Bulgarian Journal of Agricultural Science, 13 (2007), 333-339
National Centre for Agrarian Sciences

Influence of Soil Copper Pollution and *Fusarium culmorum* on the Heavy Metal Content in Wheat

I. BOGOEVA[1], E. BONCHEVA[1], R. STEFANOVA[2] and B. BLAJEV[2]
[1]*Plant Protection Institute, BG - 2230 Kostinbrod, Bulgaria*
[2]*Central Laboratory for Chemical Testing and Control, BG -1330 Sofia, Bulgaria*

Abstract

BOGOEVA, I., E. BONCHEVA, R. STEFANOVA and B. BLAJEV, 2007. Influence of soil copper pollution and *Fusarium culmorum* on the heavy metal content in wheat. *Bulg. J. Agric. Sci.,* 13: 333-339

A greenhouse experiment in pots was carried out in order to assess the effect of combination of stress factors – copper pollution and *Fusarium culmorum* pathogen on the bioavailability of heavy metals in contaminated soils and their uptake from agricultural plants. Cu ions were introduced into the soil as a solution of $CuCl_2.2H_2O$ in three concentrations: 150, 400 and 600 $mg.kg^{-1}$. The soil was artificially inoculated with *Fusarium culmorum* pathogen. Wheat (*Triticum aestivum*) plants were grown at the phenophase "5-6 leaf". Soil samples were analyzed for determination the content of elements: Pb, Fe, Zn, Cu, Cd, Mn. An ICP technique was used for determination of total and mobile forms of heavy metals in the soil, before planting the seeds. Concentration of the same elements was measured also in the stems and roots of investigated plants, after the harvesting.

A higher concentration of all determined elements was established in the roots of wheat, compared to those, in the stems. A substantial change of the heavy metal content in the infected and uninfected with *F. culmorum* plants was not determined.

Key words: Copper pollution, heavy metals, total and mobile forms of elements, pathogens, *Fusarium culmorum*, wheat

*E- mail: irenbog@yahoo.com

Introduction

Heavy metal pollution of agricultural lands and crops is a significant problem in all developed countries, including Bulgaria in this number too. In spite of strongly diminished production of industrial wastes the problem still persists, because the heavy metals accumulated from the previous years remained in the surface layer of the soil. This point is a widely recognized and studies on the harmful effects caused by heavy metals on crop plants are receiving increased attention (Ciscato et al., 1997; Cook et al., 1997; Maksymiec, 1997; Angelova et al., 2003; Anguelova et al., 2004). It takes a long time to extract them using some plants and the most important thing is the right choice of the grown agricultural crops (Zupan et al., 1997; Nvari-Izzo, 2001; Vassilev, 2002; Barocsi et al., 2003; Kos et al., 2003; Hajiboland, 2005). According to some authors (Angelova et al., 2003) the cereal crops can be divided into two groups which differ considerably in their ability to accumulate heavy metals: (i) moderate accumulator - barley, rye, triticale and (ii) high accumulator – wheat.

Elevated concentrations of heavy metals in soil may cause phytotoxicity, direct hazard to human health, indirect effects due to transmission through the food chain or contamination of ground- or surface-waters (Clijsters et al., 1999; Cuypers et al., 1999; Berglund et al., 2002; Quartacci et al., 2003). Heavy metals cannot be degraded or destroyed, but it is possible to alter their chemical form and change their solubility in water and hence availability to plants (Pulford et al., 2002; Nakova, 2002).

One of the widespread fungal pathogens in Bulgaria is *Fusarium* pathogen. They cause significant diseases in the cereal crops. To the most important species belongs *F. culmorum* and *F. graminearum*, because of their high invasiveness to the crops (Schachermayr and Fried, 2001). Almost all cereal species as wheat, triticale, rye, barley, oat and corn may be attacked and wheat is considered the most susceptible species to *Fusarium*. *Fusarium* disease in cereals may result in quantitative /reduced grain weight and number of grains per spike/ and qualitative /decreased protein, starch and gluten contents/ yield losses. The disease also diminishes seed quality. *Fusarium* fungi attacking cereals are able to produce numerous mycotoxins as secondary metabolites (Kononenko et al., 1999; Sokolova et al., 1999).

Copper is an essential micronutrient element, which plays a significant metabolic role, but in high doses it can be toxic for the plants. What is the correlation of elevated Cu concentrations in the soil with the other toxic elements is one of the main objects of this study. An investigation of the interaction between heavy metal pollution and *Fusarium* infection also would be interesting, especially for the agricultural practice.

Materials and Methods

A greenhouse experiment in pots was carried out for two years replication in order to assess the effect of combination – copper pollution and pathogen *Fusarium culmorum* on the bioavailability of heavy metals in contaminated soils and their uptake from agricultural plants. An alluvial-meadow type of soil, pH-6.6, and humus content 3.8 % $mg.kg^{-1}$ was used for the investigation. The soil was air-dried, sieved trough 2 mm sieve and sterilized at 60°C.

Cu ions were introduced into the soil as a solution of $CuCl_2.2H_2O$ in three concentrations: 150, 400 and 600 mg.kg-[1] and left for 20 days for stabilization. An uncontaminated soil was used for growing of control plants. At the end of this period the soil was infected with *Fusarium culmorum*. This pathogen was used for the experiment because of it's higher aggressiveness to the crops in comparison with *F. graminearum* (Bogoeva and Karadjova, 2005). The infection was preliminary grown on barley grains at a temperature 18-25° C. The inoculum was introduced into the soil in a quantity of 4 g.kg-[1]of soil, left for development at a temperature 20-25° C and kept wet. After pathogen's development (10–15 days later) the soil was cropped with seeds of wheat (*Triticum aestivum*) "Sadovo-1" cultivar. The experiment was prepared in two replications. During the vegetation cultures were grown at a t 20-25° C and soil humidity was maintained by watering with distilled water. Plants were grown at the phenophase "5-6 leaf" when the experiment was finished. Two parallel plant and soil samples from each variant were taken.

Analytical methods

The soil was analyzed for determination the content of metals (Pb, Cd, Fe, Zn, Cu, Mn). Soil samples (5.0 g) were air-dried, homogenized in a mortar and sieved. A fraction with particle size < 1 mm was taken for analysis. Total metal concentration was determined following HNO_3/HCl, (2.5: 7.5 ml) digestion for 3 hours over sand bath. After cooling it was transferred into a 50 ml flask and 0.1 N HCl was added to the mark. Mobile forms of metals in soil were analyzed after extraction with EDTA, following the procedure described from Trierweiler and Lindsay, 1969. After washing in water and distilled water, plants were dried and prepared for analyses. Heavy metal content in the plant material (1.0 g from infected wheat plants) was determined using the method of dry mineralization at 450°C until ashing. After cooling to a room temperature the ash was digested with 1 ml conc. HNO_3, evaporated in a sand bath. The procedure was repeated until the ash was white. It was finally dissolved in 0.2 M HNO_3 and transferred into a 25 ml flask.

An ICP-AES ("Liberty II" – Varian) technique was used for determination of elements. The working wave lengths were as follows: Cu – 324.8 nm, Pb – 220.4 nm, Cd – 228.8 nm, Fe – 259.9 nm, Zn – 213.9 nm, Mn – 257.6 nm. A commercial multielement standard solution (Merck) with concentration 100 mg/L was used as a stock solution. Total and mobile forms quantity of heavy metals was measured in the soil, before planting the seeds. Concentration of the same elements was estimated also in the stems and roots of investigated plants, after the harvesting.

Results and Discussion

The represented results are an average from 6 repetitions of analyze.

1. Soil analyses

Two forms of heavy metal ions were determined in the soil: total and mobile.

1.1 Total forms: an increase in the Cu content was established in a correlation with the enhancement of the applied dose. A substantial elevation of Fe content was influenced in the variants, treated with higher copper quantities. The content of the other measured elements was not remarkably changed in all experimental variants (Table 1).

1.2 Mobile forms: their concentrations were lower, compared to those of the common forms. Content of Cu mobile forms was increased with the growth of the applied metal quantity; concentration of Zn was slightly elevated with the enhancement of the applied copper dose. Not a substantial decrease in the content of Fe was observed, depending on the introduced Cu amount (Table 2).

2. Plant analyses

2.1. Element content in the stems (Table 3): Considerable differences in the distribution of the metals in the separate parts of the plants were ascertained. As a general tendency was determined that heavy metal quantity was at a lower level in the stems, compared to those in the roots of wheat. About the main observed element – copper, it‘s accumulation was in a very small concentration, comparing to the roots. A substantial difference between copper quantity in the infected and uninfected plants was not established. A tendency of elevation was observed in the concentration of Fe and Zn – they were increased in accordance with enhanced Cu application in the both variants – infected and uninfected plants. Mn presence was determined to be lower, compared to those in the roots. It‘s quantity was not in a correlation with the applied

Table 1

Element content - common forms in the soil, mg.kg^{-1}

Variant	Cu	Fe	Cd	Mn	Pb	Zn
	x ± SD	x ± SD	x ± SD	x ± SD	x ± SD	x ± SD
Cu = 0 /Control/	73.6±1.3	23 211±46.4	1.03±0.01	1 165±2.33	12.7±0.24	128.0±0.001
Cu=150	255.0±0.26	18 054±18.1	1.17±0.02	1 155±2.31	14.7±0.68	142.0±0.14
Cu=400	435.0±5.66	37 812±37.8	1.31±0.04	1 112±1.11	11.5±0.32	109.0±0.33
Cu=600	665.0±5.32	37 858±37.8	1.48±0.02	1 121±2.24	22.2±0.24	123.0±0.37

x – average value (mg/kg) from 6 repetitions; SD – mean standard deviation

Table 2

Element content - mobile forms in the soil, mg.kg^{-1}

Variant	Cu	Fe	Cd	Mn	Pb	Zn
	x ± SD	x ± SD	x ± SD	x ± SD	x ± SD	x ± SD
Cu = 0 /Control/	11.1±0.28	9.29±0.01	0.144±0.003	21.4±0.04	1.86±0.02	16.3±0.05
Cu=150	58.8±0.13	7.26±0.05	0.257±0.008	22.9±0,07	1.73±0.01	22.9±0.07
Cu=400	156.0±0.62	8.64±0.03	0.098±0.001	19.4±0.04	1.63±0.01	24.2±0.05
Cu=600	223.0±0.84	5.47±0.02	0.194±0.003	18.5±0.08	1.31±0.02	25.3±0.03

x – average value (mg/kg) from 6 repetitions; SD – mean standard deviation copper dose. Pb content in the stems and in the roots was in a very low quantity, and almost equivalent in the both plant parts. Cd concentration was measured to be insignificant.

2.2. Element content in the roots (Table 4): A higher concentration of all measured elements was established in the

Table 3

Element content in the stems of wheat, mg.kg^{-1}

Variant	Cu	Fe	Cd	Mn	Pb	Zn
	x ± SD	x ± SD	x ± SD	x ± SD	x ± SD	x ± SD
Pat - 0						
Cu = 0 /Control/	3.35±0.01	107.0±2.13	0.845±0.025	125.0±0.25	4.73±0.07	38.2±0.19
Cu=150	5.56±0.05	146.0±0.73	0.707±0.013	99.8±0.60	4.74±0.15	86.6±0.09
Cu=400	17.3±0.12	164.0±0.16	0.742±0.025	120.0±0.36	5.17±0.11	82.3±0.16
Cu=600	18.3±0.07	255.0±1.28	0.822±0.003	156.0±0.16	3.98±0.14	122.0±0.12
Pat +						
Cu=0	2.49±0.03	138.0±0.97	0.825±0.017	143.0±0.72	4.65±0.04	49.5±0.05
Cu=150	6.33±0.01	195.0±0.78	0.724±0.004	159.0±0.16	4.56±0.10	83.4±0.17
Cu=400	10.3±0.01	202.0±0.61	0.757±0.008	158.0±0.47	4.26±0.10	121.0±0.61
Cu=600	20.2±0.14	287.0±0.57	0.926±0.013	163.0±1.14	3.98±0.03	164.0±0.16

x – average value (mg/kg) from 6 repetitions; SD – mean standard deviation

Table 4

Element content in the roots of wheat, mg.kg^{-1}

Variant	Cu	Fe	Cd	Mn	Pb	Zn
	x± SD	x± SD	x± SD	x± SD	x± SD	x± SD
Pat - 0						
Cu = 0 /Control/	22.2±0.09	2519±10.1	1.12 ±0.036	350.0±1.75	5.94±0.13	396.0±0.40
Cu=150	88.9±0.53	2895±14.5	2.46 ±0.005	311.0±0.62	5.95±0.12	442.0±0.44
Cu=400	187.0±0.94	2075± 4.2	2.22 ±0.004	398.0±0.80	4.68±0.06	310.0±0.62
Cu=600	306.0±1.22	2705±10.8	2.41 ±0.012	363.0±2.90	5.67±0.12	490.0±0.98
Pat +						
Cu=0	13.1±0.11	1918± 3.4	2.59 ±0.013	255.0±0.26	4.45±0.08	201.0±1.00
Cu=150	91.0±0.46	2585±10.3	0.793±0.006	267.0±1.34	4.89±0.10	239.0±0.48
Cu=400	185.0±0.93	2758± 5.5	0.976±0.025	255.0±1.28	4.94±0.18	250.0±0.75
Cu=600	242.0±0.24	2884± 2.9	1.30 ±0.036	305.0±1.53	5.37±0.08	303.0±0.30

x – average value (mg/kg) from 6 repetitions; SD – mean standard deviation roots of the plants. Cu accumulation was increased with the enhancement of the applied dose in the soil. A tendency of increase in Fe presence was observed with more copper application in the soil. Higher availability of Mn was measured in the roots, compared to the stems. Elevated concentration of Zn with increased copper application and in comparison with the stems was determined. Insignificant amount of Cd was also measured.

Conclusions

As a general tendency was determined that heavy metal quantity was at a lower level in the stems, compared to those in the roots of wheat. A higher concentration of all measured elements was established in the roots of the plants. Their quantities in the stem of the studied crop were considerably lower compared to the root system which showed that their movement through the conductive system was strongly restricted. The accumulation of the heavy metals in the vegetative organs of the studied crops differed significantly. A substantial difference in Cu and other heavy metals accumulation was not determined between infected and uninfected plants. Based on this result we can conclude that *F. culmorum* pathogen did not influence element uptake in wheat.

Content of the mobile forms of all measured elements in the soil was considerably lower in comparison with the common forms. Cu concentration was determined to be in a correlation with the applied dose into the soil.

References

Angelova, V., R. Ivanova and K. Ivanov, 2003. Uptake and distribution of Pb, Zn, and Cd in cereal crops, grown in industrially polluted region. *Bulg. J.Agric. Sci.,* **9:** 665-672.

Anguelova, I., G. Anguelov, I. Atanassov and N. Bailey, 2004. Heavy metal accumulation in biomass of some crop plants. *Bulg. J.Agric. Sci.,* **10:** 107-112.

Barocsi, A., Z. Csintalan, L. Kocsany, S. Dushenkov, J. Kuperberg, R. Kucharski and P. Richter, 2003. Optimizing Phytoremediation of heavy metal contaminated soil by exploiting plant's stress adaptation. *Int. J. Phytoremediation,* **5 (**1): 13-23.

Berglund, A., M. Quartacci, L. Calucci, F. Navari-Izzo, C. Pinzino and C. Liljenberg, 2002. Alterations of wheat root plasma membrane lipid composition induced by copper stress result in changed physicochemical properties of plasma membrane lipid vesicles. *Biochimica et Biophysica Acta,* **1564:** 466-472.

Bogoeva, I. and J. Karadjova, 2005. Influencce of copper ions on *Fusarium* pathogens in wheat. Sci.Conference with Int.participation, St.Zagora, June 2-3, pp.198-201.

Ciscato, M., R.Valcke, K. Van Loven, H. Clijsters and F. Navari-Izzo, 1997. Effect of in vivo copper treatment on the photosynthetic apparatus of two *Triticum durum* cultivars with different stress sensitivity. *Physiologia Plantarum,* **100**: 901-908.

Clijsters, H., A. Cuypers and J. Vangronsveld, 1999. Physiological responses to heavy metals in higher plants; defence against oxidative stress. *Verlag der Zeitschrift fur Naturforschung*, **54:** 730-734.

Cook, C., A. Kostidou, E. Vardaka and T. Lanaras, 1997. Effect of copper on the growth, photosynthesis and nutrient concentrations of Phaseolus plants. *Photosynthetica,* **34** (2): 179-193.

Cuypers, A., J. Vangronsveld and H. Clijsters, 1999. The chemical behaviour of heavy metals plays a prominent role in the induction of oxidative stress. *Free Rad. Res.,* **31:** 39-43.

Hajiboland, R., 2005. An evaluation of the efficiency of cultural plants to remove heavy metals from growing medium. *Plant Soil Environ.,* **51** (4): 156-164.

Kononenko, O., Z. Malinovskaya, N. Soboleva, E. Tiriazeva, E. Zotova, O. Shalyganova, 1999. Distribution and production of toxins by fungy from the genus Fusarium from Moscow region. *Mikologiya i Fitopatologiya,* **33** (2): 118-124.

Kos, B., H. Greman and D. Lestan, 2003. Phytoextraction of lead, zinc and cadmium from soil by selected plants. *Plant Soil Environ.,* **49** (12): 548-553.

Maksymiec, W., 1997. Effect of copper on cellular processes in higher plants. *Photosynthetica,* **34:** 321-342.

Nakova, R., 2002. Composition of elements in bean leaves and soil treated with herbicide escort. *Scientific Conference with International Participation*, St.Zagora, **2:** pp. 122-125.

Navari-Izzo, F. and M. Quartacci, 2001. Phytoremediation of metals. Tolerance mechanisms against oxidative stress. *Minerva Biotec.,* **13**: 73-83.

Pulford, I., D. Riddell-Black and C. Stewart, 2002. Heavy metal uptake by Willow clones from sewage sludge-treated soil: the potential for phytoremediation. *Int. J. of Phytoremediation,* **4** (1): 59-72.

Quartacci, M., E. Cosi, S. Meneguzzo, C. Sgherri and F. Navari-Izzo, 2003. Uptake and translocation of copper in *Brassicaceae*. *J. Plant Nutrition,* **26** (5): 1065-1083.

Sokolova, O., V. Pavlova, Z. Dorophееva, O. Deviatkina and V. Kozhuchovskaya, 1999. Effect of Fusarium graminearum toxigenicity of *Fusarium* head blight of winter wheat. *Mikologiya i Fitopatologiya,* **33** (2): 125-129.

Schachermayr,O. and T. Fried, 2001. Fusarium head blight of cereals in Switzerland. *Revue Suisse of Agriculture,* **32** (6): 241-246.

Trierweiler, J. and W.Lindsay, 1969. EDTA-Ammonium carbonate soil test for zinc. *Soil Sci.Soc.Amer.Proc.,* **33**: 49-54.

Vassilev, A., 2002. Metal phytoextraction; state of the art and perspectives. *Bulg. J.Agric. Sci.,* **8:** 125-140.

Received January, 13, 2007; accepted April, 23, 2007.