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EFFECT OF ZINC AND COPPER SOIL CONTAMINATION ON THE TRANSPIRATION INTENSITY AND STOMAL INDEX OF WINTER CROP WHEAT SEEDLINGS

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Abstract

In the study, the physiological response of wheat plants to the effect of metal salts was examined by measuring gaseous exchange transpiration parameters, such as transpiration intensity, stomatal conductivity and stomata count per 1mm^2 of the leaf under- and upperside. The study was carried out using the gley soil [black earth] of granulometric composition of loam collected from the plough-humus horizon of 0-10 cm depth. The soil was contaminated with different pollutant doses, i.e. 0 (control), 0.05, 0.5, 5 and 50 mmol·kg^{-1}. Each combination was seeded with 10 wheat grains.

Two-factor analysis of variance was used, with the type of applied salt ($ZnSO_4$ and $CuSO_4$) being the first factor and its dose the second one. The significance of factors was examined with Tukey's test ($p<0.05$). A delayed germination of seeds was observed in relation to the control in soil samples contaminated with 5 mmol·kg^{-1} (both salts). The seedlings examined showed large differentiation in their response to Zn and Cu; in the case of $CuSO_4$, transpiration was much lower than under the effect of $ZnSO_4$. The transpiration intensity was the lowest after application of copper salts in a concentration of 5 and 50 mmol·kg^1, whereas the highest one was observed in zinc concentration of 5 mmol·kg^{-1}.

The soil contamination with a dose of 0.05 and 50 mmol·kg^{-1} resulted in a significant decrease of stomatal conductivity in relation to the control. The examined heavy metals significantly affected the stomatal count only on the leaf underside, where it increased at the highest concentration of heavy metals, i.e. 50 mmol·kg^{-1}.

Key words: cooper, transpiration, stomatal conductivity, stomata count.

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WPŁYW ZANIECZYSZCZENIA GLEBY CYNKIEM I MIEDZIĄ NA INTENSYWNOŚĆ TRANSPIRACJI ORAZ INDEKS SZPARKOWY SIEWEK PSZENICY OZIMEJ

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Abstrakt

Reakcję fizjologiczną roślin pszenicy na działanie soli metali badano mierząc parametry wymiany gazowej, takie jak: intensywność transpiracji, przewodność szparkowa oraz liczba aparatów szparkowych na 1 mm^2 dolnej i górnej epidermy blaszki liściowej. Badania przeprowadzono z użyciem czarnej gleby o składzie granulometrycznym gliny, pobranej z poziomu orno-próchniczego z głębokości 0-10 cm. Glebę zanieczyszczono różnymi dawkami polutantów: 0 – kontrola; 0,05; 0,5; 5; 50 $mmol \cdot kg^{-1}$. Do wszystkich wariantów wysiano po 10 nasion pszenicy. Zastosowano dwuczynnikową analizę wariancji: pierwszym czynnikiem był rodzaj zastosowanej soli ($ZnSO_4$, $CuSO_4$), a drugim dawka. Istotność czynników testowano testem Tukeya na poziomie 0,05. Zaobserwowano opóźnione kiełkowanie nasion w stosunku do kontroli w glebie zanieczyszczonej dwoma rodzajami soli w ilości 5 $mmol \cdot kg^{-1}$. Stwierdzono, że badane rośliny różnie reagowały na zastosowany metal. W przypadku soli miedzi transpiracja roślin była znacznie mniejsza niż w przypadku soli cynku. Intensywność transpiracji była najmniejsza po zastosowaniu soli miedzi w stężeniu 5 i 50 $mmol \cdot kg^{1}$, natomiast największe natężenie transpiracji wykazano po zastosowaniu soli cynku w stężeniu 5 $mmol \cdot kg^{-1}$.

Zanieczyszczenie gleby dawką 0,05 i 50 $mmol \cdot kg^{-1}$ spowodowało znaczne zmniejszenie przewodności szparkowej w stosunku do kontroli. Badane metale ciężkie wpłynęły istotnie na liczbę aparatów szparkowych jedynie na dolnej epidermie blaszki liściowej, gdzie ich liczba zwiększyła się po zastosowaniu dawki 50 $mmol \cdot kg^{-1}$ metali ciężkich.

Słowa kluczowe: miedz, cynk, transpiracja, przewodność szparkowa, indeks szparkowy.

NTRODUCTION

Industry and intensive agriculture cause increased environmental pollution with heavy metals. High level of contamination occurs most frequently in the vicinity of emitters (GORLACH 1991, FABER, WARTA 1981). According to the State Inspectorate for Environmental Protection (1997), soil contamination with copper and zinc results first of all from the impact of metallurgical, electrochemical and chemical industries, whereas zinc pollution is additionally caused by coal, waste and refuse combustion as well as heavy road traffic.

Heavy metals available to plants can affect their growth and physiology in different way. While in threshold quantities, they frequently stimulate physiological processes, they will become toxic after exceeding permissible amounts, first of all disturbing the stability of cytoplasmatic membranes and cell structures (GORLACH 1991, HARBORNE 1997, STROIŃSKI 2002).

The study aimed at determining the effect of different zinc and copper concentrations in soil on the transpiration intensity, stomatal conductivity and stomatal index of wheat seedlings.

MATERIAL AND METHODS

In 2004-2005, an experiment was carried out under laboratory conditions with cv. Tonacja winter crop wheat involving 10-hour-long invariable light intensity PAR at 400 $\mu mol \cdot m^{-2} \cdot s^{-2}$ and mean air temperature of 17–19°C. Soil mass per one pot was 0.5 kg. The substrate consisted of typical black earth, loamy in granulometric composition, collected from the plough-humus horizon of 0–10 cm depth in the Experimental Station of Agricultural University in Szczecin in Dołuje. Soil reaction (pH_{KCl}) was 6.4 and the hydrolytic acidity was 1.2 $me \cdot 100\ g^{-1}$. The content of soil zinc equalled 28.70 $mg \cdot kg^{-1}$, whereas that of copper to 9.60 $mg \cdot kg^{-1}$.

The soil was contaminated with heavy metals in the form of $CuSO_4$ and $ZnSO_4$ salts in the amounts of 0.05, 0.5, 5 and 50 $mmol \cdot kg^{-1}$ of soil. The control was soil free of addition of zinc and copper salts. Each combination was seeded with 10 wheat grains of cv. Tonacja. The experiment was carried out in four replications. After germination, measurements were made of the intensity of transpiration process ($H_2O \cdot m^{-2} \cdot s^{-1}$) and of the stomatal conductivity ($mmol \cdot m^{-2} \cdot s^{-1}$) using a gas analyser LCA-4. Stomata were counted with an optical microscope Olympus CX-41on the upper and the lower leaf epidermis, converting them into an area of 1 mm^2.

The results underwent statistical elaboration by means of two-factor analysis of variance, with a type of the applied salt type ($ZnSO_4$ and $CuSO_4$) being the first factor and Zn and Cu dose the second one. The significance was examined with Tukey's test at a significance level of 0.05.

RESULTS AND DISCUSSION

The soil contamination with copper and zinc salts clearly modified the values of physiological parameters examined, i.e. of transpiration intensity, stomatal conductivity and stomatal index. Heavy metals added to a substrate in larger doses resulted in a clear osmotic stress, thus impeding the water intake from substrate by germinating seeds and causing a weaker sprouting of seedlings. According to Stolarska et al. (2006), soil contamination with copper and zinc salts results in delaying of wheat grain germination, which is brought about by the suppression of water access from a substrate to germinating seeds and then to seedlings. The accepted limit value of copper content in soils under agricultural use is 150 mg·kg^{-1}, whereas that of zinc is 300 mg·kg $^{-1}$ (*Ordinance of the Minister of Environmental Protection of 9 September 2002*). In contaminated soils, these doses can be several-fold exceeded (TRZASKOŚ, DZIDA 1995). In the own research, a delayed germination of wheat grains was observed with the soil contamination with zinc and copper salts in the amounts of 5 and 50 mmol·kg^{-1} in relation to the control. This is confirmed by studies of PRZYBULEWSKA and STOLARSKA (2004), who state that among the symptoms of excessive zinc concentrations in plants are suppression of seed germination and slower seedling growth.

This experiment demonstrated that reduction of transpiration intensity in wheat leaves was nearly proportional to the soil contamination with copper salts (Fig. 1).

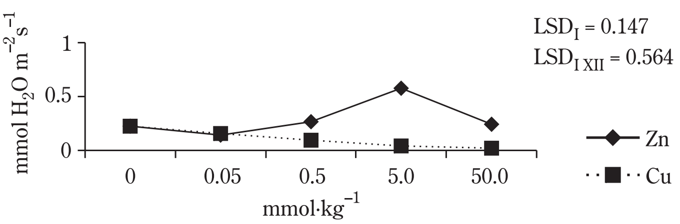

Fig. 1. Influence of varied zinc and copper rates in substrate on transpiration at winter crops wheat sedling seedling

Rys. 1. Wpływ różnych stężeń cynku i miedzi na intensywność transpiracji siewek pszenicy ozimej

According to PRZYBULEWSKA and STOLARSKA (2004), cereal plants are unusually susceptible to increased concentration of that chemical element is soil. The effect of $ZnSO_4$ in a dose of 0.05 mmol·kg^{-1} was not significant. The rise of zinc sulphate dose resulted in a considerable increase of the intensity of transpiration process, not until a large concentration of that zinc salt was used (50 mmol·kg^{-1}), which clearly inhibited this process (Figure 1). Similar results were obtained by BRUNE et al. (1994). According to PRZYBULEWSKA and STOLARSKA (2004), cereals are one of the plants that are most susceptible to the concentration of that metal in soil. PANDEY and SHARAMA (2002) report that a large content of heavy metals in soil considerably decreases the intensity of transpiration process in plants.

The wheat seedlings clearly showed greater differentiation of transpiration intensity depending on a metal type than on its doses, since it was demonstrated that after application of 5 and 50 mmol·kg^{-1} copper salt the transpiration intensity was significantly lower than after applying zinc salt in the amount of 5 mmol·kg^{-1} (Figure 1).

The stomatal conductivity of wheat seedlings resulting from the soil contamination with copper and zinc salts was shaped alike (Figure 2). However, the ex-

Table 1
Tabela 1

Influence of zinc and copper salts (mmol · kg^{-1} of soil)
on transpiration of winter crops wheat seedling
Wpływ soli cynku i miedzi (mmol · kg^{-1} gleby) na
intensywność transpiracji siewek pszenicy ozimej

Dose – Dawka	Average – Średnia
50	0.121 a
0.05	0.150 a
0.5	0.181 a
0	0.226 a
5	0.288 a

Metal	Average – Średnia
Cu	0.096 a
Zn	0.291 b

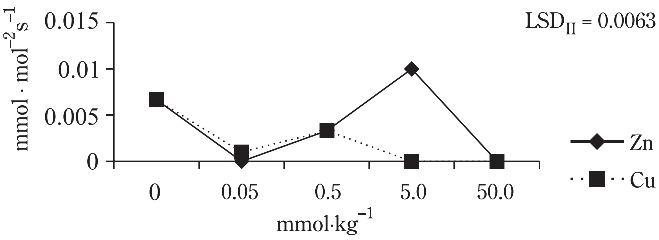

Fig. 2. Influence of varied zinc adn cooper rates in substrate stomatal conductance at winter crops wheat seedling
Rys. 2. Wpływ różnych stężeń cynku i miedzi na przewodność szparkową siewek pszenicy ozimej

amined plants clearly responded to a dose of heavy metal rather than to its type. A decrease in stomatal conductivity was found after application of 0.05 and 50 mmol · kg^{-1} doses in relation to the control (Table 2). Similar results were obtained by Burzyński and Kłobus (2004), who found that at a dose of copper salt of 0.02 mmol · kg^{-1} the stomatal conductivity was similar to that in control plants, whereas a rise of metal dose to 0.05 mmol · kg^{-1} considerably reduced this index. According to Monnet et al. (2001), a very low concentration of copper salts resulted in strong reduction of stomatal conductivity versus the control. Slight elevation of salt concentration increases the stomatal conductivity, while a very strong concentration of that chemical element in soil suppresses it (Monnet et al. 2001).

Table 2
Tabela 2

Influence of zinc and copper salts (mmol·kg^{-1} of soil)
on stomatal conductance of winter crops wheat seedling
Wpływ soli cynku i miedzi (mmol·kg^{-1} gleby)
na przewodność szparkową siewek pszenicy ozimej

Dose – Dawka	Average – Średnia
0.05	0.000 a
50	0.000 a
0.5	0.003 ab
5	0.005 ab
0	0.006 b

Metal	Average – Średnia
Cu	0.002 a
Zn	0.004 a

Table 3
Tabela 3

Influence of zinc and copper salts (mmol·kg^{-1} of soil) on stomatal apparatus in leaf underside and leaf upperside of winter crops wheat seedling
Wpływ soli cynku i miedzi (mmol·kg^{-1} gleby) na liczbę aparatów szparkowych dolnej i górnej strony liścia siewek pszenicy ozimej

Leaf upperside – Strona górna		Leaf underside – Strona dolna	
Dose – Dawka	average – średnia	dose – dawka	average – średnia
0	396 a	0.5	249 a
50	417 a	5	270 a
0.05	434 a	0.05	271 a
5	469 a	0	412 ab
0.5	497 a	50	453 b

Leaf upperside – Strona górna		Leaf underside – Strona dolna	
Metal	average – średnia	metal	average – średnia
Cu	442 a	Cu	319 a
Zn	443 a	Zn	343 a

The size of a heavy metal dose had a significant effect on the stomata count only on the leaf underside. After application of heavy metals in a dose of 50 mmol·kg^{-1}, the number of stomata was significantly larger than when exposing plants to lower doses of that metal, i.e. 0.05, 0.5 and 5 mmol·kg^{-1} (Table 3). However, no significant differences were found in relation to the control. It is difficult to explain such a response of wheat seedlings to the increased concentration of zinc and copper salts, as available reference data are short of findings that refer to their effect on the number of stomata.

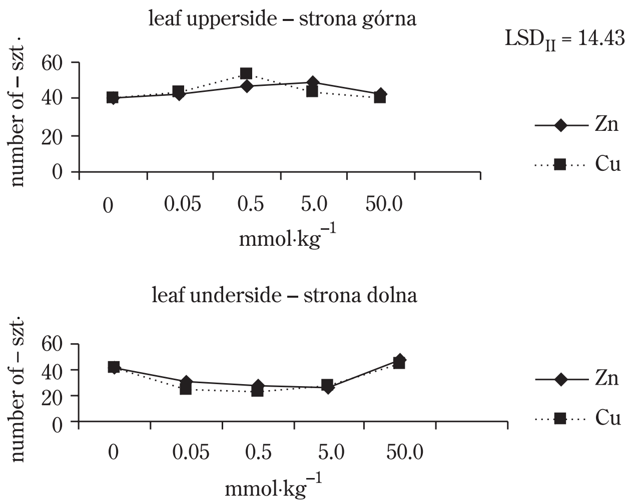

Fig. 3. Influence of varied zinc and copper rates in substrate on stomatal apparatus in leaf underside and leaf upperside of wheat seedling winter crops wheat seedling

Rys. 3. Wpływ różnych stężeń cynku i miedzi na liczbę aparatów szparkowych dolnej i górnej strony liścia siewek pszenicy ozimej

CONCLUSIONS

1. The intensity of transpiration was more evidently slowed down after application of copper salts in the amounts of 5 and 50 mmol·kg^{-1} than after application of zinc salts in a dose of 5 mmol·kg^{-1}. Most probably, the wheat seedlings considerably suppressed transpiration after application of copper salts in order to use water in the photosynthesis process rationally.

2. The soil contamination with zinc and copper salts in the amounts of 0.05 and 50 mmol·kg^{-1} resulted in a significant decrease of stomatal conductivity in relation to the control object.

3. After soil contamination with heavy metals in a dose of 50 mmol·kg^{-1}, the mean stomata count on the leaf underside was over twice as high as that in control plants.

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