content in soil, roots, straw and grain of winter wheat has been established (r=0,89-0,98).

Table 2 Correlation coefficients between indices of water-soluble Cu compounds in the soil and in the investigated parts of plants of winter wheat (without the use of Phosphoenterin)

	Soil	Roots	Straw	Grain
Soil	-			
Roots	0,98	-		
Straw	0,97	0,93	-	
Grain	0,97	0,98	0,89	-

The use of the microbial preparation Phosphoenterin for seed-presowing inoculation contributed to the reduction of the content of water-soluble copper compounds in the rhizosphere and various parts of winter wheat, which is also proved by the direct close connection in the correlation analysis (Tab. 3).

Thus, it can be maintain, that with increase in the Cu content in the rhizosphere of winter wheat, occurred increase of content this element in the rest parts of wheat plants both with and without inoculation.

Table 3 Correlation coefficients between the content of water-soluble Cu compounds in the soil and the studied parts of the plant (presowing inoculation)

	Soil	Roots	Straw	Grain
Soil	-			
Roots	0,94	-		
Straw	0,98	0,95	-	
Grain	0,99	0,94	0,97	-

Thus, the correlation analysis showed the presence of direct, close, reliable relationships between the content of water-soluble Cu compounds in the rhizosphere, roots, straw and winter wheat grain (r=0.89-0.98). The positive effect of the microbial preparation Phosphoenterin (presowing inoculation of winter wheat seeds) on the migration of water-soluble forms of Cu in the soil-plant system was established. In the rhizosphere of inoculated plants their accumulation decreased by 29-39%, roots-by 7-28%, straw – by 7-39%, grain – by 6-40% in comparison to the control variant.

It is known, that one of the most important indicators of grain quality is the content of protein and gluten. The results indicate that use of Phosphoenterin for seeds-presowing inoculation contributed to the increase in protein content in wheat grain, growing in plots of natural background: up to 15.2% against 13.4% in the control (Tab. 4). There were no significant effects of seed-presowing inoculation on protein content in wheat grain, growing on contaminated $CuSO_4$ plots.

Table 4 - Effect of inoculation on quality indicators of winter wheat grain at different levels of soil pollution

Variants	Contents, %	
	Protein	Gluten
Control (less HM)		
Control	13.4	23.9
Inoculation	15.2	28.4
HM (5 MPC)		
Control	12.8	25.0
Inoculation	12.7	25.3
HM (10 MPC)		
Control	12.0	24.8
Inoculation	12.9	28.2
HM (20 MPC)		
Control	12.8	25.4
Inoculation	12.5	25.1

The positive effect of presowing inoculation of seeds on the increase of gluten content in winter wheat grain grown on the natural background plots also has been established: up to 28.4% against 23.9% on the control variant. In addition, it is necessary to note the positive impact Phosphoenterin on the amount of gluten in wheat grain when the soil contamination at the level of 10 MPC: it increased to 28.2% (against 24.8% in the variant without inoculation).

Thus, the positive effect of presowing inoculation of seeds on the main indicators of quality of winter wheat grain (protein and gluten content increased to 15.2% and 28.4% against 13.4% and 23.9% under control, respectively), growing on the natural background areas. There was no significant influence of presowing inoculation of seeds on the protein and gluten content in wheat grain grown on contaminated $CuSO_4$ plots.

According to obtained results, the use of the microbial preparation Phosphoenterin also showed a positive effect on the grain productivity of winter wheat: it increased on average in three years compared to the control by 0.16 t/ha (6.4%) in the natural background areas and by 0.25-0.38 t/ha (12% -22%) in soil contamination plots.

CONCLUSIONS

1. The positive effect of the microbial preparation Phosphoenterin on the migration of water soluble forms of Cu in the soil–plant system at cultivated of winter wheat under the conditions of model field experiments has been established. The accumulation of water soluble forms of Cu in the rhizosphere of inoculated plants decreased by 29-39 %.
2. The correlation analysis showed the presence of direct, close, reliable links between the content of water soluble Cu compounds in the rhizosphere, roots, straw and winter wheat grain (r=0.89-0.98).
3. The positive influence of Phosphoenterin on grain quality indicators of winter wheat showed: protein and gluten contents increased to 15% and 28% against 13% and 24% in control variant accordingly.

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