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Effect of Ca/Mg ions ratio on copper accumulation, photosynthetic activity and growth of Cu^{2+}-treated *Salix viminalis* L. 'Cannabina'

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Abstract

The aim of the study was to the assess the influence of Ca/Mg ions ratio on the photosynthetic activity of *Salix viminalis* L. 'Cannabina' plants cultivated in medium enriched with $Cu(NO_3)_2$. The experiment was conducted in controlled conditions in a phytotron for 21 days; hence the early plant response was tested. Plants were cultivated with different Ca/Mg ions ratios, *i.e.* $(4:1)_l$, $(4:1)_h$, and 1:10. Plants were additionally treated with $Cu(NO_3)_2$ at 1, 2, and 3 mM concentration in cultivation medium. Net photosynthetic rate, stomatal conductance and transpiration were measured after the first, second and third week of cultivation. Additionally, chlorophyll content, leaf morphology, root biomass and copper accumulation in leaves and roots were investigated. The investigations revealed differences in plant response to particular treatments – differences in Cu accumulation for particular Ca/Mg ions ratios were detected. It seems that plants are adapted to high Cu^{2+} concentrations, when 1:10 Ca/Mg ions ratio is applied. The highest Cu accumulation in roots was noted for plants fertilized with 1:10 Ca/Mg ions ratio, together with high Cu translocation to above-ground plant organs, which suggests its higher potential in phytoremediation.

Additional keywords: Ca/Mg ions ratio; copper, net photosynthesis rate; phytoremediation; *Salix* sp.; stomatal conductance; transpiration rate.

Introduction

Cu is a heavy metal occurring in mine soils and in many industrial areas. It is a highly mobile element in soils (McBride 1994) and an essential element necessary for plant growth and development. Cu plays an important role in plant metabolism, such as enzymatic activities implicated in respiration and photosynthesis (Woolhouse and Walker 1981, Marchner 1995, Faust and Christians 2000). Nevertheless, high levels of Cu^{2+} in medium may induce many negative processes, which result in leaf chlorosis, reduced root and shoot growth, and premature leaf senescence (Vangronsveld and Clijsters 1994, Chen and Kao 1999, Kamenova-Jouhmenko *et al*. 2003, Toller *et al*. 2005).

Plants show variability in tolerance to heavy metals, being able to exclude or accumulate and store them in particular organs, tissues, modified cells or subcellular compartments. Some plant species, called hyperaccumulators, can accumulate large amounts of heavy metals, and can be used in phytoextraction technologies to achieve lower levels of soil pollution (Salt *et al*. 1998).

Accumulation of heavy metals can be modified by the presence of high concentrations of other cations that displace heavy metal ions from binding sites in the cell wall and the outer plasma membrane surface, with evidence that Ca^{2+} ions can be more effective than Mg^{2-} (Kinraide 1994). Earlier investigations revealed that Ca^{2+} could cause a Cu detoxification effect in bean plants at the initial growth stage, while in older plants Ca accumulation supported the induction of the senescence process (Maksymiec and Baszyński 1998), similarly as Cu did, which suggests the possibility of displacement of Ca^{2+} by Cu^{2+} (Maksymiec 1997).

Hyperaccumulation of Ni by *Alyssum bertolonii* was highly reduced, with higher than physiological concentrations of both Ca^{2+} and Mg^{2+} (Vergnano Gambi *et al*. 1992). In hydroponic experiments, Mg^{2+} increased plant

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Abbreviations: Chl – chlorophyll; DM – dry mass; *E* – transpiration rate; FM – fresh mass; g_s – stomatal conductance; LA – leaf area; P_N – net photosynthetic rate; PPFD – photosynthetic photon flux density.
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tolerance to high Cu^{2+} concentrations, but such a situation was not observed in an ecotype adapted to lime soil (Lombini *et al.* 2003).

Interactions between heavy metals and mutual Ca/Mg ions ratios in the phytoremediation process have been described (Ramos *et al.* 2002). Similar observations were reported between microorganisms and the presence of other soil components (Kamnev and Lelie 2000, Feroci *et al.* 2005, Mihucz *et al.* 2008). The presence of different Ca/Mg ratios influences the efficiency of heavy metal accumulation and significantly influences plant growth. Lombini *et al.* (2003) found that plant resistance to Cu^{2+} is connected with low Ca/Mg ions ratio, due to internal detoxification of Cu by means of more efficient chelation and compartmentation.

Heavy metals affect many physiological processes and changes in numerous biochemical compounds, physiological parameters and growth. Earlier experiments revealed a decrease in plant growth when treated with heavy metals (Shvetsova 1998), and a decrease of chlorophyll (Chl), abscisic acid and plant water potential (Rauser and Dumbroff 1981, Angelov *et al.* 1993, Bishnoi *et al.* 1993). Some investigations revealed the influence of heavy metals on stomatal conductance (Rauser and Dumbroff 1981), which might affect the whole plant photosynthetic process. The mechanisms of response of various plant species remain still unknown, which might be partly connected with specific experimental designs, but some investigations have pointed to the involvement of electron transport in light reactions (Giardi *et al.* 1997) and enzyme activity in dark reactions (Van Assche and Clijsters 1990, Chugh and Sawhney 1999). Controlled experiments usually revealed a decrease of photosynthesis rate as a result of heavy metal treatments (Clijsters and Van Assche 1985, Krupa and Baszyński 1995, Pietrini *et al.* 2010). It is possible to examine tolerance to heavy metals in plants by measuring the reduction of the growth rate (Hunt 1978) and impairment of the main physiological functions, such as photosynthetic activity and chlorophyll content (Tognetti *et al.* 2004, Borghi *et al.* 2008).

The effect of heavy metals on photosynthesis is connected with some other elements due to their interactions, *e.g.* Cu^{2+} can substitute for Ca^{2+}. Ca is a very important element in normal functioning of the photosynthetic apparatus, especially at the lumen side of PSII (Ganothakis and Yocum 1990), and the Calvin cycle (Kreimer *et al.* 1988). *In vitro* experiments showed the possibilities of Ca^{2+} substitution by Cu^{2+}, leading to a decrease of PSII activity due to energy losses in the form of heat (Krieger and Weis 1993). The toxic effect of Cu^{2+} on the photosynthetic apparatus of young bean plants was partially connected with Ca^{2+} or Ca^{2+} channels. In older plants, longer Cu^{2+} action caused disturbances at the acceptor site of PSII when higher Ca^{2+} content was present in the nutrient solution. This was due to opening of the Ca^{2+} channels, which intensified the direct inhibitory effect of Cu^{2+} on photosynthetic activity (Maksymiec and Baszyński 1999).

There is still little knowledge about photosynthetic activity of plants treated with heavy metals at different Ca/Mg ions ratios. Hence, our investigations will fill this gap, and provide information for further considerations on whether plants decrease/increase their photosynthetic activity according to different copper concentrations with different Ca/Mg ions ratio applications. This could be crucial for further investigations aiming at phytoremediation techniques with *Salix* sp. cultivated with the addition of Ca^{2+} and Mg^{2+}. Furthermore, it is known that tolerant plants should reveal an increase, or maintenance at a stable level, of the Chl content in response to treatment with metals (Baszyński *et al.* 1982, Stiborova *et al.* 1986). Hence, it is crucial to measure photosynthetic activity parameters together with Chl content. According to Borghi *et al.* (2008), changes in the photosynthesis rate and Chl content in response to Cu^{2+} treatment were associated with a similar response of biomass production.

Materials and methods

Experimental design: One-year-old cuttings of *Salix viminalis* L. cv. 'Cannabina' from 3-year-old rootstock were harvested at the end of November and stored in a moisture box at 5ºC. Before the experiment the standardized stocks (25 cm in length and all similar in diameter, 1.5 cm) were incubated for 10 days for rooting in Knop's medium [4.23 mM of $Ca(NO_3)_2$, 2.5 mM of KNO_3, 1.84 mM of KH_2PO_4, 1 mM of $MgSO_4 \cdot 7H_2O$ and 0.045 mM of $FeSO_4 \cdot 7H_2O$]. Nutrient solution was adjusted to pH 5.19 and electrical conductivity of nutrient solution was 1.27 mS cm^{-1}. Concentrations of the most significant ions were as follows: 6.63 mM of NO_3^-, 1.84 mM of PO_4^{3-}, 4.32 mM of K^+, 4.23 mM of Ca^{2+}, 1.04 mM of Mg^{2+}, 0.045 mM of Fe^{2+} and 1.059 mM of SO_4^{2-}. After that, cuttings (one rod per pot and 4 plants per each Cu concentration) were cultivated in hydroponic pots (13×15 cm, diameter × height), stabilized by ultrapure river sand at 3 different Ca/Mg ions ratios ($1{:}^1/_4$ – $C_{Ca} = 1.06$ mM and $C_{Mg} = 0.26$ mM; 1:10 – $C_{Ca} = 1.06$ mM and $C_{Mg} = 10.4$ mM) and as the reference (control) Ca/Mg 4:1 ratio ($C_{Ca} = 4.23$ mM and $C_{Mg} = 1.04$ mM). For this paper purposes 1:1/4 Ca/Mg ratio is labeled as $(4{:}1)_l$ – lower concentrations, and 4:1 is labeled as $(4{:}1)_h$ – higher concentrations. Ca^{2+} and Mg^{2+}, in the form of $Ca(NO_3)_2$ and $MgSO_4$, were added with other macroelements of Knop's medium. The cultivation medium contained copper soluble salt, *i.e.* $Cu(NO_3)_2 \cdot 3\ H_2O$ at 0 (control), 1, 2, and 3 mM. The experiment was conducted for 21 days in a controlled climate chamber (temperature 18 ± 1°C and air relative humidity 84–87%), equipped with a fluorescent lamp (*MASTER TL-D Secura 58W/830 1SL*, *Koninklijke Philips Electronics N.V.*, The Netherlands) with 16-h photoperiod and photon (radiation) flux of 235 μmol s^{-1} m^{-2} measured at the top of the plant. At the beginning of the experiment, young shoots were approximately 8–10 cm high. Plants were watered with distilled water to maintain a constant medium level. Cultivation pots with concave inserts facilitated constant mixing of the solution.

Photosynthetic activity investigations: Fully developed leaves were chosen for investigations of photosynthetic activity. The measurements were conducted after the first, second, and third week of the experiment. The handheld photosynthetic system *Ci 340aa* (*CID Biosciences Inc.*, Camas, USA) was used to evaluate net photosynthesis (P_N), stomatal conductance (g_s) and transpiration rate (E). For these purposes, constant conditions of measurements in the leaf chamber were maintained: CO_2 inflow concentration [330 μmol(CO_2) mol^{-1}], photosynthetic photon flux density (PPFD) 1,000 μmol(photon) m^{-2} s^{-1}, chamber temperature 30°C, relative humidity 40 ± 3%. Investigations were conducted during midday light conditions, between 10:00 and 15:00 h.

Chl content and leaf growth parameters: Chl (*a*+*b*), *a* and *b* contents were extracted with dimethyl sulfoxide according to the combined methods of Shoaf and Lium (1976) and Hiscox and Israelstam (1978). The measurements were made after the second and third week of the experiment. Mean leaf length, cumulative area and root biomass were measured at the end of the expriment.

Cu content in plant organs: Roots and young shoots were weighed (wet mass), dried at 105 ± 5°C for 24 h (constant mass), acclimated in desiccators, weighed again and moisture content was calculated. *Salix viminalis* L. samples were ground in a vibration laboratory mill. Cu content was analysed in the milled fraction after passing through sieves of diameter 0.5–1 mm.

Concentration of Cu was analysed by flame atomic absorption spectrometry (FAAS) with an *AA 280FS* spectrometer (fast sequential capability and deuterium background correction) and electrothermal atomic absorption spectrometry (ETAAS) with an *AA280 Zeeman* instrument (*Agilent Technologies*, Mulgrave, Victoria, Australia)) (fast transverse Zeeman background correction and *GTA120* graphite furnace).

Hollow-cathode lamps (HCL), from *Varian* and *Perkin Elmer*, were exclusively used (lamps for one element only). The apparatus optimization procedure was performed.

Results were validated on the basis of certified reference materials NIST 1575a (pine needles) and NCS DC 73348 (bush branches and leaves) every tenth sample and by inductively coupled plasma optical emission spectrometry (ICP-OES) with *Vista MPX* apparatus (*Varian*, Australia) in randomly selected samples. As additional verification of results, analysis of selected samples by accredited laboratories and comparison of the results were performed.

Statistical analysis: The data were analysed with the statistical software *STATISTICA 9.1*. Results were analysed with a factorial *ANOVA* with "week of cultivation", "Mg/Ca ions ratio" and "Cu^{2+} level" fixed factors. *Tukey*'s test was employed to analyse differences between measured parameters. A graphical presentation of *Tukey*'s test results is provided in the present study.

Results

In the case of $(4:1)_l$ Ca/Mg ions ratio, statistically significant (p=0.05) decrease of P_N was observed for 0 mM Cu^{2+} concentration in the second week of the experiment, while in the case of the three Cu^{2+} treatments a slight increase (not statistically significant) or maintenance at a similar level was observed. Similar levels of P_N after the third week of the experiment for 3 mM Cu^{2+} and statistically significant decrease (p=0.05) for 1 and 2 mM Cu^{2+} treatments were noted, while an increase (statistically significant, p=0.05) was seen for the control. Similar tendencies were noted for stomatal conductance and transpiration rate parameters, excluding E for 3 mM Cu^{2+} treatment in the third week, where statistically significant increase (p=0.05) was recorded (Fig. 1*A*,*B*,*C*).

Plants cultivated with 1:10 Ca/Mg ions ratio revealed the highest level of P_N, g_s and E in control plants (statistically significant difference, p=0.05, excluding the last week of P_N). Plants treated with 1 and 2 mM Cu^{2+} revealed lower levels (especially in the first two weeks of cultivation). An increase of P_N at the end of the experiment was noted, although not statistically significant. Simultaneously, for the 3 mM Cu^{2+} treatment the P_N level increased in second week of experiment (statistically significant, p=0.05), than decreased in the last week, but it was comparable with the control plants. A similar tendency was observed for the rest of the parameters connected with the photosynthetic activity. But g_s and E for 3 mM Cu^{2+} increased significantly in the last week (Fig. 1*D*,*E*,*F*).

A decrease (statistically significant, p=0.05) of P_N was observed in the second week of the experiment for application of Ca/Mg at 4:1 ions ratio for control and 1 mM Cu^{2+} treatments, while after the third week higher values (statistically different, p=0.05) were noted. Similar tendencies were observed for 2 and 3 mM Cu^{2+} treatments, although mostly not statistically significant (Fig. 1*G*). Stomatal conductance and transpiration rate gave similar results for control plants, while, for plants treated with Cu^{2+} lower values were noted in first two weeks, statistically different (p=0.05) in comparison to

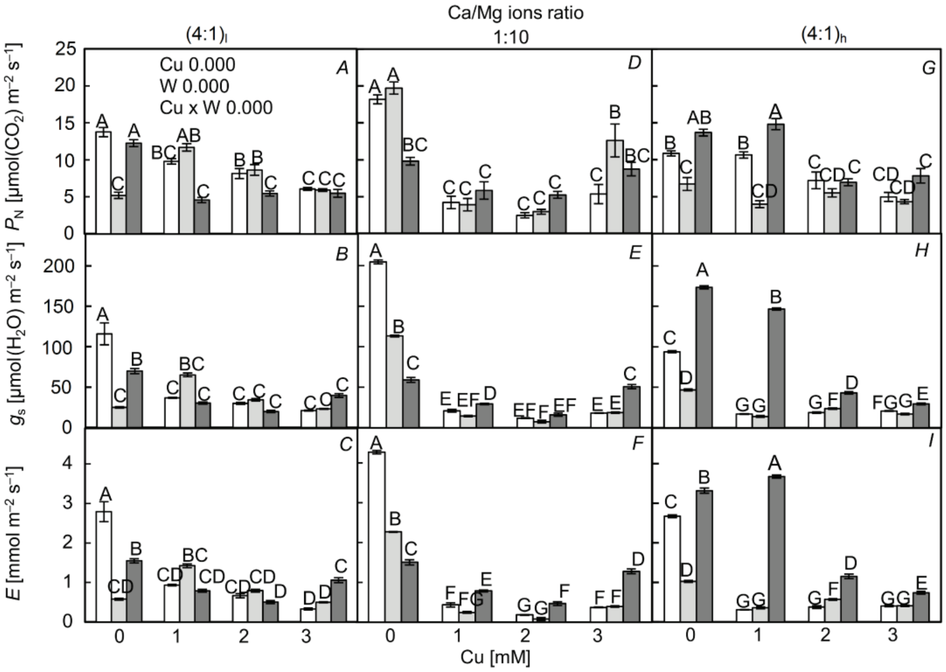

Fig. 1. Net photosynthetic rate (P_N), stomatal conductance (g_s) and transpiration rate (E) measured for *Salix viminalis* L. plants cultivated in medium with Ca/Mg ions ratio of (4:1)$_l$; 1:10; (4:1)$_h$ and enriched with Cu^{2+} soluble salt (means ± SE; n = 4; *different letters* denote significant differences between means at p=0.05). White columns – results of measurements in 1st week of experiment, light grey – 2nd week, dark grey – 3rd week. The p-values of *ANOVA* analysis of Cu^{2+} application level (Cu), week of measurement (W) and interaction (Cu × W) are presented in each graph. (p=0.000 for interaction Cu × W × Ca/Mg for all measured parameters).

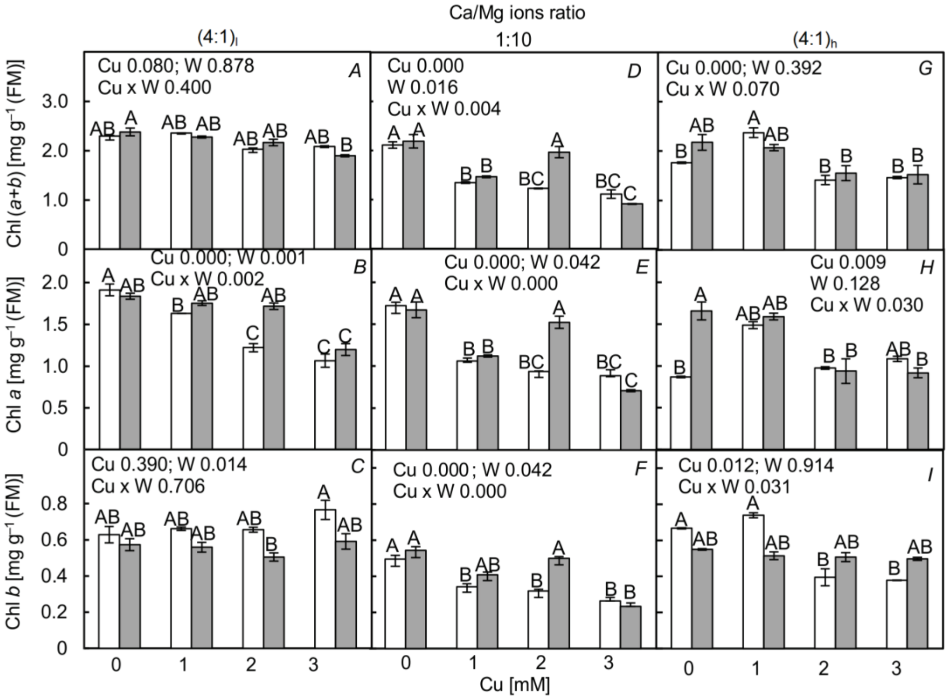

Fig. 2. Chlorophyll (Chl) (*a*+*b*), *a* and *b* contents in leaves of *Salix viminalis* L. plants cultivated in medium with Ca/Mg ions ratios of (4:1)$_l$; 1:10; (4:1)$_h$ and enriched with Cu^{2+} soluble salt (means ± SE; n = 4; *different letters* denote significant differences between means at p=0.05). White columns – results in the 2nd week, light grey columns – 3rd week. The p-values of *ANOVA* analysis of Cu^{2+} application level (Cu), week of measurement (W) and interaction (Cu × W) are presented in each graph. (p=0.000 for interaction Cu × W × Ca/Mg for all Chl forms).

Table 1. Means ± SE of chlorophyll *a/b* ratios in *Salix* leaves treated with different Cu^{2+} levels and Ca/Mg ions ratios.

Cu [mM]	Ca/Mg ions ratio					
	$(4:1)_{\mathrm{l}}$		1:10		$(4:1)_{\mathrm{h}}$	
	2nd week	3rd week	2nd week	3rd week	2nd week	3rd week
0	3.051 ± 0.109	3.218 ± 0.147	3.489 ± 0.357	3.085 ± 0.108	1.301 ± 0.019	3.029 ± 0.076
1	2.467 ± 0.002	3.137 ± 0.047	3.113 ± 0.345	2.761 ± 0.025	2.187 ± 0.013	3.095 ± 0.053
2	1.932 ± 0.032	3.401 ± 0.072	2.948 ± 0.159	3.058 ± 0.099	2.501 ± 0.026	1.839 ± 0.019
3	1.405 ± 0.019	2.046 ± 0.027	3.352 ± 0.046	3.031 ± 0.073	2.865 ± 0.090	1.877 ± 0.019

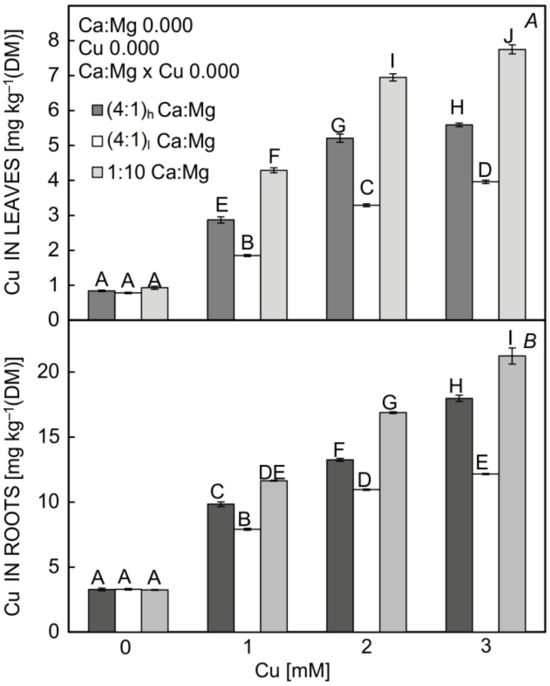

Fig. 3. Cu accumulation in *Salix viminalis* L. leaves and roots harvested after three weeks of cultivation on medium at different Ca/Mg ions ratios and enriched with Cu^{2+} soluble salt (Means ± SE; *n* = 4; *different letters* denote significant differences between means at *p*=0.05). The *p*-values of *ANOVA* analysis of Cu^{2+} application level (Cu), Ca/Mg ions ratio (Ca/Mg) and interaction (Ca/Mg × Cu) are presented in each graph.

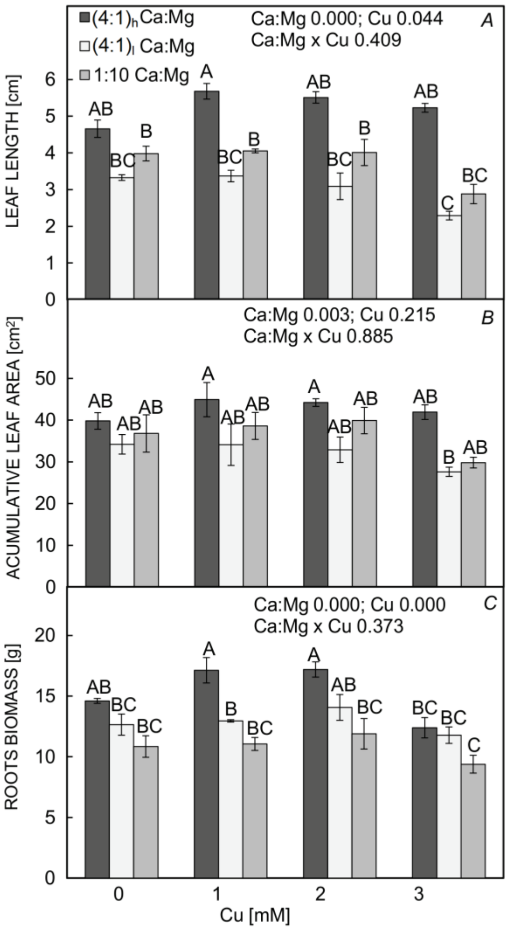

Fig. 4. Parameters of leaf and root growth of *Salix viminalis* L. harvested after three weeks of cultivation in medium at different Ca/Mg ions ratios and enriched with Cu^{2+} soluble salt (means ± SE; *n* = 4; *different letters* denote significant differences between means at *p*=0.05). The *p*-values of *ANOVA* analysis of Cu^{2+} application level (Cu), Ca/Mg ions ratio (Ca/Mg) and interaction (Ca/Mg × Cu) are presented in each graph.

results of third week (Fig. 1*H*,*I*).

A comparable level of Chl (*a*+*b*) contents was noted in plants with $(4:1)_{\mathrm{l}}$ Ca/Mg ions ratio for all Cu treatments and control, while lower values of Chl *a* content were noted in the case of plants treated with 3 mM Cu^{2+}. Higher Chl (*a*+*b*) and *a* contents (statistically significant, *p*=0.05) were noted in control plants cultivated with 1:10 Ca/Mg ions ratio. Lower concentrations (statistically significant, *p*=0.05) were again observed in plants treated with 3 mM Cu^{2+}. This was true for all Chl contents, although not always statistically significant. There were not such clear tendencies for $(4:1)_{\mathrm{h}}$ Ca/Mg ions ratio (Fig. 2). The Chl *a*/*b* ratio was the highest in plants cultivated with 1:10 Ca/Mg ions ratio in comparison to almost all the Ca/Mg ions ratios and Cu combinations (Table 1).

The highest (statistically significant, *p*=0.05) Cu accumulation in leaves was seen for plants cultivated with 1:10 Ca/Mg ions ratio and the lowest for plants with $(4:1)_{\mathrm{l}}$ Ca/Mg ions ratio. Cu concentrations in roots were approximately three times higher than in leaves. However, the pattern of concentrations was the same as for leaves (Fig. 3). The leaf area did not vary between Cu concentrations; the Ca/Mg ions ratio influenced leaf growth more. It seems that the highest leaf increase was noted for plants with $(4{:}1)_h$ ratio, which was treated as a reference, and the lowest values were noted for plants cultivated with $(4{:}1)_l$ Ca/Mg ions ratio. However, these differences were not always significant at level $p=0.05$. In the case of root biomass, the lowest values (not always significantly different) were observed for plants cultivated with 1:10 Ca/Mg ions ratio (Fig. 4).

Discussion

Cu concentration in aboveground plant parts usually varies between 2 and 20 mg kg^{-1}, regardless of Cu concentration in soil (Wallnöfer and Engelhardt 1984). Our results are in agreement with these results. In contrast, accumulation in the range 20–60 mg kg^{-1} was found in *B. pendula* (Kopponen *et al.* 2001) and *S. viminalis* (Punshon *et al.* 1995), when Cu^{2+} salts were added to a substrate or medium. According to Kabata-Pendias and Pendias (2001), Cu accumulation becomes toxic for sensitive plants if it reaches 15–20 mg kg^{-1}. In our experiment, Cu accumulation did not reach these values, probably due to the short time of the experiment. The Cu concentration in roots was three times higher than in aboveground parts, and translocation was higher in plants cultivated at higher Cu^{2+} concentration in the medium. Copper is treated as hardly mobile element in plant organs, when concentration is very low in the medium, but when it increases the transport is more effective (Siedlecka *et al.* 2001, Baldantoni *et al.* 2004). Our results are in agreement with these observations.

Low Cu sorption by roots and transport to leaves by plants cultivated at $(4{:}1)_l$ Ca/Mg ions ratio were noted. However, no differences in P_N at the end of the experiment between the three Cu^{2+} treatments and a higher level in control plants were observed, as well as low values (although not statistically significant) of leaf morphological parameters. So it suggested that low copper sorption and translocation to aboveground parts did not protect the plants against the negative effect of Cu^{2+} level in medium. On the other hand, the highest Cu accumulation was revealed in plants cultivated with 1:10 Ca/Mg ions ratio, while photosynthesis activity in control plants and at 3 mM Cu^{2+} treatment was at a comparable level at the end of the experiment. The strong decrease of P_N in control plants in the last week of the experiment might be explained as the effect of deficit of Cu^{2+} in the medium. However, the similar level of photosynthetic activity in control and in 3 mM Cu^{2+}-treated plants, as suggested by Lombini *et al.* (2003), could be explained as low Ca/Mg ions ratio is more favourable to internal detoxification of Cu by means of efficient chelation and compartmentation. So, low Ca/Mg ions ratio increased Cu^{2+} uptake but did not worsen the toxicity effect. However, we observed the lowest (although not always significant) root biomass in plants cultivated with 1:10 Ca/Mg ions ratio, while Lombini *et al.* (2003) observed variation between *Silene* cultivars. They suggested that low Ca/Mg ions ratio enhanced the resistance to Cu^{2+} of the cultivar which is already resistant to this element. Plants cultivated in the medium at $(4{:}1)_h$ Ca/Mg ions ratio, which is treated as a reference, revealed a similar levels of P_N at the end of the experiment when 2 and 3 mM Cu^{2+} were applied, while a similar level for control and 1 mM were noted. Moreover, the highest values of root biomass and leaf morphology were noted for all Cu^{2+} concentrations in plants cultivated with $(4{:}1)_h$ Ca/Mg ions ratio. Lombini *et al.* (2003) reported that stronger root elongation of *Silene* plants cultivated with high Ca/Mg ions ratio was noted only in the cultivar with low resistance to Cu^{2+}. They also noted not so high concentration and translocation of Cu to aboveground plant organs for all *Silene* cultivars exposed to high Ca/Mg ions ratio.

As mentioned before, there is still little knowledge about the effect of heavy metals on tree species' photosynthetic activity. Some investigators did not prove differences in plant response to heavy metals' presence in soil (Singh and Agrawal 2007), although a decrease of P_N during the growing season was observed for all heavy metals. Hattab *et al.* (2009) reported a decrease of *Pisum sativum* photosynthetic activity together with an increase of heavy metal concentration. A similar tendency was observed by Pietrini *et al.* (2010) in the case of poplar. This might suggest differences between tree species in response to heavy metals. However, our earlier investigations with *Betula* sp. treated with two different heavy metal levels revealed higher photosynthetic activity in plants where higher concentrations were applied (Borowiak, unpublished data).

For better evaluation of the effect of Cu on the photosynthetic process, Chl content in leaves was also investigated. It is known that interveinal chlorosis of leaves is one of the earliest symptoms of the Cu toxicity (Taylor and Foy 1985). This is due to the inhibition of Chl biosynthesis and a subsequent drop of Chl content, caused by a damage of thylakoid membranes (Maksymiec 1997). Cu in leaves negatively interferes with the electron transport chain and the oxygen evolving complex of PSII (Romanowska 2000). Simultaneously, it has been discovered that Chl content in tolerant plants (*Populus* × *canadensis*) did not decrease, or even increased the response to Cu^{2+} levels. A similar tendency was noted for P_N – an increase for tolerant *P.* × *canadensis* and a decrease for sensitive *P. alba* (Borghi *et al.* 2008). Nevertheless, an experiment with *Salix* species and different applications of Cu^{2+} revealed no effect on Chl content (Hakmaoui *et al.* 2006). In our experiment, we did not observe clear tendencies in Chl content. Usually it is closely related to photosynthetic activity and Cu^{2+} concentration. Hence, we mainly observed a decrease or maintenance at a similar level of Chl content due to increase of Cu^{2+} concentration. This was especially true for Chl *b*.

Ca is a very important macronutrient for proper functioning of trees (McLaughlin and Wimmer 1997). It is also found as a second messenger in the signal transduction between environmental factors and plant responses (Marschner 1995). Magnesium is also a very important element for plants, as it is a component of Chl. Investigations with spruce plants fertilized with Ca^{2+}, K^+ and Mg^{2+} revealed a significant increase in periclinal cell division compared with control trees (Dünisch and Bauch 1994). The increase of Ca content could result in an increase of stem diameter, total fresh mass and root dry mass of spruce plants (Österås and Greger 2003). Elevated Ca^{2+} levels could result in changes of the properties of wood formed in trees (Eklund and Eliasson 1990). As mentioned before, there is still a lack of detailed information about the interaction of Ca^{2+} and Mg^{2+}, and its impact on heavy metal uptake by plants. Österås and Greger (2003) demonstrated that Cd^{2+} and Cu^{2+} treatments gave a smaller diameter of 2-year-old spruce trees compared to control ones. The toxic effect on wood formation was suggested to be related to a deficiency of Ca in wood and bark, due to low Ca concentration. The application of Cu^{2+} could decrease wood formation, probably due to competition of Cu^{2+} *vs.* Ca^{2+}. Moreover, if we do not provide proper Ca^{2+} nutrition, the continuous drop of Ca could cause a negative effect on wood formation (Österås and Greger 2003). On the other hand, high Ca^{2+} concentration could cause negative effects on trees (Dünisch *et al.* 1998). In our study, we observed differences in plant response to different Cu^{2+} concentrations combined with different Ca/Mg ions ratios in the medium. It seems that Ca plays an important role in the wood creation process, and Mg in the photosynthetic process, hence we noted a positive effect in the case of 1:10 Ca/Mg ions ratio.

As previously described, Cu^{2+} treatment caused growth inhibition, leaf area reduction and a decrease of root biomass (Lidon and Henriques 1993, Maksymiec 1997). The degree of growth reduction as a response to Cu^{2+} application was species/clone dependent (Borgegård and Håkan 1989, Punshon *et al.* 1995). Furthermore, no reduction in leaf area or significant variation in growth parameters for *P.* × *euramericana* plants growing in medium containing low Cu^{2+} concentrations was noted (Borghi *et al.* 2007). Kuzovkina *et al.* (2004) even noted a significant stimulation of plant growth at low Cu^{2+} treatments, while inhibition was observed at higher Cu^{2+} concentrations. In our investigations, the lowest leaf area was observed for plants fertilized with $(4:1)_l$ Ca/Mg ions ratio, where the lowest Cu accumulation in leaves was noted.

Overall, the experiment revealed the influence of different Ca/Mg ions ratios on photosynthetic activity of *Salix* sp. treated with Cu^{2+}. The highest Cu accumulation in roots was noted for plants fertilized with 1:10 Ca/Mg ions ratio, together with high Cu translocation to aboveground plant organs, which suggests its higher potential in phytoremediation. Together with high Cu accumulation in plant organs cultivated with 1:10 Ca/Mg ions ratio, a similar level of photosynthesis activity was revealed in control plants and with 3 mM of Cu^{2+} at the end of the short experiment, probably due to adaptation to higher Cu^{2+} concentration in the medium.

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