*Acta Agrophysica, 2015, 22(2), 173-182*

# PHYSIOLOGICAL ACTIVITY OF COMMON OSIER (*SALIX VIMINALIS* L.) UNDER COPPER-INDUCED STRESS CONDITIONS

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A b s t r a c t. The study examined the influence of copper salt at concentrations ranging from 50 to 250 mg dm$^{-3}$ on the physiological reaction of the Bjor and Jorr varieties of common osier. The content of assimilation pigments, the intensity of $CO_2$ assimilation, transpiration, stomatal conductance for $CO_2$ and water vapour, coefficients of the relative water content and water saturation deficit were determined. The intensity of $CO_2$ assimilation, transpiration and the assimilation pigment content in the tested common osier cultivars varied with relation to the copper salt dose in the medium. A decrease in the intensity of the tested physiological parameters was found together with an increase in the copper salt dose in the medium. $CO_2$ assimilation and transpiration of the Bjor and Jorr osier was significantly limited by the stomata. The addition of copper salts to the medium resulted in an increase in the water saturation deficit in leaves of the tested common osier varieties.

K e y w o r d s : common osier, copper, $CO_2$ assimilation, transpiration, assimilation pigments

## INTRODUCTION

Environmental pollution requires undertaking intensive treatments aimed at restoring the natural environment to its original condition. Heavy metals are among the substances which have a negative influence on the environment. Copper is one of such substances; it is an element which is necessary for the plant's life and it is strongly toxic at the same time if it occurs in excess in the environment. It activates a lot of enzymes in a plant and it is a component of catechol oxidase, ascorbate oxidase, superoxide dismutase, plastocyanin and copper flavoproteins (Szatanik-Kloc *et al.* 2010). In addition, it participates in the processes of photosynthesis, respiration, protein formation and in the transformation of nitrogen compounds and carbohydrates. It participates in the metabolism of cell membranes, influences their permeability and also the water balance (Konieczyński and

Wesołowski 2008). Copper belongs to a group of metals which have the ability to catalyse the Fenton-catalysed Haber-Weis reaction and its excess can directly contribute to an increase in the concentrations of reactive oxygen species in cells (Kehrer 2000). Copper toxicity involves the reduction of the most important vital processes in plants, both at the physiological and biochemical level (Groppa *et al.* 2001, Wang *et al.* 2004) and it inhibits root growth (Gajewska and Skłodowska 2010).

The effectiveness and duration of remediation using phytoremediation does not only depend on the concentration of pollutants in the soil, but also on the proper selection of plants for a given type of pollution (Zemleduch and Tomaszewska 2007). Research conducted in the area of chemistry, physiology and biochemistry allowed the acquisition of more accurate knowledge on the mechanisms and processes responsible for toxin collection and accumulation by plants and also on the use of physiological and biochemical parameters for the assessment of usefulness of plants in the remediation of degraded areas. In this way, it is possible to use the natural properties of plants and to plan optimal pollution remediation methods in a degraded area (Zemleduch and Tomaszewska 2007).

Phytoremediation is a method of reclamation of areas contaminated with copper. From the point of view of phytoremediation of large industrial protection zones and urban areas, it is the Salix species plants, a large number of which are considered to be resistant to soil contamination with heavy metals, that seem to be particularly predisposed to do well under such conditions (Landberg and Greger 1996, Wrzosek *et al.* 2008). Physiological and biochemical parameters are used for the evaluation of plants useful in reclamation of degraded areas.

The aim of the study was to verify whether an increased copper ion content in the medium influences the selected physiological parameters of common osier (*Salix viminalis*) Bjor and Jorr varieties. The verification of osier as a particularly valuable species for phytoremediation, and understanding of the physiological grounds of its resistance to stress induced by an increased ion content in the medium, will allow to determine, on a preliminary basis, the usefulness of these forms in managing soils polluted with this element.

## MATERIAL AND METHODS

Common osier (*Salix viminalis* L.) var. Bjor and Jorr was used as the experimental material. 120 cuttings of willow obtained from the last year's shoots were used for the studies. Laboratory studies were carried out as a hydroponic culture with different doses of copper. Willow cuttings were divided into four groups of the same number and placed in containers filled with Hoagland full nutrient solution. Copper sulphate was added to the nutrient solutions 14 days after the cuttings were placed in the hydroponics, when the plants had taken roots and had shot. Three levels of contamination of the nutrient solutions with copper salts were applied: 50, 150 and 250 mg $dm^{-3}$. Plants placed in Hoagland full nutrient solution were the control object. The determination of physiological parameters was carried out three times: on the 7th, 14th and 21$^{st}$ day after the day the rates of copper salts were applied. The measurements of the parameters of gas exchange: $CO_2$ assimilation (A), transpiration (E), stomatal conductance for water vapour ($g_s$), stomatal conductance for $CO_2$ ($g_c$) were made (the measurement was replicated three times) on leaves using a gas analyser TPS-2, working in an open system with a chamber of PLC-4 type. In the analyser cuvette the following conditions were established: a permanent inflow of carbon dioxide, humidity equal to the humidity of the environment, and lighting equal to 2053 PAR (µmol $m^{-2}$ $s^{-1}$), supplied by means of a light unit attached to the cuvette. On the basis of the obtained results of the assimilation and transpiration rates, photosynthetic effectiveness of the use of water ($\omega_F$) was calculated. The determination of assimilation pigments (chlorophyll "a" and "b" and carotenoids) were carried out on the same leaves on which the parameters of gas exchange were determined. The content of chlorophyll was defined using the method of Arnon *et al*. (1956) modified by Lichtenthaler (1987), whereas the content of carotenoids was determined by means of the Hager and Meyer-Bethenrath (1966) method. The indices of the relative water content (RWC) and the deficit of water saturation (WSD) were determined according to Bandurska (1991). The statistical analysis of the studies was carried out using Duncan's test at the level of significance $LSD_{0.05}$. Pearson linear correlation coefficients between the analysed variables characteristic of gas exchange were also determined.

## RESULTS AND DISCUSSION

Based on the results obtained, it can be concluded that stress induced by the presence of large doses of copper in the medium caused a change in the tested physiological parameters.

The toxic impact of copper salts on the tested physiological parameters was found in both tested forms of common osier. The highest dose of the copper salts used caused a reduction in chlorophyll “a” and “b” by approx 37.5% in the Bjor variety, while in Jor the reduction was by almost 60% and 53%, respectively, as compared to the control plants (Tab. 1). The carotenoid content in the leaves of the Bjor and Jorr varieties of common osier with a copper salt dose in an amount of 250 mg $dm^{-3}$ amounted to 52% and 43.5% of the content of this pigment in the control plant leaves (Tab. 1). A decrease in the content of chlorophyll “a” and “b” in plants under the influence of large doses of copper was observed in Jasiewicz *et al.* (2004), Smolik and Malinowska (2006, 2009). When excessive amounts of copper occur in the soil, this element is accumulated in large quantities in the roots. Also, Cu transport to the above-ground parts of the plants is slow due to the occurrence of a strong conductance barrier for this element from the roots to the stem (Jurkowska *et al.* 1996, McBirde and Martinem 2000). Inhibition of chlorophyll synthesis is a symptom of the impact of various heavy metals, also including excess copper in the plant. According to Stiborova *et al.* (1986), an excessive Cu content in the plant inhibits synthesis of especially chlorophyll "a" and "b" and reduces their content in photosynthetic cells. According to Szatanik-Kloc *et al.* (2010), copper ions are accumulated in chloroplasts if high concentrations of this element occur, which interferes with the synthesis of photosynthetic pigments and enzyme activity.

**Table 1.** Toxic impact of copper on the content of photosynthetic pigments (mg $g^{-1}$ fresh weight), $CO_2$ assimilation (μmol $m^{-2}$ $s^{-1}$), transpiration (mmol $m^{-2}$ $s^{-1}$) and water use photosynthetic efficiency ($\omega_f$) of common osier varieties Bjor and Jorr

| Dose of Cu (mg $dm^{-3}$) | Chlorophyll a (% of control) | Chlorophyll b (% of control) | Carotenoids (% of control) | $CO_2$ assimilation (% of control) | Transpiration (% of control) | Water use photosynthetic efficiency ($\omega_f$) |
|---|---|---|---|---|---|---|
| | | | Cv. Bjor | | | |
| 0 | 1.34 ± 0.07 (100) | 0.56 ± 0.07 (100) | 0.75 ± 0.09 (100) | 3.32 ± 0.08 (100) | 1.12 ± 0.08 (100) | 2.96 |
| 50 | 0.98 ± 0.10 (73.1) | 0.46 ± 0.03 (82.1) | 0.66 ± 0.05 (88.0) | 3.14 ± 0.04 (94.6) | 0.86 ± 0.07 (76.8) | 3.65 |
| 150 | 1.03 ± 0.09 (76.8) | 0.34 ± 0.05 (60.7) | 0.56 ± 0.04 (74.7) | 2.97 ± 0.05 (89.5) | 0.83 ± 0.07 (74.1) | 3.58 |
| 250 | 0.84 ± 0.08 (62.7) | 0.35 ± 0.03 (62.5) | 0.39 ± 0.04 (52.0) | 2.67 ± 0.05 (80.4) | 0.68 ± 0.09 (60.7) | 3.93 |
| 0.248 | 0.135 | n.s. | n.s. | n.s. | | |
| | | | Cv. Jorr | | | |
| 0 | 1.83 ± 0.08 (100) | 0.68 ± 0.07 (100) | 1.08 ± 0.08 (100) | 7.29 ± 0.11 (100) | 1.27 ± 0.12 (100) | 6.13 |
| 50 | 0.81 ± 0.05 (44.3) | 0.36 ± 0.04 (52.9) | 0.58 ± 0.04 (53.7) | 6.29 ± 0.08 (86.3) | 1.20 ± 0.08 (94.5) | 5.24 |
| 150 | 0.83 ± 0.09 (45.3) | 0.35 ± 0.03 (51.5) | 0.48 ± 0.06 (44.4) | 4.80 ± 0.07 (65.8) | 0.98 ± 0.08 (77.2) | 4.89 |
| 250 | 0.74 ± 0.05 (40.4) | 0.32 ± 0.01 (47.1) | 0.47 ± 0.05 (43.5) | 3.67 ± 0.08 (50.3) | 0.77 ± 0.06 (60.6) | 4.77 |
| 0.394 | 0.168 | 0.241 | 1.611 | n.s. | | |

A decrease in the intensity of the process of photosynthesis and transpiration was found together with an increase in the copper salt dose in the medium. The intensity of the $CO_2$ assimilation process in the Bjor and Jorr varieties in the presence of the largest dose of copper salts in the medium amounted to 80.5% and 50.3%, respectively, as compared to the control plant (Tab. 1). The copper salt dose of 250 mg dm$^{-3}$ reduced the intensity of the transpiration process in both osier varieties by 39% as compared to the control plant. Similar reactions of plants to copper salt were observed by Smolik and Malinowska (2006, 2009).

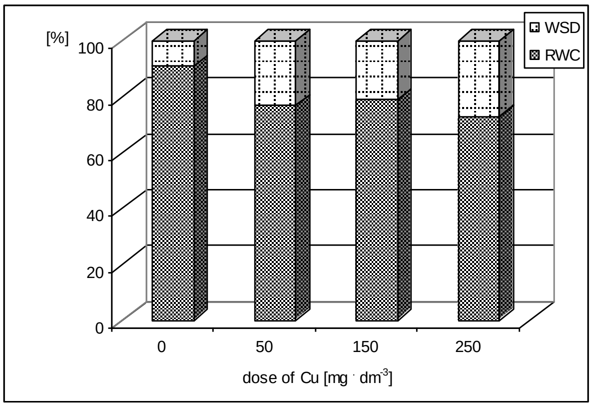
A decrease in the intensity of $CO_2$ assimilation and transpiration is probably caused by a toxic impact of copper on these processes. Excessive amounts of copper in the plant limit the process of photosynthesis by inhibiting the transport of electrons. In addition, copper influences the metabolism of cell membranes by increasing the $K^+$ ion secretion by exacerbating the water balance of plants and contributing to a decrease in the transpiration process (Ruszkowska and Wojcieska-Wyskupajtys 1996, Maksymiec 1997, Küpper *et al*. 2002). Woźny (1995) indicates that heavy metals, which also include excess copper in the plant, may reduce the water potential in the leaves by limiting the hydration of the plant, thus also inhibiting transpiration. Szatanik-Kloc *et al*. (2010) think that plants die in the presence of phytotoxic $Cu^{2+}$ concentrations as a result of a reduced content of other micro- and macronutrients and related changes in plant metabolic processes.

Photosynthetic effectiveness of water use is often the decisive indicator of plant productivity under stress conditions (Górny and Garczyński 2002). The calculated index varied depending on the copper salt doses used. The effectiveness of the use of water for the $CO_2$ assimilation process in the Jorr varieties decreased together with an increase in copper salt concentrations in the soil. A decrease in the effectiveness of this parameter by 22% was observed for the dose of 250 mg dm$^{-3}$ as compared to control plants. Whereas, in the Bjor varieties, an increase in the effectiveness of water use by almost 33% as compared to the control plant was observed at the highest dose of Cu salt. An increase in the value of this parameter mostly results from the low intensity of transpiration of the tested osier variety (Tab. 1).

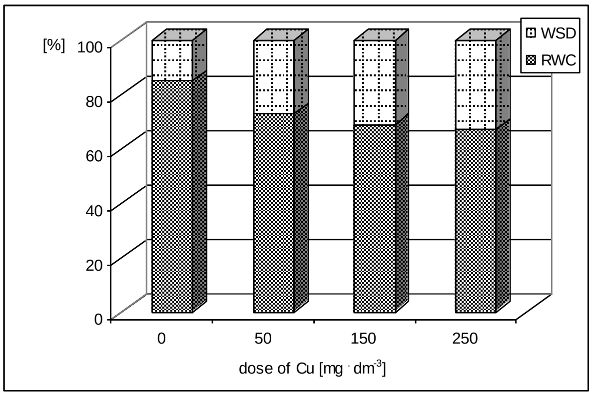
The relative water content (RWC) coefficient and the water saturation deficit coefficients are, amongst other things, indicators of changes in the water balance. Increasing doses of copper salts reduced water content in leaves in both tested varieties of common osier. In both tested osier varieties, the greatest decrease in the relative water content (by 18%) was observed after the application of the largest dose of copper salt, as compared to the control plants (Fig. 1).

When exposed to stress, plants produce excessive amounts of osmoregulators and other substances. The changes observed in the physiological parameters tested may result from both stress induced by an increased number of copper ions in the medium and repair parameters (Wójcik and Tukendorf 1995, Starck 2002).

Cv. Bjor




Cv. Jorr




RWC – relative water content, WSD – water saturation deficit

**Fig. 1.** Water indices (%) of common osier varieties Bjor and Jorr, in relation to the copper ions dose in the medium

Based on gas exchange parameters, an analysis was conducted of the linear correlation between $CO_2$ assimilation (A), transpiration (E) and the concentration of carbon dioxide in intercellular spaces ($c_i$), stomatal conductance for $CO_2$ ($g_c$) and stomatal conductance for water vapour ($g_s$). In the research conducted, $CO_2$ assimilation and transpiration of the Bjor and Jorr osier varieties was largely limited by the stomata. Significant relationships between $CO_2$ assimilation occurred in the tested varieties of osier both under control conditions and under copper salt contamination. The relationship between these parameters increases together with an increase in the copper salt dose (Tab. 2).

**Table 2.** Correlation between $CO_2$ assimilation, transpiration and stomatal conductance of common osier varieties Bjor and Jorr

| Varieties | Variables (y) | Variables (x) | Correlation coefficients (r) |
|---|---|---|---|
| Dose of Cu 0 (mg $dm^{-3}$) | | | |
| Bjor | A | $g_c$ | r = 0.327* |
| | E | $g_s$ | r = 0.433* |
| Jorr | A | $g_c$ | r = 0.397* |
| | E | $g_s$ | r = 0.508* |
| Dose of Cu 50 (mg $dm^{-3}$) | | | |
| Bjor | A | $g_c$ | r = 0.452* |
| | E | $g_s$ | r = 0.539* |
| Jorr | A | $g_c$ | r = 0.425* |
| | E | $g_s$ | r = 0.574* |
| Dose of Cu 150 (mg $dm^{-3}$) | | | |
| Bjor | A | $g_c$ | r = 0.562* |
| | E | $g_s$ | r = 0.611* |
| Jorr | A | $g_c$ | r = 0.697* |
| | E | $g_s$ | r = 0.715* |
| Dose of Cu 250 (mg $dm^{-3}$) | | | |
| Bjor | A | $g_c$ | r = 0.541* |
| | E | $g_s$ | r = 0.628* |
| Jorr | A | $g_c$ | r = 0.746* |
| | E | $g_s$ | r = 0.803* |

A – $CO_2$ assimilation, E – Transpiration, $g_c$ – Stomatal conductance for $CO_2$, $g_s$ – Stomatal conductance for water vapour, *–Significant for 0.05.

The analysis of correlation coefficients for the relationships between transpiration and stomatal conductance for water vapour revealed the highest significant correlation coefficient in Jor with the highest applied dose of Cu salt (Tab. 2). The linear dependence between transpiration and stomatal conductance in common osier under stress conditions was shown by Wróbel and Gregorczyk (2004). Plants close stomata in response to various kinds of stress. It takes place by means of a complicated mechanism with phosphorylation of certain proteins, an increase in the abscisic acid content, which leads to $H_2O_2$ accumulation and the activation of calcium channels in stomatal cell membranes, which results in reduced intensity of transpiration (Mott and Parkhurst 1991, Laloi *et al.* 2004). Slowing down the physiological processes, including $CO_2$ assimilation, transpiration, and stomatal conductance in examined varieties of *Salix viminalis* L., allowed the cultivars to stay in good form under stress condition, making them especially useful in reclamation.

The obtained results of the tested physiological parameters can be useful for the assessment of resistance of the tested varieties of common osier to stress induced by an increased number of copper ions in the medium and of their usefulness in the remediation of areas degraded as a result of human activities.

## CONCLUSIONS

1. The applied doses of copper salts in the willow varieties under study decreased the intensity of $CO_2$ assimilation and transpiration and the assimilation pigments content compared to control plants.
2. $CO_2$ assimilation and transpiration of the Bjor and Jorr varieties of common osier growing in the medium with the addition of copper ions was significantly reduced by stomata.
3. The addition of copper salts to the medium resulted in a decrease in the relative water content (RWC) coefficient and an increase in the water saturation deficit in the leaves of the tested common osier varieties.

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# AKTYWNOŚĆ FIZJOLOGICZNA WIERZBY WICIOWEJ (*SALIX VIMINALIS* L.) W WARUNKACH STRESU WYWOŁANEGO MIEDZIĄ

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S t r e s z c z e n i e. Badano wpływ soli miedzi w zakresie stężeń 50-250 mg·dm$^{-3}$ na reakcję fizjologiczną wierzby wiciowej odmiany Bjor i Jorr. Oznaczono zawartość barwników asymilacyjnych, intensywność asymilacji $CO_2$, transpiracji, przewodność szparkową dla $CO_2$ i pary wodnej, wskaźniki względnej zwartości wody i deficytu wysycenia wodą. Intensywność asymilacji $CO_2$, transpiracji oraz zawartość barwników asymilacyjnych u badanych odmian wierzby wiciowej była różnicowana przez dawkę soli miedzi w pożywce. Stwierdzono spadek natężenia badanych parametrów fizjologicznych wraz ze wzrostem dawki soli miedzi w pożywce. Asymilacja $CO_2$ i transpiracja wierzby wiciowej odmian Bjor i Jorr była w istotny sposób ograniczona szparkowo. Dodatek soli miedzi do pożywki spowodował wzrost deficytu wysycenia wodą w liściach badanych odmian wierzby.

S ł o w a k l u c z o w e : wierzba wiciowa, miedź, asymilacja $CO_2$, transpiracja, barwniki asymilacyjne