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Effect of experimentally polluted water on the morphological characteristics of 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 (Calcic chernozem and Rendzina). The influence of experimentally polluted water was investigated on the morphological characteristics of the leaves of the two varieties. Manganese and ammonia have positive effect, while diluted concentrations of copper and magnesium have negative impact. The biggest changes in the morphological characteristics have been observed in the length of the leaves. Sadovo variety is more resistant to the impact of pollutants and has better performance which is important for the agricultural practice.*

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

Abbreviations: FAO – Food and agriculture organisation, BSS – Bulgarian state standard

Introduction

Increasing the pollution of soil and water with heavy metals necessitates examination of their effects on plants. Their resistance and the opportunities for their use as sensitive or accumulative bioindicators are of importance for the conservation of biocenosis and monitoring system.

The impact of wastewater on agricultural species has undoubtedly not only ecological, but also practical importance. Most of the symptoms associated with environmental stresses in plants are linked with changes in growth, differentiation and physiological aspects such as photosynthesis, ions uptake and transport (Orcutt and Nilson, 2000; Cseh, 2002). Even though root systems are exposed to the presence of heavy metals or any other contaminants in growth media, the ions quickly move to the shoot via apoplastic pathway (Bell et al., 1991) though it depends on the nature of metal and the plant species (Kabata-Pendias and Pendias, 1992). Some authors have reported suppression of plant growth at elevated concentrations of elements such as Mn, Cu, Zn in the soil (Dospatliev et al., 2008; Zaprjanova et al., 2009).

Felix-Henningsen et al. (2010) studied a possible uptake of heavy metals by food crops from highly-polluted chernozem soils and an irrigation experiments were carried out using spinach and summer wheat. They found that high concentrations of Cu and Zn in topsoils and the uptake of these elements in crops cause limitations in yield and crop quality.

The aim of this study is to investigate the influence of experimentally polluted water on the morphological characteristics of the leaves of two varieties of wheat grown on different soil types (Calcic chernozem and Rendzina).

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Material and methods

Grains of wheat variety „Sadovo" and „Yantar" were planted in vessels on Rendzina soil by FAO (Humus carbonate by BSS) and Calcic chernozem, by FAO (Carbonic chernozem by BSS) soil. To trace the morphological response of pollutants on plants parallel to the control (distilled water) they are watered with different polluted water. Contaminated water is used: $(Nh_4)_2$ SO_4 with concentration 600mg/l (Nh), $CuSO_4$ with concentration 600mg/l (Cu), $MgSO_4$ with concentration 600mg/l (Mg), $MnSO_4$ with concentration 600mg/l (Mn), $(Nh_4)_2$ SO_4 + $CuSO_4$ + $MgSO_4$ + $MnSO_4$ with concentration 600mg/l (Mix), $(Nh_4)_2$ SO_4 with concentration 300mg/l (Nh 1/2), $CuSO_4$ with concentration 300mg/l (Cu 1/2), $MgSO_4$ with concentration 300mg/l (Mg 1/2), $MnSO_4$ with concentration 300mg/l (Mn 1/2), $(Nh_4)_2$ SO_4 + $CuSO_4$ + $MgSO_4$ + $MnSO_4$ with concentration 300mg/l (Mix 1/2). At the end of the trial (45 days), plants were fixed in 70% C_2H_5OH.

The classical comparative morphological method is used. From the morphological features were selected those most significant for the purposes of bioindication by variations in growth performance metric: length of leaves (l), width of leaves (d), number of leaves (S).

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

Results and discussion

The contents of mineral elements in irrigated water used for the cultivation of cultural plants have various effect on the

morphological, physiological indicators and productive opportunities. The effect is dependent to a large extent on the concentration of mineral elements, the specifics of their physiological effects on exchange processes in plants, and synergistic or antagonistic interactions in mixed minerals. An essential role also has the soil with its chemical, physical and biological properties, which increases or decreases the effect of the mineral impact. Significant role in the determination of the impact of mineral elements on plants has the specific response and adaptability of crops or varieties which have a specific mechanism of absorption and adequate reactions.

Analysis of morphological parameters of the variety „Sadovo" with Rendzina soil.

High content of mineral elements in the irrigated water in growing wheat as e.g. biotest, has a different impact on the morphological parameters. Substantial influence have the mineral elements and variability in parameters. In wheat variety Sadovo, farmed on the soil Rendzina, the length of the leaves is 28.08 mm on average, with a variation of 14.7 to 40.4 mm (Table 1). The longest length of the leaves is with Nh1/2, where the difference is very well demonstrated ($P < 0.01$). The smallest is the length in the presence of Cu in the full doses, as the difference to the control is statistically proven at $P < 0.05$. High levels of copper in the water have negative impact not only on the length of the leaves but also on their diameter. Favourable impact on the length and number of leaves has Nh1/2, which is statistically proven ($P < 0.01$). Manganese had the greatest positive impact on the diameter of the leaves, as the difference to the control is very well demonstrated ($P < 0.001$).

The high level of magnesium in the water has led to an increase in the number of leaves ($P < 0.001$), which is a positive trend, facilitating the enhancement of leave index of plants. A positive effect on the number of leaves is observed also with Nh1/2 ($P < 0.01$).

Analysis of morphological parameters of the variety „Sadovo" with soil calcic chernozem.

While growing wheat on calcic chernozem smaller values of the morphological parameters of the leaves were detected. The average length of the leaves is 23.86 mm with fluctuation of 9.80 to 37.00 mm (Table 2). The biggest length of the leaves is with Mn and a mixture of all mineral substances ($P < 0.01$). In contrast, the calcic chernozem copper carbonate has a stronger negative impact on the length of the leaves ($P < 0.01$) as the strongest suppressive action was observed at the lowest dose. The most significant positive effect is found in water with Nh to control, which is statistically proven ($P < 0.01$). The diameter of the leaves is the largest with Nh1/2 ($P < 0.001$) and the smallest in the treatment with Cu1/2. The number of leaves is the largest when Mix ½ ($P < 0.001$) was used.

Analysis of morphological parameters of the variety „Yantar" with Rendzina soil.

In the variety "Yantar" grown on Rendzina soil, the average length of the leaves is 27.66 mm, with variation from 10.00 to 39.40 mm (Table 3). The biggest length is with Mn 1/2 ($P < 0.001$) and Nh1/2 ($P < 0.01$). The smallest length is with Mg1/2 and Cu1/2. Lower values for the length of the leaves grown under the influence of copper content in the water confirmed the negative and suppressive action of copper on the growth of leave length. The diameter of the leaves has the highest value after the use of Nh1/2 ($P < 0.01$). The number of leaves is increased with Mn1/2 and Cu ($P < 0.01$). The shortest length and diameter of the leaves are observed after the treatment with Mg1/2. In the variety "Yantar" similar tendencies of the negative influence of certain mineral elements and positive of others are observed, which demonstrated the different

Table 1. Leaf morphological parameters of variety Sadovo on Rendzina soil, n=110

Mineral elements	Parameters		
	Length	Diameter	Number
Mg	31.34[a]	0.33**	3.8***
Mg1/2	30.81[a]	0.28[a]	3.0[a]
Mn	30.41[a]	0.36***	3.1[a]
Mn1/2	26.13[a]	0.29[a]	3.1[a]
Cu	23.58[a]*	0.24[a]	3.0[a]
Cu1/2	27.19[a]	0.27[a]	3.2[a]
Nh	25.34[a]	0.24[a]	3.1[a]
Nh1/2	32.83**	0.30[ab]	3.4**
Mix	28.23[a]	0.31**	3.0[a]
Mix1/2	26.21[a]	0.34**	3.0[a]
Control	26.78[a]	0.25[a]	3.0[a]
Average	28.08	0.29	3.2
Min	14.70	0.20	3.0
Max	40.40	0.50	5.0
SD	5.60	0.06	0.4
SE	0.50	0.01	0.0

Statistical significance at *-P<0.05 **; P<0.01; ***P<0.001

Table 2. Leaf morphological parameters of variety Sadovo on calcic chernozem soil, n=110

Mineral elements	Parameters		
	Length	Diameter	Number
Mg	22.29[a]	0.27[a]	3.0[a]
Mg1/2	19.83[a]	0.28[a]	3.0[a]
Mn	27.65**	0.29*	3.1[a]
Mn1/2	24.89[a]	0.27[a]	3.0[a]
Cu	26.27[a]	0.28[a]	3.0[a]
Cu1/2	15.61**	0.25[a]	3.0[a]
Nh	29.32**	0.3**	3.0[a]
Nh1/2	22.81[a]	0.33***	3.0[a]
Mix	27.44**	0.31**	3.1[a]
Mix1/2	24.54[a]	0.27[a]	3.5***
Control	21.79[a]	0.23[a]	3.1[a]
Average	23.86	0.28	3.1
Min	9.80	0.10	3.0
Max	37.00	0.40	4.0
SD	6.10	0.06	0.3
SE	0.58	0.01	0.0

Statistical significance at *-P<0.05 **; P<0.01; ***P<0.001

Table 3. Leaf morphological parameters of variety Yantar on Rendzina soil, n=110

Mineral elements	Parameters		
	Length	Diameter	Number
Mg	28.41[a]	0.41[a]	3.1[a]
Mg1/2	20.87**	0.33[a]	3.2[a]
Mn	28.98[a]	0.41[a]	3.3[a]
Mn1/2	33.5***	0.40[a]	3.5*
Cu	30.64[a]	0.41[a]	3.6**
Cu1/2	23.42[a]	0.35[a]	3.0[a]
Nh	29.27[a]	0.37[a]	3.1[a]
Nh1/2	32.73**	0.43**	3.3[a]
Mix	25.46[a]	0.41[a]	3.0[a]
Mix1/2	24.65[a]	0.38[a]	3.0[a]
Control	26.35[a]	0.36[a]	3.1[a]
Average	27.66	0.39	3.2
Min	10.00	0.20	3.0
Max	39.40	0.50	4.0
SD	6.07	0.07	0.4
SE	0.57	0.01	0.0

Statistical significance at *-P<0.05 **; P<0.01; ***P<0.001

influence of mineral substances on the growth of plants.

The analysis of the obtained results on the effects of different quantities of mineral substances in the water on the two varieties of wheat grown on two different soil types indicates the specific influence of soil, which shows that the effect of the mineral impact is different. The comparison of two soil types showed that on humus carbonate soil wheat has improved characteristics of the leaf parameters, which is statistically proven (P < 0.001, Table 4). This shows that the negative effect of mineral contaminants is less expressed in the soil type which has higher fertility and larger buffer capacity.

The comparison between the planned varieties indicates that there is a varietal-specific reaction against the influence of soil contaminants. Variety "Sadovo" has better characteristics of green leafy parameters in comparison with a variety "Yantar", which indicates that it is more resistant to the effects of the pollutants. The concentration (full or half dose) of mineral elements as contaminants does not have a significant impact on the number and diameter of the leaves and has a negligible impact on the length of the leaves.

The summarized analysis of the mineral substances in the water indicates that of all chemical elements Cu1/2 has the most negative impact, which affects to a greater extent the length of the leaves and their diameter. A positive impact is detected under the influence of Mn, Mn1/2 and Nh1/2 on the length and diameter of the leaves. The difference is statistically proven at P < 0.01-0.001. A positive impact on the number of leaves is observed under the influence of Mg (P < 0.01).

Our comparative study on the influence of mineral elements as contaminants in water on two varieties of wheat on soil type humus carbonate shows that the two factors – variety and mineral elements have different impact on the morphological parameters of the leaves of wheat. On the leaf length the most influential was the interaction

Table 4. Average for all investigated combinations N=330

Investigated Factors	Parameters		
	Length	Diameter	Number
Soil type			
Rendzina	27.86***	0.33***	3.17**
Calcic chernozem	23.85***	0.28***	3.07**
Average	26.53	0.32	3.14
Variety			
Sadovo	27.66**	0.38***	3.2*
Yantar	25.96**	0.28***	3.11*
Average	26.53	0.32	3.14
Doses			
Full	27.64**	0.33	3.15
Half	25.73**	0.32	3.15
Average	26.69	0.32	3.15
Mineral Elements			
Mg	27.35	0.33**	3.3**
Mg1/2	23.84	0.30	3.07
Mn	29.01**	0.35***	3.17
Mn1/2	28.17**	0.32	3.20
Cu	26.83	0.31	3.20
Cu1/2	22.07	0.29	3.07
Nh	27.98	0.30	3.07
Nh1/2	29.45**	0.35***	3.23
Mix	27.04	0.34**	3.03
Mix1/2	25.13	0.33*	3.17
Control	24.97	0.28	3.07
Average	26.53	0.32	3.14

Statistical significance at *-P<0.05 **; P<0.01; ***P<0.001

between variety and mineral elements – 52.90% (Table 5). Next are the mineral elements while the variety as a factor has only a minor impact. The diameter of the leaves is the most affected by the variety factor – 65.47%, which shows that this parameter is above all varietal-specific features. Stronger impact on the number of leaves has the interaction between variety and mineral elements. Variety does not have a significant impact either.

The summaries (main component) analysis for the distribution of the effect of the influence of mineral elements in the irrigation water and their categorization as primary and secondary factors on morphological parameters of wheat shows that the first factor describes 72.48 % from the impact (Figure 1). To this group are Cu1/2, Mg1/2, Nh. As a second factor describing 18.30 % refer Mg, Mn, Nh1/2.

This trend of impact is also confirmed by the two-way cluster analysis for a group of mineral elements and the effects of the impact. From the observed 3 morphological parameters, the mineral elements in the water have the strongest influence on the length of the leaves (Figure 2). The least is the impact on the number of leaves.

With the indicator length of leaves 3 groups are formed. First group – Cu1/2 and Mg1/2 with the lowest values of the length of the leaves. The second group – Cu, Nh, Mix1/2 – with average. A third

Table 5. Influence of factors, varieties (Sadovo and Yantar) and elements on Rendzina soil

Parameters/Factors	SS	DF	MS	F	p	%
Length						
Variety	9.5	1	9.5	0.366	0.546152	0.40
Elements	1110.9	10	111.1	4.277	0.000021	46.70
Variety*Elements	1258.3	10	125.8	4.845	0.000003	52.90
Diameter						
Variety	0.49637	1	0.49637	112.419	0.000000	65.47
Elements	0.18502	10	0.01850	4.190	0.000028	24.40
Variety*Elements	0.07675	10	0.00767	1.738	0.074501	10.12
Leaf number		SF				
Variety	0.114	1	0.114	0.95	0.331069	1.09
Elements	4.636	10	0.464	3.87	0.000082	44.62
Variety*Elements	5.636	10	0.564	4.71	0.000005	54.25

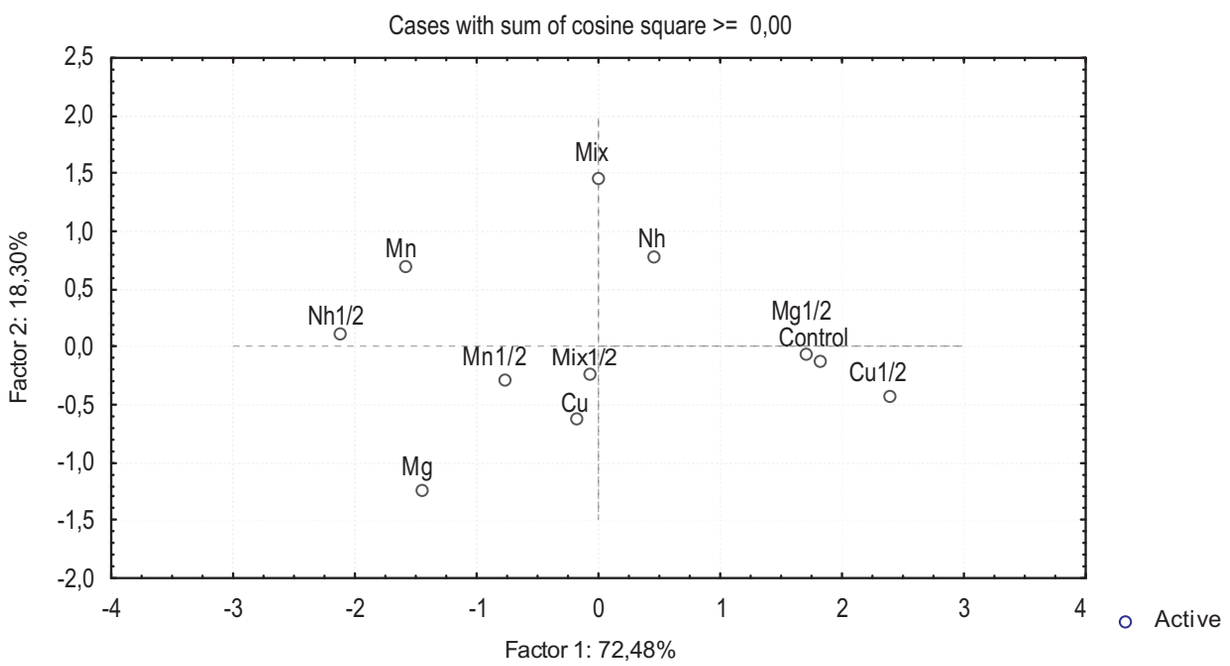

Figure 1. PC analysis for distribution of elements in irrigation water.

group – Mn, Mn1/2, Nh1/2, Mg – with the greatest length of the leaves. The third group has a positive impact whereas the second and especially the first group have negative impact.

The analysis on the variation of parameters of leaves under influence of mineral substances in the water confirms that the number and diameter of the leaves are considerably less ranging parameters compared to the length of the leaves as an indicator (Figure 3). The length of the leaves (mean values) under the influence of Cu1/2 and Mg1/2 are significantly lower, which confirms their negative impact. The highest are the values with Nh1/2 and Mn, which confirms their positive effect.

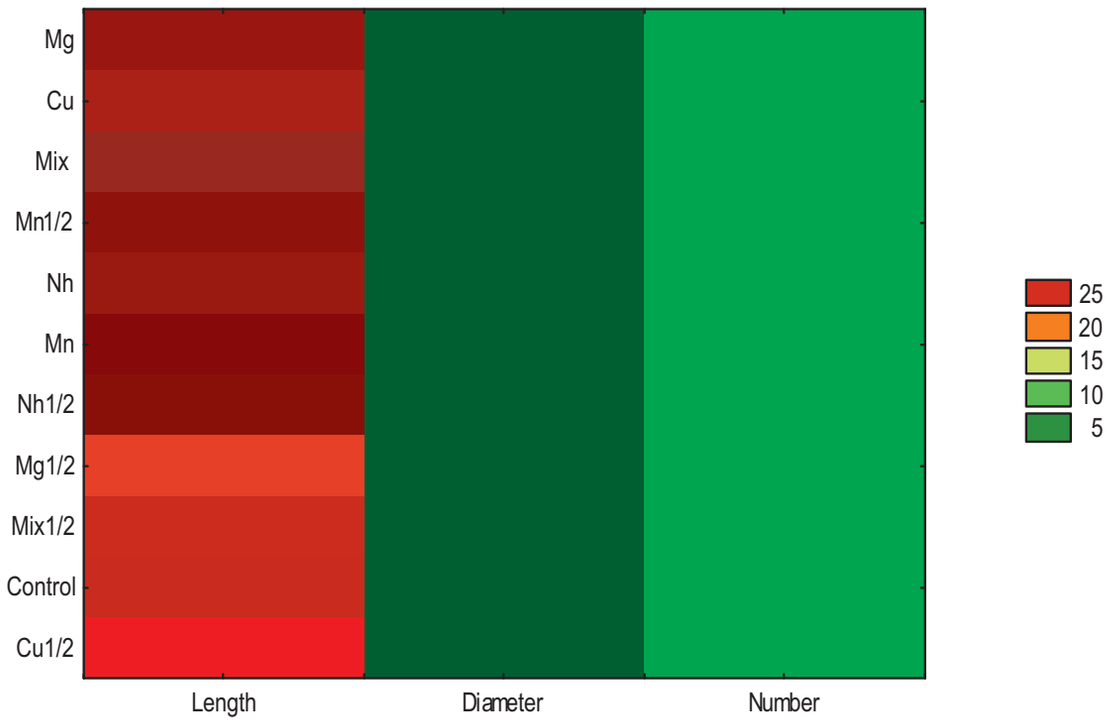

Figure 2. Cluster analyse Two-Way Joining Results - grouping of elements

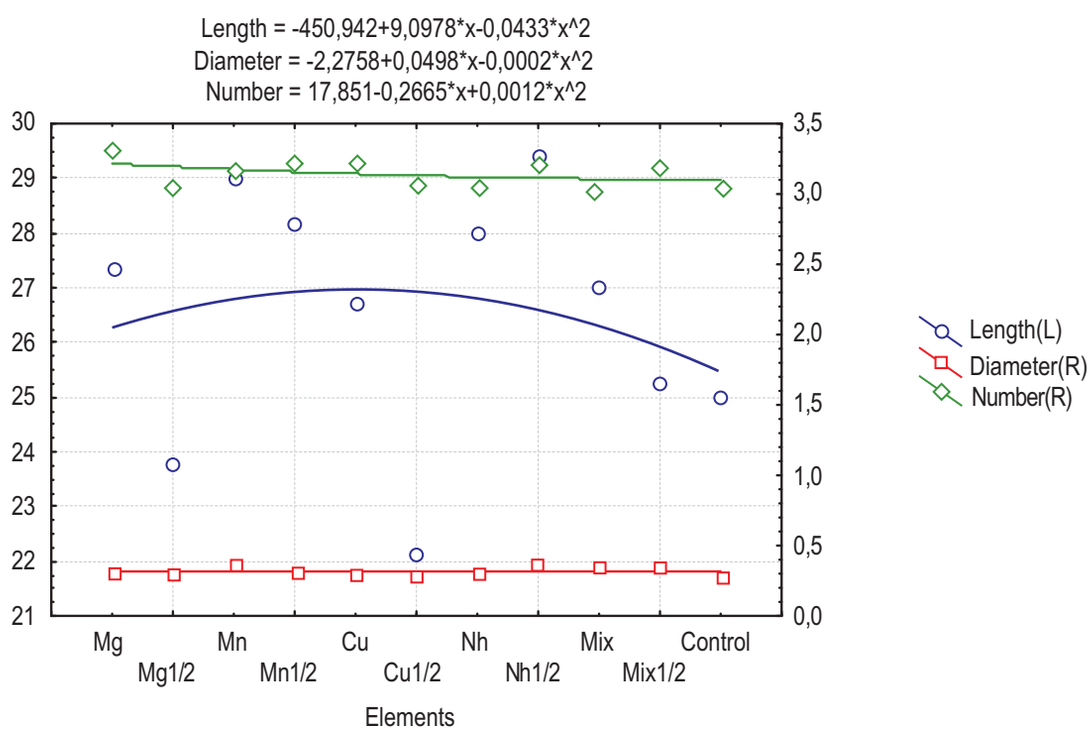

Figure 3. Influence of elements on the variation of parameters

Conclusion

The content of mineral substances in the water in high concentrations has a stronger impact on the length of the leaves of wheat as morphological parameter. The mineral elements have a different impact on the morphological parameters. Positive effect have manganese and ammonia, while diluted concentrations of copper and magnesium have negative impact.

Rendzina soil provides better characteristics of the leaf parameters and because of the buffer capacity the negative influence of mineral elements in the water as contaminants is reduced. Wheat varieties have different sensitivity and resistance to the influence of mineral substances in the water. Sadovo variety is more resistant to the impact of pollutants and has better performance, which is important for the agricultural practice.

Parameters of the leaves vary differently under the influence of mineral substances. On the length and number of leaves the strongest influence has the interaction between variety and mineral elements. On the diameter of the leaves the strongest influence has the variety as a factor.

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