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Agriculture and Environment

Effect of experimentally polluted water on the stomatal characteristics on the leaves of two varieties of *Triticum aestivum* L. grown on different soil types

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Abstract. *Two varieties of wheat were grown on different soil types (chernozem carbonate and humus-carbonate). The influence of experimentally polluted water was investigated on the stomatal characteristics on the leaves* of the *two varieties, and copper, ammonium and magnesium sulfate had inhibitory effect. Wheat variety "Yantar" had greater dimensions of the stomata, than variety "Sadovo" in the same soil. Variety "Sadovo" grown on carbonate chernozem had larger stomata compared to the same in humus-carbonate soil.*

Keywords: polluted water, soil, stomata, *Triticum aestivum*

Introduction

Heavy metal pollution is a serious problem worldwide. Low doses of them are necessary for mineral nutrition of the plants, but elevated concentrations become toxic (Bogoeva, 2008). Increased concentrations of heavy metals in soil may cause phytotoxicity: suppression of growth, reducing crop productivity and ultimately - death of plants (Parekh et al., 1990; Steffens, 1990; Verkleij and Schat, 1990; Chlopecka, 1996; Dospatilev et al., 2007). Many studies find that the sensitivity of plants on the influence of toxic ions in the soil depends on the soil type, and particularly on soil pH and concentration of nutrients (Bojarczuk et al., 2002). Elements such as Mn, Cu, Zn, Fe are necessary for living organisms, but become toxic when concentrations exceed the limits. Accumulation of heavy metals in plants provokes growth retardation, reduced photosynthetic activity, wilting and chlorosis, and ultimately a low yield (Bogoeva, 2008). Many authors prove that toxic ions of aluminium, zinc, copper, lead, cadmium are common environmental pollutants and they are often accumulated in the degraded soils of industrial regions (Bojarczuk et al., 2002; Zaprjanova et al., 2010). The data about the localization of heavy metals and their effect on the plant's anatomy and on tissue differentiation are scanty (Setia and Bala, 1994; Shaw, 1995; Mor et al., 2002).

In the bioindication, the mainly used anatomical indices are these of the leaves: thickness of cuticle, alterations in stomatal apparatus, system of intercellular spaces and veins (Ilykun, 1978; Nikolaevskiy, 1979). In the national strategy of monitoring (1999), the stomatal method is included in the group of the rapid methods.

The aim of this study is to investigate the influence of experimentally polluted water on the stomatal characteristics on the leaves of two varieties of wheat, grown on different soil types (chernozem carbonate and humus-carbonate).

Material and methods

Grains of wheat variety „Sadovo"and „Yantar" were planted in vessels on humus-carbonate soil and carbonate chernozem soil. To trace the anatomical response of pollutants on plants parallel to the control (distilled water) they are watered with different polluted water. About variety „Sadovo" with soil carbonate chernozem, parallel to the control plants are watered with $(NH_4)_2SO_4$ with concentration 600mg / l (Nh 600); $CuSO_4$ with concentration 300mg / l (Cu 300). About variety „Sadovo" with humus-carbonate soils (parallel to the control) plants are watered with $MgSO_4$ with concentration 600mg / l (Mg 600); $CuSO_4$ with concentration 600mg / l (Cu 600). About the variety „Yantar" with humus-carbonate soils (parallel to the control) plants are watered with $MnSO_4$ with concentration 300mg / l (Mn 300); $CuSO_4$ with concentration 300mg / l (Cu 300). At the end of the trial (45 days), plants were fixed in 70% C_2H_5OH. A modern-epidermal stomatal analysis of the leaf lamina is studied. The selection of the epidermal-stomatal features is based on their ecological plasticity and their ability to bring quantitative information about the metric features in mathematical language. From fixed with 70% alcohol sheet material, semi microscopic preparations are prepared. The following stomatal features on both leaf surfaces (Dorsal, Ventral) are analyzed: length of stomata (L), width of stomata in the polar parts (D), width of stomata in the equatorial part (D1).

Anatomical studies of the epidermis are made by microscope Pzo War chara, with lens 40 and a screw micrometer eyepiece.

Statistica 7 for Windows was used to analyze the data for statistical significance of differences among the evaluated parameters.

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Results and discussion

Analysis of stomatal parameters of the variety „Sadovo" with soil carbonate chernozem.

Applied $(NH_4)_2SO_4$ (Nh 600) and $CuSO_4$ (Cu 300) have a negative impact on the anatomical structure of wheat leaves compared to the control. The negative influence is higher mainly concerning the length of the stomata on both dorsal and ventral leaf surfaces (Table 1). The reduction of the L stomata is statistically significant at P<0,05. Treatment with Cu 300 led to increasing the width at the polar of the dorsal (D and D1) surface and the width of the equatorial ventral (D1) surface. The differences with the control group are statistically significant at P< 0,05.

The parameter Dorsal L stomata correlated significantly positively with: Ventral L (r = 0.66); Ventral D (r = 0.41); Dorsal D (r = 0.17) and negatively with Ventral D1 (r=-0.23). The parameter Ventral L correlated significantly positively with Ventral D (r = 0.56). The parameter Ventral D1 correlated with Dorsal D (r = 0.23) and Dorsal D1 (r = 0.22). All correlations are statistically significant at P< 0.05.

Analysis of stomatal parameters of the variety „Sadovo" with humus-carbonate soil.

The size of wheat (*Triticum aestivum* cultivar „Sadovo") stomata cultivated on humus-carbonate soil is significantly smaller compared to these grown on carbonate chernozem soil. Applied $MgSO_4$ and $CuSO_4$ have a negative effect on the anatomical parameters of wheat leaves compared to control, concerning the length of stomata on both surfaces. The degree of reduction of stomata parameters in humus carbonate soil is significantly lower compared to Nh 600 and Cu 300 in carbonate chernozem soil.

The width in the equatorial part of the stomata of the leaves' dorsal and ventral (D and D 1) parts are larger after treatment with copper sulfate in increased amount of 600 mg, compared to magnesium sulfate and control (Table 2). The parameter Dorsal L correlated significantly positively with Ventral L (r = 0.45); Dorsal D (r = 0.32) and Ventral D (r = 0.23). The parameter Dorsal D correlated positively with Ventral L (r = 0.35); Dorsal L (r = 0.32); Ventral D (r = 0.22). The parameter Ventral L correlated positively with Dorsal D (r = 0.35); Ventral D (r = 0.31) and correlated negatively with Ventral D1 (r =-0.26). All correlations among the parameters are statistically significant at P<0.05.

Analysis of stomatal parameters of the variety „Yantar" with humus-carbonate soil.

The dimensions of the stomata in *Triticum aestivum* variety „Yantar" with humus-carbonate soils are greater than the variety „Sadovo" in the same soil parameters and smaller than the variety „Sadovo" with carbonate chernozem soil. Treatment with $CuSO_4$ (Cu 300) reduced the length of the stomata on both surfaces in bigger extent compared to control and $MnSO_4$ (Mn 300). The width at the polar of the dorsal (D and D1) surface and the width of the equatorial ventral D 1 area increased after treatment with copper sulfate compared to control and manganese sulfate (Table 3). The effect of Cu to the control regarding to above mentioned characteristics are statistically significant at P<0,01. The parameter Dorsal L correlated

Table 1. Influence of Nh 600 and Cu 300 on stomatal parameters of wheat "Sadovo" cultivated on carbonate chernozem soil, n = 100

	Dorsal L stomata	Dorsal D	Dorsal D1	Ventral L stomata	Ventral D	Ventral D1
Nh 600	313.65[a]	71.15	44.6[a]	321.57[a]	66.25[a]	42.55
Cu 300	309.47[a]	77.62	46.32[a]	309.22[a]	65.73[a]	46.62
Control	380.21	74.37	40.87	388.75	77.21	37.95
Average	334.44	74.38	43.93	339.85	69.73	42.37
Min	210	47	21	215	36	20
Max	470	100	78	490	98	70
SD	51	9.6	10	58.5	11.4	8.7
SE	3	0.5	0.6	3.3	0.65	0.5

* Differences among the elements are statistically significant at P<0,05 if they have no equal letter

Table 2. Influence of Mg 600 and Cu 600 on stomatal parameters of wheat "Sadovo" cultivated on humus carbonate soil, n = 100

	Dorsal L stomata	Dorsal D	Dorsal D1	Ventral L stomata	Ventral D	Ventral D1
Mg 600	315.51[a]	70.84[a]	39.5	319[a]	67.5[a]	39.84
Cu 600	316.28[a]	74.19	46.71	320.48[a]	67.61[a]	43.03
Control	328.8	70.14[a]	42.19	337.91	66.73[a]	36.79
Average	320.20	71.72	42.80	325.80	67.28	39.89
Min	228	45	22	200	35	20
Max	430	91	70	434	98	63
SD	33.7	8.4	7.9	40	10	7.2
SE	1.9	0.5	0.4	2.3	0.5	0.4

* Differences among the elements are statistically significant at P<0,05 if they have no equal letter

Table 3. Influence of Mn 300 and Cu 300 on stomatal parameters of wheat "Yantar" cultivated on humus carbonate soil, n = 100

	Dorsal L stomata	Dorsal D	Dorsal D1	Ventral L stomata	Ventral D	Ventral D1
Mn 300	365.16	69.49[a]	44.89[a]	383.49	68.01[a]	42.34[a]
Cu 300	343.43	74.85	44.66[a]	347.16	66.84[a]	44.97
Control	379.48	70.04[a]	40.92	364.11	70.38	41.05[a]
Average	362.69	71.46	43.49	364.92	68.41	42.79
Min	223	51	21	240	50	25
Max	490	92	75	462	95	63
SD	49.6	7.8	7.42	42.3	8.11	6.7
SE	2.8	0.45	0.43	2.4	0.46	0.38

* Differences among the elements are statistically significant at P<0,05 if they have no equal letter

positively with Ventral L (r = 0.49) and Ventral D (r =0.23). The parameter Ventral L correlated with Ventral D (r = 0.29). Correlations are significant at P<0.05.

Summarized data from the three experiments with examined 4 polluters (Mg, Cu, Mn and Nh) in different concentrations for establishing the impact of different polluted waters on wheat stomatal characteristics demonstrated big sensitivity and negative impact for stomata Figure 1. All polluters decreased the parameters of stomata. The biggest negative effect is observed by treatments with Nh 600, Cu 600, Mg 600. The effect of polluters on the Dorsal and Ventral D and D 1 structures is lower compared with the length of stomata. Differences with the control are not expressed in such a big extent and there is even positive effect for some polluters. Cu 300 has positive effect on Dorsal D and D1 and Ventral D structures and negative for Ventral D1 structure. These demonstrated that Dorsal and Ventral D and D structures do not react adequately and in the same extent to the polluters as compared with the length of stomata. Maybe this is due to the specific structure and specific reaction ability of these structures.

PC Analyze defined that copper and ammonium defined a group describing 58,57 % of the effects (Figure 2). The higher negative effect is observed with Mn 300.

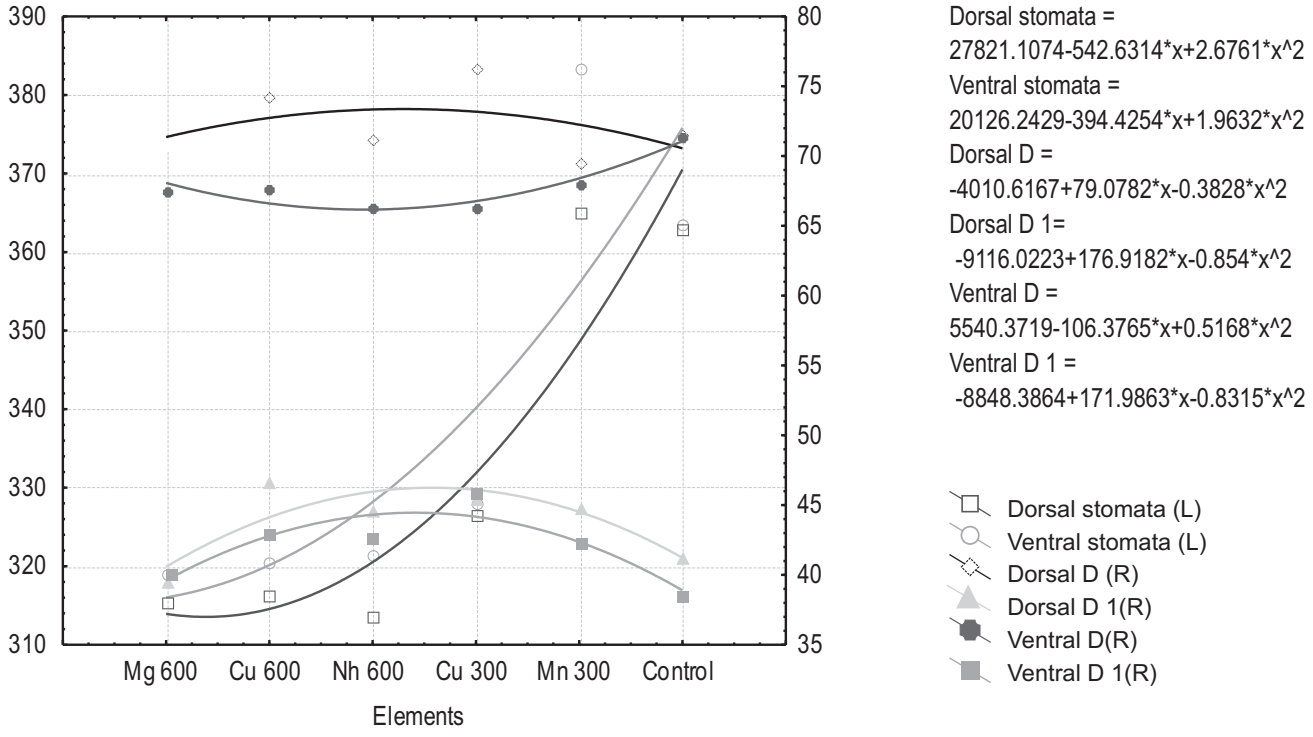

Figure 1. Effect of pollutea water on wheat stomatal characteristics

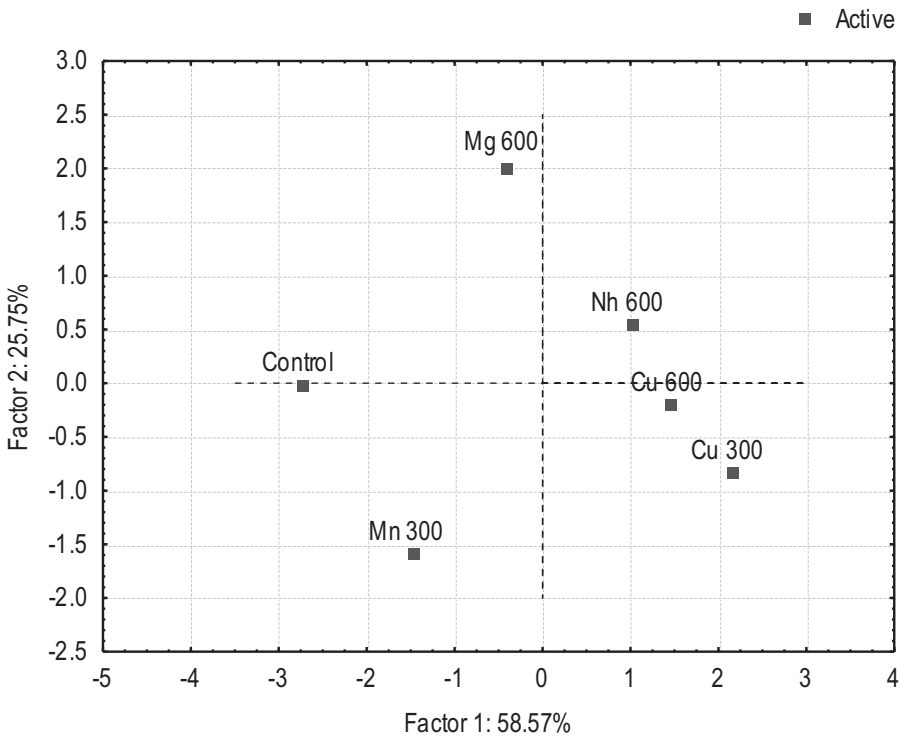

Figure 2. PCA effect of polluters on wheat anatomical characteristics. Cases with sum of cosine square >=0.00

Conclusion

Copper, magnesium and ammonium sulfate have an inhibitory effect on stomatal parameters of wheat. The length of stomata is more sensitive to treatment with polluted water compared to other dorsal and ventral D and D1 characteristics of wheat. Varieties differences - the size of stomata in the same soil are bigger in wheat variety „Yantar" than variety „Sadovo". Variety „Sadovo" is more resistant to contaminants, which is important for agricultural practice. Edaphic factor affects the stomata. The same variety wheat (Sadovo) was larger when it was grown on carbonate chernozem compared to the one grown on humus-carbonate soil.

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