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Changes in the degree of cellulose polymerisation of *Salix viminalis* willow seedlings after supplementation with Cu and Pb

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Abstract: *Changes in the degree of cellulose polymerisation of Salix viminalis willow seedlings after supplementation with Cu and Pb*. The study examined the degree of polymerisation and polydispersity of cellulose of willow seedlings in a hydroponic cultivation supplemented with copper and lead. Differences were demonstrated in the cellulose chain lengths as well as in the content of individual fractions of molecular weights when the medium was supplemented with copper and lead.

Keywords: cellulose, degree of polymerization, hydroponic cultivation, heavy metals

INTRODUCTION

Willow is a plant which is extremely well adapting to the environment polluted with heavy metals. According investigations conducted by Mocek (1996) for several cultivars of willow growing near the copper plant in Legnica the highest content of heavy metals was found in leafs and roots. Rods being utilitarian parts of the plant contained the lowest amount of heavy metals. Despite of the high amount of the elements in the plant there was not observed slowing down of the growth and development of bushes. It proves high adaptation ability of willows.

The contamination of the natural environment with heavy metals may cause changes in the chemical composition of wood. The investigations performed for willows growing in a plantation near the A-2 road (Brzozowska and Prądzyński 1995) showed the distinct decrease of cellulose content from 51% in six years old rods to 37% in rods sampled in the first year after planting. There was also observed decrease of lignin of 3% as well as insignificant increase of extractive substances.

Values that characterise cellulose structure as a polymer include the degree of polymerisation and its polydispersity. Knowledge of these values is necessary to assess the strength of cellulose fibre as well as the entire timber material as biopolymer. Reduced degree of polymerisation results in deterioration of timber properties (Prosiński 1984).

MATERIALS AND METHOD

One year old *Salix viminalis* cuttings came from a 2-year old rootstock harvested in the end of November and stored in moisture box at 5 ºC. Before experiment the standardized stocks (20 cm of length and similar diameter: diameter of shoots – 6-8 mm, diameter of pith – 3.8-4.0 mm) were incubated for 10 days for rooting in 50% concentration of Knop medium (10 cm^3 of 10% $Ca(NO_3)_2$, 2.5 cm^3 of 10% KNO_3, 1.2 cm^3 of 10% KCl, 10 cm^3 of 2.5% KH_2PO_4, 5 cm^3 of 5% $MgSO_4$ and 0.25 cm^3 of 0,25% $FeCl_3$ in 1 dm^3 of acidified water at pH=3.94). After that cuttings were put individually into hydroponic pots and stabilized by the ultra pure

river sand. Experiment was conducted 21 days and the reference systems was combination without heavy metals in Knops medium. Two solution with different Ca/Mg ratio were used as follows: 20:1 and 1:10. The each plant was incubated in 0.5 dm^3 of 0.5 mM solution of each of tested 2 heavy metals: Cu and Pb. Solution of individual heavy metals (Cu^{2+}, Pb^{2+}) was prepared on the base of analytical grade nitrates (V) dissolved in de-ionized water (Milli-Q) acidified to 0.08% nitric acid content (Ultrapure).
The experiment was performed in a controlled climate chamber equipped with fluorescent lamp (MASTER TL-D Secura 58W/830 1SL) providing a photon (radiation) flux of 220 µE sec^{-1} m^{-2} (µmol sec^{-1} m^{-2}) in the top of the plant for 16 hours of 20±1°C and humidity of 81-84%. Loss of water was compensated by pure water. The growth medium was not replaced during experiment with metals and no bactericide was used.
Analysis of molecular weights, degree of polymerization and polydispersity of cellulose were performed by gel permeation chromatography (GPC/SEC). GPS System consist of HP 1050 Hewlet Packard liquid chromatographer equipped with differential refractometric detector HP 1047A, manual injector (Model 7125 - Rheodyne Inc.) and column set: 3x PLgel Mixed A, 20µm + guard (Polymer Laboratories Ltd.). Chromatographic parameters were: solvent - DMAC/0.5% LiCl, flow rate - 1.0 ml min^{-1}, column set - N`= 10158 (plates/metr), Rs=1.65, concentration - 0.05%, injection value - 100 µl, temperature - 80 ºC and paek start/end - 17.60/26.92. Calibration was performed using barrow polystyrene standards. Values of molecular weights were calculated using universal calibration methods. Parameters K and a (Mark-Houwink equation) were as follows: cellulose: K=2.78 10^{-5} [dL g^{-1}] and a=0.957 and polystyrene standards: K=17.35 10^{-5} [dL g^{-1}] and a=0.642.

RESULTS

Initial investigations were carried out at the Institute of Wood Technology and Department of Plant Physiology of the Poznań University of Life Sciences regarding the impact of the addition of some heavy metals on changes in the wood of *Salix viminalis* during hydroponic cultivation. On the basis of the obtained results, two metals - copper and lead – were selected for further experiments with the aim to examine the influence of their addition to the medium on the quality of cellulose in the timber of seedlings. Bearing in mind the fact that changes in cellulose quantities occurred only in specific combinations, analyses of the most interesting of them were performed with the aim to assess the degree of cellulose polymerisation and polydispersity and to determine the distribution of the molecular weight and mean molecular weights. The following three experimental combinations were analysed:

1. Control combination 0, in order to determine initial parameters (samples subjected to no treatment),
2. Addition of $Pb_{0.5}$ (at Ca/Mg 20:1) - due to the highest cellulose content,
3. Addition of $Cu_{0.5}$ (at Ca/Mg 1:10) - due to the lowest cellulose content.

Table 1.Characteristic of cellulose for *Salix* cuttings incubated in selected combinations

Parameter		Unit	Combinations		
			0 (control)	$Cu_{0.5}$ (Ca/Mg 1:10)	$Pb_{0.5}$ (Ca/Mg 20:1)
			Molecular weights averages		
M_p		g mol^{-1}	71796	67464	64703
M_n			40566	34813	35836
M_v			184720	176052	144771
M_w			190307	181481	148840
DP_w		-	**1180**	**1120**	**920**
PDI (M_w/M_n)		-	4.7	5.2	4.2
			Content of fractions, M [%]		
low	M<20000		13	16	15
medium	20000<M<200000		60	58	63
high	M>200000		27	26	22

M_p – amount of highly polymerised molecules; M_n - number-average molecular weight; M_v – viscosity-average molecular weight; M_w - weight-average molecular weight; DP_w - degree of polymerization, PDI – polydispersity;

The obtained results collated in Table 1 show a decline in the degree of cellulose polymerisation. The highest drop in the degree of cellulose polymerisation was observed in samples in which both the highest and lowest cellulose contents were determined. In the sample with the addition of $Pb_{0.5}$ (Ca/Mg 20:1), in which the lowest cellulose content of 39.75% was recorded (Waliszewska et al., 2010), the degree of its polymerisation amounted to 920, while in the reference sample DP_w, it amounted to 1120, which means that it was close to 1200, i.e. the value characteristic for the cellulose from wood obtained with the assistance of Seifert's method. In the case of samples incubated in $Cu_{0.5}$ (Ca/Mg 1:10) solution, the degree of cellulose polymerisation dropped to 1120, at the highest degree of PDI polydispersity amounting to 5.2. On the other hand, the lowest degree of cellulose polydispersity of 4.2 was determined in samples subjected to the action of $Pb_{0.5}$ (Ca/Mg 20:1) solution which was also accompanied by the lowest degree of cellulose polymerisation amounting to 920. In willow samples incubated in both $Cu_{0.5}$ (Ca/Mg 1:10) and $Pb_{0.5}$ (Ca/Mg 20:1) solutions, the percentage proportion of individual fractions of molecular weights, in relation to the control sample, showed an increase in the proportion of the fraction with molecular weight of M<20 000 and a decrease in the proportion of the M>200 000 fraction. In the case of the sample treated with the $Pb_{0.5}$ (Ca/Mg 20:1) solution, the proportion of the fraction of the highest molecular weight amounted to 22%, while in the control sample – 27%.

It is difficult to state unequivocally what the probable cause of shortening of cellulose chains was. It is known that hemicelluloses and pectins are characterised by a greater capacity to bind heavy metal ions than cellulose and lignin. Cellulose molecules, forming characteristic chains, are arranged in a regular manner giving cell walls a precisely definite, orderly structure. Between cellulose chains, there are free spaces filled with water and pectins and, hence, the observed considerable ease of accumulation of metal ions in these spaces. It is the opinion of the authors that the presence of heavy metal ions in these places, combined with acid hydrolysis triggered off by the action of acid metal salts, can be the main causes of shortening of cellulose chains. At the same time, variations in the degree of chain shortening, depending on the type of the metal found in the medium, indicate a slightly different influence of each of them on changes in the plant structure.

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Streszczenie*: Zmiany stopnia polimeryzacji celulozy sadzonek wierzbowych Salix viminalis w wyniku dodatku Cu i Pb.* W pracy zbadano zmiany stopnia polimeryzacji i polidyspersyjności celulozy sadzonek wierzbowych w uprawie hydroponicznej z dodatkiem miedzi i ołowiu. Wykazano różnice w długości łańcuchów celulozowych oraz zawartości poszczególnych frakcji mas cząsteczkowych przy dodatku miedzi i ołowiu do pożywki.

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