



ORIGINAL RESEARCH

Changes in the chemical composition and the structure of cellulose and lignin in elm wood exposed to various forms of arsenic

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Abstract The chemical composition of wood depends on many factors, including plant growth conditions. Among the many dangerous elements it was decided in this study to select arsenic in various forms: As(III), As(V) and cacodylic acid (DMAA), as well as various concentrations and mutual combinations. The experiment was conducted on 1-year old elm seedlings planted in pots with an addition of Knop medium and arsenic. Supplementation of the substrate with each arsenic form caused changes in contents of cellulose and lignin as well as extractive substances

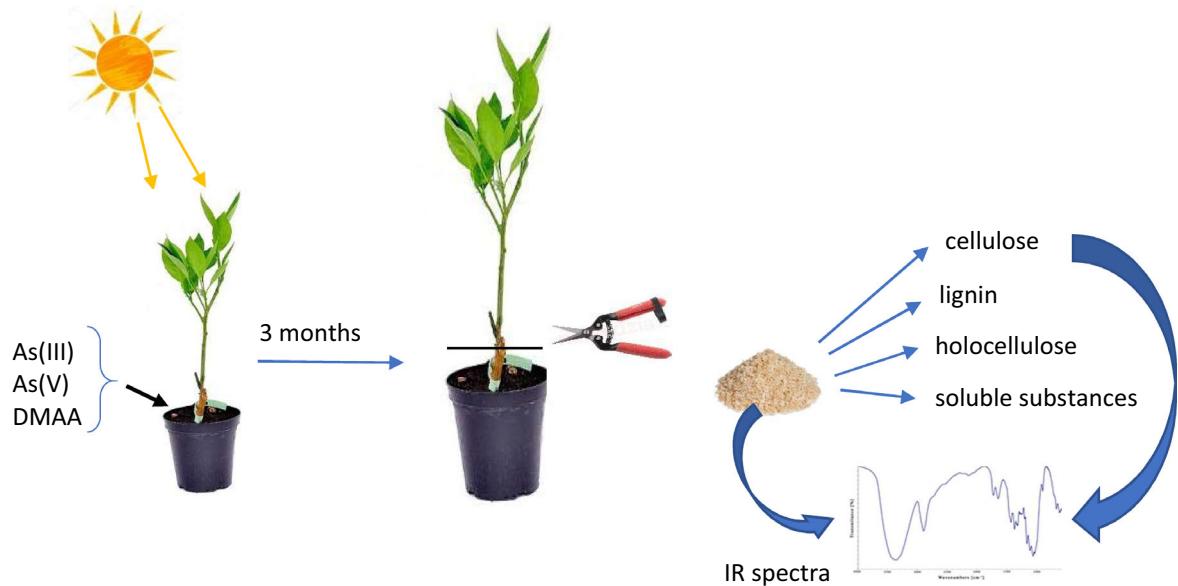
and those soluble in 1% NaOH. Arsenic also led to changes in the structure of basic wood components. The FTIR analysis showed the greatest changes under the influence of As(V), As(III)/As(V)/DMA. Despite high arsenic concentration in the substrate 1-year old elm seedlings survived the period of the experiment, thus providing valuable results and broadening our knowledge on the response of trees to the environment contaminated with arsenic.

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Graphical abstract



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Introduction

Cellulose is the main component of plant cell walls and ranks very high in terms of its contents in the plant world. Wood contains approx. 40–45% cellulose (Rowell et al. 2005), while cellulose content in cotton may be as high as 95% (Prosiński 1984). It is a natural chain polymer composed of D-glucopyranose residues that are bound by β -(1 → 4)-glucosidic bonds (Rowell et al. 2005; Fengel and Wegener 1989). Cellulose chains in various materials differ in their lengths. The number of D-glucopyranose constitutional units in the chain defines the degree of polymerisation (DP). In turn, the ratio of crystalline areas to amorphous areas in the structure of a given fibre is defined as crystallinity of cellulose. The degree of polymerization and cellulose crystallinity (CrI) are some of the most useful characteristics of renewable and biodegradable materials, because they play an important role in their physical, mechanical, and chemical properties. Knowledge on the degree of polymerisation is of great importance for the assessment of

strength of a given fibre. DP of native cellulose varies depending on plant material, ranging from 9000 to 10,000, and possibly may be as high as 15,000 (Pettersen 1984).

Next to cellulose, lignin is the most abundant and important polymeric organic substance found in the plant world. Lignin is a phenolic substance consisting of an irregular array of variously bonded hydroxy- and methoxy-substituted phenylpropane units. Lignin content in wood falls within a wide range from 20 to 40% (Fengel and Wegener 1989).

The primary structural components of wood include also hemicelluloses, i.e. polysaccharides with a low degree of polymerisation (average DP of 100–200) and more branched chains. Their content in wood is 15–30% (Rowell et al. 2005). Hemicelluloses are soluble in alkali and are easily hydrolysed by acids.

Amounts of individual wood components depend on many factors, e.g. the plant species, age, plant part and growth conditions. For example, contents of cellulose and lignin as well as other wood components change under the influence of air pollution (Waliszewska 2002; Waliszewska and Prądzynski 2002; Judzentiene et al. 2007). Anthropogenic environmental pollution, particularly in industrialised areas, is huge worldwide. Thousands of tons of heavy metals

are released to the environment. In 2015 in Poland the total emissions of certain toxic heavy metals amounted to 43.5 Mg (tons) arsenic, 10.6 Mg mercury, 13.5 Mg cadmium, 1407.1 Mg zinc and 415.6 Mg copper (GUS 2017). Arsenic as an element is found in the environment in various forms differing in their degree of toxicity: arsenite [As(III)], arsenate [As(V)], arsenious acids (HAsO_3^{2-} ; H_2AsO_3^- ; H_3AsO_3); arsenic acids (HAsO_4^{2-} ; H_2AsO_4^- ; H_3AsO_4) monomethylarsenate (MMA), dimethylarsinate (DMAA), etc. (Kumaresan and Riyazuddin 2001; Singh et al. 2015). Information on the toxicity of these compounds and their effect on growth and development of living organisms (plants, animals, humans) is of paramount importance (Jedynak et al. 2010). Some plants are capable of adapting to different growth conditions by accumulating harmful compounds in their organisms (Budzyńska et al. 2018). It obviously has an effect on their health condition or even survival rates. These substances may cause changes in the structure of basic structural compounds, such as e.g. cellulose or lignin, while they may also reduce the increment of biomass and alter morphological characteristics (Makgalaka-Matlala et al. 2008; Jiang and Singh 1994; Melo et al. 2009; Gomes et al. 2012; Carbonell et al. 1998).

The aim of this study was to investigate contents and changes in the structure of specific structural components (cellulose, lignin, holocellulose, extractives and substances soluble in alkalies) in wood of elm (*Ulmus laevis* Pall) under the influence of various arsenic forms: As (III), As (V), and DMAA (cacodylic acid) as well as their combinations in modified Knop medium. Results made it possible to verify previous reports (Krutul et al. 2006, 2010, 2014; Mleczek et al. 2018) that some forms of pollution causes a decrease in cellulose content, an increase in the amount of components dissolved in alkali, and an increase in lignin content.

Theory

The theoretical assumption for the experiment was that plants adapt to new environmental conditions and the aim was to gain knowledge on the influence of As on the chemical composition of elm wood.

Materials and methods

Characteristics of material

One-year old seedlings of white elm (*Ulmus laevis*) were collected from the forest nursery of the Pniewy Forest Division ($52^{\circ}29'04''\text{N}$; $16^{\circ}15'28''\text{E}$) in March 2016. The plants were weighed and measured before the experiment. Seedlings were selected to ensure uniformity of their weight and height. The mean biomass was 16.6 g and mean height was 63.9 cm (Budzyńska et al. 2017).

Experiment design

The pot experiment was conducted in a greenhouse for a period of three months. The mean values of temperature, humidity and CO_2 concentration were 23.6°C , 39% and 446 ppm, respectively. Elm seedlings were cultivated in hydroponic pots (diameter \times height, 18 \times 19 cm) so that the experimental systems were set up with four plants cultivated in each separate pot (Table 1).

Plants were stabilised using approx. 1.2 kg of ultrapure quartz sand per pot ($\text{pH} = 7.2$, content of $\text{SiO}_2 > 97\%$) with a particle size of 1–3 mm. Modified Knop solution (0.8 L per pot) prepared strictly according to Barabasz et al. (2010) was enriched with the following As forms: As(III), As(V) and DMAA (dimethylarsinic acid or cacodylic acid) applied as sodium (meta) arsenite (AsNaO_2), sodium arsenate dibasic heptahydrate ($\text{Na}_2\text{HAsO}_4 \cdot 7\text{H}_2\text{O}$) and dimethylarsinic acid ($\text{CH}_3)_2\text{As(O)OH}$). Particular As forms were added to the final concentrations at 0.06 or 0.6 mM L^{-1} at the start of the experiment (Table 1).

Preparation of the samples

Following the experimental plant growth period elm shoots were harvested, leaves and roots were removed and stems were seasoned at the laboratory until air dry. After reaching moisture content of approx. 7% shoots (with bark) were cut manually and next ground in a laboratory grinding mill by Retsch. Screens were used to separate the analytical fraction of 0.1–0.4 mm, which was used in the study.

Table 1 Characteristics of the experimental design

Sample	System	Addition of As species (mM L ⁻¹)
Elm 1—control	Control	0
Elm As 2	As(III)	0.06
Elm As 3	As(V)	0.06
Elm As 4	DMAA	0.6
Elm As 5	As(III)/As(V)	0.06/0.06
Elm As 6	As(III)/DMAA	0.06/0.06
Elm As 7	As(V)/DMAA	0.06/0.6
Elm As 8	As(III)/As(V)/DMAA	0.6/0.06/0.06

Determination of chemical composition

Analyses of the raw material chemical composition were performed according to the TAPPI standard for plant material and the following parameters were determined:

- cellulose content according to Seifert using a mixture of acetylacetone and dioxane (Seifert 1960);
- lignin content (TAPPI – T 222 om-06) using concentrated sulfuric acid;
- holocellulose content using sodium chlorite (TAPPI – T 9 wd-75);
- amount of substances soluble in organic solvents according to Soxhlet (TAPPI – T 204 cm-07);
- substances soluble in 1% NaOH (TAPPI – T 212 om-07).

Hemicellulose content was calculated theoretically from the difference in the contents of holocellulose and cellulose.

Determination of polymerisation degree

Results are means of three measurements and were calculated in relation to dry matter of the material.

Cellulose obtained applying the method proposed by Seifert was used in analyses of its degree of polymerisation and polydispersity. Cellulose was dried at a temperature of 60 °C. Such prepared cellulose was analysed using gel chromatography to determine the degree of polymerisation according to the method proposed by Ekmanis (1987). In this method cellulose is dissolved in DMAc (dimethylacetamide) and 0.5% LiCl. Cellulose dissolution was performed in several stages:

1. Activation of the capillary structure in water (24 h);
2. Exchange in the capillary system of water onto DMAc;
3. Dissolution in DMAc/8% LiCl;
4. Dilution of the produced solution with pure DMAc;
5. Filtration of the solution through the PTFE membrane.

Analyses were conducted in an Agilent gel chromatograph with an RI Wyatt detector (flow rate 1.00 ml/min; temperature of column 80 °C, column 3xPLgel MixedA, 300 mm, 20 µm (Agilent), calibration—polystyrene standards; solvent 0.5% LiCl/DMAc; 1260 Iso Pump (Agilent Technologies). The polystyrene standards were used to calculate molar mass of cellulose according to Mark-Houwink and Sakurada. Parameters for polystyrene $\alpha = 0.642$, $K = 17.35 \times 10^{-5}$ cm³/g (Timpa 1991). Parameters for cellulose $\alpha = 0.957$, $K = 2.78 \times 10^{-5}$ cm³/g (Bikova and Treimanis 2002).

The mean degree of polymerisation was calculated according to formula (1):

$$DP = \frac{M_w}{162} \quad (1)$$

where DP—degree of polymerisation; M_w—weight average molecular mass; 162—molecular mass of cellulose constitutional unit.

In turn, polydispersity of cellulose was calculated from the formula:

$$P = \frac{M_w}{M_n} \quad (2)$$

where P—polydispersity of cellulose; M_w—weight average molecular mass; M_n—number average molecular mass.

Fourier transform infrared (FTIR) spectroscopy

The Fourier transform spectra were obtained using the KBr (Sigma-Aldrich, Steinheim, Germany) pellet technique (1 mg cellulose/200 mg KBr or 2 mg lignin/200 mg). Spectra were registered using the Nicolet iS5 spectrometer (Thermo Scientific, Madison, USA) at the range from 4000 to 450 cm⁻¹ at a resolution of 4 cm⁻¹ recording 16 scans. Different measurements for each sample were evaluated and the average value was considered.

FTIR analysis of cellulose

The spectra were baseline corrected and normalised and next used to calculate the total crystalline index (TCI, H₁₃₇₂/H₂₉₀₀) (Nelson and O'Connor 1964), lateral order index (LOI, A₁₄₂₉/A₈₉₇) (Carrillo et al. 2004) and hydrogen bond intensity (HBI, A₃₄₀₀/A₁₃₂₀) (Oh et al. 2005). The basic spectra were transposed to their second derivative (D2) using the OMNIC 9 software (Company, version, City, Country) and next the energy of the hydrogen bonds (E_H) was calculated from equation Eq. (3) (Ciolacu et al. 2010; Poletto et al. 2014).

$$E_H = \frac{1}{k} \frac{v_0 - v}{v_0} \quad (3)$$

where v_0 —standard frequency corresponding to free OH groups (3650 cm⁻¹); v is the frequency of the bonded OH groups; and k is a constant (1/k = 2.625 × 102 kJ).

The length of hydrogen bonds (R) was determined on the basis of the frequency shift Δv of the OH absorption band using Eq. (4) (Poletto et al. 2014; Pimentel and Sederholm 1956):

$$\Delta v = 4430 \times (2.84 - R) \quad (4)$$

where $\Delta v = v_0 - v$; v_0 is the monomeric OH stretching frequency, which is taken to be 3600 cm⁻¹; and v is the stretching frequency observed in the infrared spectrum of the sample.

FTIR analysis of lignin

In the IR spectra peak absorbances were determined by the base line method for each peak separately. The condensation indices (CI) of lignin were calculated

from the FTIR spectra using equation Eq. (5) (Faix 1991a, b):

$$CI = \frac{\sum \text{minima between } 1500 \text{ and } 1050 \text{ cm}^{-1}}{\sum \text{maxima between } 1600 \text{ and } 1039 \text{ cm}^{-1}} \quad (5)$$

Statistical analysis

The experimental data were analysed using the Dell™ Statistica™ 13.1 software with the analysis of variance (ANOVA). Significant differences between mean values for the investigated properties of elm samples from trees growing under different conditions were determined using Tukey's HSD test. The comparison tests were performed at a 0.05 significance level. Identical superscripts (a, b, c) denote no significant difference between mean values for the investigated properties.

Results and discussion

When analysing contents of individual components presented in Table 2 we may observe that various forms of arsenic applied in this experiment had a significant effect on changes in structural components in elm wood. The amount of cellulose in the elm control was 35.92%. Since they were 1-year old seedlings this is the content comparable to that reported by Waliszewska and Prądzynski (2002) for 1-year old willow rods (37.5–38.7%). In young woody plants growing under standardised, unpolluted environmental conditions the content of cellulose increases with age, reaching 40–50% at mature age (Fengel and Wegener 1989; Prosiński 1984). That experiment showed a marked effect of arsenic contained in the substrate, which caused a decrease in the contents of the primary wood component in all cases. In comparison to the control the differences in cellulose contents in samples growing on the substrate containing arsenic ranged from 1.21% in the case of elm As 7 (Table 2) to 3.68% in the case of elm As 8. Significance of differences in cellulose contents in wood was also confirmed by statistical analysis. The greatest decrease in cellulose contents in elm wood compared to the control was caused by the presence of a mixture of various arsenic forms in the substrate, i.e. As(III)/AS(V)/DMAA at 0.6/0.06/0.06 mM L⁻¹ (elm

Table 2 Content of selected components of elm wood after experiment with arsenic

Sample	System	Content (%)	Cellulose	Lignin	Holo-cellulose	Hemi-celluloses	Extractives	Substances soluble in 1% NaOH
Elm 1—control	Control	35.92 ^d	0.51	23.75 ^c ± 0.53	72.26 ^c ± 0.77	36.34 ^{ab} ± 1.03	9.15 ^b ± 0.31	33.52 ^c ± 0.32
Elm As 2	As (III)	34.68 ^{cd} ± 0.15	26.21 ^e ± 0.06	72.44 ^c ± 0.54	37.76 ^a ± 0.64	9.52 ^b ± 0.15	35.64 ^{ac} ± 2.32	
Elm As 3	As (V)	32.99 ^{ab} ± 0.40	25.57 ^{de} ± 0.37	69.75 ^{ab} ± 0.43	36.76 ^a ± 0.03	10.23 ^{bc} ± 0.38	37.16 ^{ab} ± 0.28	
Elm As 4	DMAA	33.94 ^{bc} ± 0.81	24.81 ^{abd} ± 0.22	70.33 ^{ab} ± 0.46	36.39 ^a ± 0.37	11.34 ^{ac} ± 0.31	36.58 ^{ab} ± 0.33	
Elm As 5	As(III)/As(V)	32.31 ^a ± 0.19	24.66 ^{ab} ± 0.28	68.97 ^a ± 0.59	36.66 ^a ± 0.66	13.35 ^d ± 0.57	41.99 ^d ± 0.49	
Elm As 6	As(III)/DMAA	34.71 ^{cd} ± 0.30	24.42 ^{abc} ± 0.33	69.17 ^{ab} ± 0.99	34.46 ^b ± 0.76	12.23 ^{ad} ± 0.29	36.68 ^{ab} ± 0.22	
Elm As 7	As(V)/DMAA	32.82 ^{ab} ± 0.04	25.10 ^{bd} ± 0.20	70.92 ^{bc} ± 0.35	38.10 ^a ± 0.31	11.75 ^a ± 0.81	38.43 ^b ± 0.47	
Elm As 8	As(III)/As(V)/DMAA	32.24 ^a ± 0.74	24.23 ^{ac} ± 0.15	68.57 ^a ± 0.67	36.33 ^{ab} ± 1.38	11.42 ^{ac} ± 0.32	41.58 ^d ± 0.82	

Mean values ($n = 3$) ± standard deviations; identical superscripts (a, b, c...) denote no significant difference ($p < 0.05$) between mean values in column according to Tukey's HSD test (ANOVA)

As 8). In turn, the smallest differences in cellulose contents (1.21%) were observed in sample elm As 5, which was growing on the substrate supplemented with As(III)/As(V) at 0.06/0.06 mM L⁻¹. Addition of 0.6 mM L⁻¹ DMAA to the substrate (sample elm As 4) resulted in a reduction of cellulose contents by slightly less than 2%. In turn, supplementation of the mixture with As(V)/DMAA added to the substrate at 0.06/0.6 mM L⁻¹ (elm As 7) caused a decrease in the content of cellulose by 3.1% (Table 2). The effect of the polluted environment on changes in the contents of cellulose in pine wood was observed by Krutul et al. (2006), who reported 21.8% and 23.0% cellulose contents in wood depending on the distance of the growing tree from the source of pollution. Also Waliszewska and Prądzynski (2002) recorded a reduction of cellulose content from 37.5 to 35.8% in rods of *Salix mullatin* and from 38.5 to 36.9% for *S. purpurea* in wood of 1-year old willow rods in comparison to rods aged several years. According to Krutul et al. (2014), environmental pollution generated by the sulfur mining industry caused a decrease in cellulose content of about 30% in bark from the butt-end section of the trunk. The same authors reported changes in cellulose contents in wood, branches and roots of birch growing in the polluted environment (Krutul et al. 2011).

In the case of lignin its content followed a different trend than the content of cellulose. In wood of elm trees growing at an addition of arsenic to the substrate in all the cases the amount of lignin was greater in comparison to the level in wood of the control. Lignin content in the control was 23.75% (Table 2), while in the samples growing in the substrate supplemented with various forms and amounts of arsenic the content of lignin ranged from 24.23% in the case of elm As 8 to 26.21% in the case of elm As 2. The difference in lignin contents in wood of elm trees growing with the addition of As(III) at 0.06 mM L⁻¹ to the substrate in comparison to the control (no As added) amounted to 2.46%. It is a significant difference in the contents, which was confirmed by statistical analysis. Addition of As(V) at 0.06 mM L⁻¹ to the substrate caused an increase in lignin contents in elm wood (elm As 3) by 1.82% in comparison to the amount of lignin in the control. Supplementation of the substrate with DMAA at 0.6 mM L⁻¹ (elm As 4) caused an increase in lignin contents in wood by slightly over 1%; however, it needs to be stressed that the tree growth period was

short and it is also a significant difference in the contents of this structural component. An increase in the amount of lignin in wood of pine trees growing in a polluted environment was reported by Krutul et al. (2006). Those authors found a considerable effect of pollution produced by the Kozienice power station on an increase in lignin contents. Environmental pollution with particulate matter from the Kozienice power plant also caused an increase in lignin content in wood of a birch tree growing 21 km from the pollution source (Krutul et al. 2011). According to Waliszewska (2002), pollution in the area of the motorway resulted in an increase of lignin contents by approx. 2.3–2.6% in wood of willows aged several years.

In the control elm sample the level of holocellulose was 72.26%. Only in the case of elm growing on the substrate supplemented with As(III) at 0.06 mM L⁻¹ statistical analysis showed a non-significant difference in comparison to the contents of holocellulose in the control (Table 2). In the other samples considerable differences were found in holocellulose content in relation to the control. The lowest amounts of holocellulose (68.57%) were recorded in wood of the elm growing on the substrate supplemented with a mixture of As(III)/As(V)/DMAA at 0.6/0.06/0.06 mM L⁻¹. Comparable levels of holocellulose, i.e. 68.97% and 69.17%, were found in samples denoted as elm As 6 and elm As 5, respectively. In wood of the elm growing on the substrate with an addition of 0.6 mM L⁻¹ DMAA and that supplemented with As(V)/DMAA at 0.06/0.6 mM L⁻¹ the level of holocellulose was almost identical, i.e. 70.33% and 70.92%, respectively. In wood of elm As 3 at substrate supplementation with 0.06 mM L⁻¹ As(V) the level of holocellulose was 69.75%.

Analyses of hemicellulose contents in the tested samples showed very similar levels in all the experimental variants. Only in the case of elm As 5 and elm As 7 differences of 1.88% and 1.76% were recorded in comparison to the control. In the other cases the levels of these compounds ranged from 36.33 to 37.76% (Table 2). Statistical analysis confirmed slight differences in the contents of hemicellulose in elm wood between individual experimental variants. Similarly slight changes (approx. 2%) in the contents of hemicellulose in pine wood under the influence of pollution were presented by Krutul et al. (2006). In turn, Waliszewska (2002) gave hexose contents in wood of 1-year willow rods growing under different

conditions (at a motorway and in a sewage treatment plant) from 4.36 to 4.53%, while in identical willows from a plantation growing in an unpolluted environment it was 4.73%. That author stated a limited effect of pollution on the content of hexoses in willow wood.

The lowest content of extractives, amounting to 9.15%, was recorded in wood of the control elm. In all the other variants greater amounts of these compounds were recorded (Table 2). In samples with an addition of arsenic to the substrate the content of compounds extracted with ethanol ranged from 9.52% in the case of substrate supplementation with 0.06 mM L⁻¹ As(III) to 13.35% in the case of As(III)/DMAA added to the substrate at 0.06/0.6 mM L⁻¹. In wood of elms growing on the substrate with DMAA added at 0.6 mM L⁻¹ (elm As 4) and on substrate supplemented with As(III)/As(V)/DMAA at 0.6/0.06/0.06 mM L⁻¹ (elm As 8) the content of extractives was comparable, amounting to 11.34% and 11.42%, respectively. Slightly higher levels of these substances (11.75%) were recorded in wood of elm As 7. This similarity was also confirmed by statistical analysis. Addition of the As(III)/As(V) mixture to the substrate at 0.06/0.06 mM L⁻¹ (elm As 5) caused an increase in the level of compounds extracted with ethanol by over 3% in comparison to the control sample. Similar observations were presented by Krutul et al. (2010). When testing oak wood from a polluted area they recorded an increase in the levels of extractives by approx. 2% in all tree sections (butt-end, middle and top), both in sapwood and heartwood. Also Waliszewska and Prądzynski (2002) reported an approx. 1–2% increase in the levels of extractives in willows aged several years, growing at a motorway.

Very interesting results were provided by the analysis of substances soluble in diluted alkalies. Similarly as in the case of extractives, the lowest amount of compounds soluble in 1% NaOH (33.52%) was recorded in the control. The level of these compounds greater by almost 8.5% was detected in wood of elm tree denoted elm As 6, which was growing on the substrate supplemented with As(III) and DMAA at 0.06/0.06 mM L⁻¹. In comparison to the control the smallest increase in the content of substances soluble in alkalies (by 2.12%) was caused by substrate supplementation with arsenic As(III) at 0.06 mM L⁻¹. Also the addition of 0.06 mM L⁻¹ As(V) to the substrate (elm As 3), the addition of DMAA at 0.6 mM L⁻¹ (elm As 4) and that of the

As(III)/As(V) mixture at 0.06/0.06 mM L⁻¹ (elm As 5) caused an increase in the contents of substances soluble in alkalies by 3.64%, 3.06% and 3.16%, respectively (Table 2). In the case of substrate supplementation with As(V)/DMAA at 0.06/0.6 mM L⁻¹ the level of these compounds increased by 4.91%, while the As(III)/As(V)/DMAA mixture added at 0.6/0.06/0.06 mM L⁻¹ caused an increase in the recorded levels by over 8% (elm As 8). Significant differences in the contents of these compounds were confirmed by statistical analysis.

Similar observations, i.e. an increase (by approx. 14%) in the level of substances soluble in 1% NaOH in the stem, branches and bark of birch growing in a polluted environment, were presented by Krutul et al. (2014).

The ratios of holocellulose to lignin content (H/L) and that of cellulose to lignin (C/L) in the analysis of wood undergoing degradation are indicators of advancement of wood decomposition processes (Zborowska et al. 2004, 2007). The value of the H/L ratio in healthy wood is high and exceeds 3. In the experiment with the addition of various arsenic forms to the substrate in all the cases values of this index decreased to 2.73 (elm As 3), 2.76 (elm As 2), 2.79 (elm As 6) and in four cases to 2.83 (Table 3). When analysing the ratio of cellulose to lignin contents C/L we may also observe a decrease in its values in comparison to the control. In wood growing on the substrate with no arsenic added this index was 1.51, while in the other experimental variants this index decreased and ranged from 1.29 to 1.42. The lowest values of H/L and C/L were observed at the addition of

0.06 mM L⁻¹ As(V) to the substrate. The reduction in the values of H/L and C/L may indicate decomposition of polysaccharide compounds under the influence of arsenic.

FTIR spectroscopy has been used as a simple technique providing information on the chemical structure of wood (Poletto et al. 2012b), cellulose and lignin samples treated with various forms of arsenic (Fig. 1).

The bands at 3400 cm⁻¹ (vibrations of the hydrogen bonded OH) for wood exposed to the effect of arsenic forms were slightly narrower in comparison to the control sample. This confirms changes occurring in the intermolecular interactions.

In the “fingerprint region” (1800–600 cm⁻¹) (Pandey and Pitman 2003) for all the samples exposed to the action of various arsenic forms relative absorbance of all the bands decreased. The greatest changes were observed for the As(V), As(III)/As(V)/DMAA samples (Fig. 1).

Changes in relative absorbance for wood samples subjected to the influence of various arsenic forms were found both in bands associated with vibrations in the aromatic ring of lignin (1660 cm⁻¹, 1605 cm⁻¹, 1506 cm⁻¹) and stretching vibrations of cellulose and hemicellulose at (1432 cm⁻¹, 1320 cm⁻¹, 1158 cm⁻¹ and 895 cm⁻¹).

In view of the complexity of wood structure a definite interpretation of spectra is hindered due to the overlapping of similar groups of atoms originating from different wood components. For this reason a detailed analysis of structural changes was performed on isolated basic components of the lignocellulose material, i.e. cellulose and lignin.

Results of FTIR analyses for cellulose are presented in Table 4. In order to assess changes within the hydrogen bond and in the crystalline and amorphous regions of cellulose two basic spectral regions were analysed. They were the hydroxyl stretching region at 4000–2700 cm⁻¹ and the fingerprint region at 1800–600 cm⁻¹.

The ratio between the heights of the bands at 1372 and 2900 cm⁻¹, proposed by Nelson and O'Connor (1964) as the total crystalline index TCI (Carrillo et al. 2004), is proportional to the crystallinity degree of cellulose (Poletto et al. 2012a; Popescu et al. 2011). This index was used to evaluate the crystallinity of cellulose. The obtained results are presented in Table 4. The greatest increase in the values of the

Table 3 The ratio of holocellulose content to lignin (H/L) and cellulose to lignin (C/L)

Sample	System	H/L	C/L
Elm 1—control	Control	3.04	1.51
Elm As 2	As(III)	2.76	1.32
Elm As 3	As(V)	2.73	1.29
Elm As 4	DMAA	2.83	1.37
Elm As 5	As(III)/As(V)	2.83	1.42
Elm As 6	As(III)/DMAA	2.79	1.31
Elm As 7	As(V)/DMAA	2.83	1.31
Elm As 8	As(III)/As(V)/DMAA	2.83	1.33

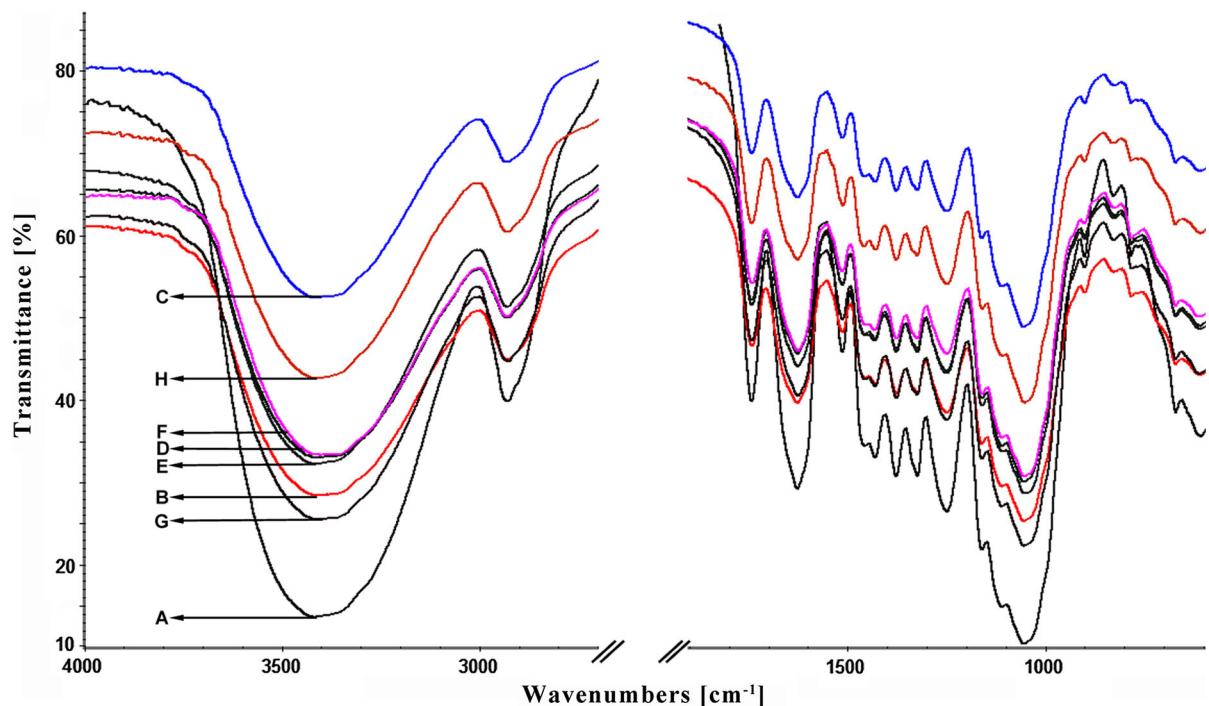


Fig. 1 FTIR spectra of elm wood studied: A—control, B—As(III), C—As(V), D—DMAA, E—As(III)/As(V), F—As(III)/DMAA, G—As(V)/DMAA and H—As(III)/As(V)/DMAA

Table 4 Infrared crystallinity ratios (LOI and TCI) and hydrogen bond intensity of the studied cellulose (HBI)

Sample	System	IR crystallinity ratio		HBI
		H 1372/H2900 (TCI)	A 1429/A897 (LOI)	
Elm 1—control	Control	0.319 ± 0.010	1.171 ± 0.128	1.245 ± 0.022
Elm As 2	As (III)	0.353 ± 0.011	1.215 ± 0.125	1.567 ± 0.011
Elm As 3	As (V)	0.363 ± 0.020	1.287 ± 0.123	1.534 ± 0.031
Elm As 4	DMAA	0.347 ± 0.010	1.144 ± 0.107	1.191 ± 0.011
Elm As 5	As (III)/As (V)	0.327 ± 0.031	1.196 ± 0.118	1.196 ± 0.012
Elm As 6	As (III)/DMAA	0.301 ± 0.010	1.248 ± 0.104	1.245 ± 0.010
Elm As 7	As (V)/DMAA	0.339 ± 0.020	1.099 ± 0.111	1.156 ± 0.050
Elm As 8	As (III)/As (V)/DMAA	0.292 ± 0.010	1.251 ± 0.108	1.189 ± 0.013

TCI index for cellulose was recorded for cellulose samples (elm As 3) at the addition of As(V), while the lowest values of the TCI index for cellulose (elm As 8) were reported for the As(III)/As(V)/DMAA mixture systems.

The band at 1430 cm^{-1} is designated a crystalline region in cellulose, while the band at 898 cm^{-1} is assigned to the amorphous region in cellulose (Åkarholm et al. 2004). The absorbance ratio between the areas of these bands is used as the lateral order

index (LOI) (Carrillo et al. 2004; Oh et al. 2005). This index correlated to the overall degree of order in cellulose (Carrillo et al. 2004).

An increase in the LOI values was observed for samples cellulose (elm As 3) exposed to an addition of both As(V) and the mixture systems of As(III)/DMAA (elm As 6) and As(III)/As(V)/DMAA (elm As 8). The recorded increase in the values of this index results from greater susceptibility to degradation in the case of amorphous regions in cellulose.

The hydrogen bond intensity (HBI) in cellulose of the investigated arsenic system ranged from 1.156 to 1.567. Considering the chain mobility and bond distance, the hydrogen bond intensity (HBI) of cellulose is closely related to the crystal system and the degree of intermolecular regularity (Oh et al. 2005). Similar observations for LOI and HBI were reported by Waliszewska et al. (2018), who found an increase in their values during the vegetation period of young plants.

In the FTIR analysis the hydroxyl stretching region was the most informative to explain hydrogen bonding patterns. The bands described below may generally be observed only in the spectra of the second derivative, which increase apparent resolution and enhance slight differences in the spectrum (Table 5). The energy of hydrogen bonds (E_H) and hydrogen bond distances (R) for the entire system are shown in Table 5. The average value of E_H for 3567 cm^{-1} significantly decreased only for cellulose sample (elm As 5) for the As (III)/As(V) mixed system. However, for the As(III)/DMAA (elm As 6) and As(III)/As(V)/DMAA systems (elm As 8) a greater energy value of bonds was found, which may indicate a higher number of intramolecular hydrogen bonds in cellulose. These high values of energy for hydrogen bonds are associated with lower hydrogen bond distances (Waliszewska et al. 2018). The energy of hydrogen bonds at 3423 cm^{-1} are similar for all samples.

In order to more precisely characterise structural changes in cellulose taking place under the influence of various arsenic forms the degree of polymerisation (DP), mean molecular mass and the degree of polydispersion were analysed. It seems advisable to assess changes in the degree of polymerisation, since next to the degree of crystallinity this is a parameter, which determines physical and chemical properties of

cellulose. A significant decrease of the degree of polymerisation from 1415 for the control sample (elm 1) to 1086 was observed for the As III/As V system (elm As 5).

The GPC analysis (Table 6) shows that the weight average molecular mass (M_w) of 229,233 for the control cellulose (elm 1) drops significantly to 175,853 for samples of the As (III)/As(V) system (elm As 5).

The greatest changes for this system were also observed for the number average molecular mass (M_n). The degree of polydispersion (M_w/M_n) showed a marked downward trend for cellulose from trees growing in the As(III)/As(V) system (elm As 5). Conducted analyses provided a surprising result for the samples supplemented with DMAA (elm As 4, elm As 6, elm As 8). The apparent lack of a decrease in DP may result from the presence of DMAA in the system.

A reduction of the degree of polydispersion from 6.5 to 5.3 for sample (elm As 5) subjected to the action of inorganic forms, i.e. As(III) and As(V), suggests that longer polymer chains are more susceptible to various types of degradation than shorter chains (Strobin et al. 2003). Shares of high molecular mass celluloses decrease markedly for these systems. At the same time an increase was recorded for the contents of fractions with smaller molecular masses, presented in Table 7.

Apart from cellulose the effect for the addition of various inorganic and organic arsenic forms was also observed for lignin. This was confirmed both by quantitative analysis (Table 2) and qualitative analysis (Fig. 2). These changes are connected with the fact that inorganic (arsenite (III)—As(III) and arsenate (V)—As(V)) and organic (dimethylarsinic acid—DMAA(V)) arsenic forms significantly influence the profile of phenolic compounds (e.g. ferulic, sinapic, p-coumaric, caffeic, trans-cinnamic acids) produced

Table 5 Energy of hydrogen bonds (E_H) and hydrogen distance (R) for studied cellulose obtained from plants treated using different systems

Sample	System	E_H 3567 cm^{-1} (kJ)	R(Å)	E_H 3423 cm^{-1} (kJ)	R(Å)
Elm 1—control	Control	6.113	2.832	16.325	2.800
Elm As 2	As (III)	6.041	2.831	16.253	2.800
Elm As 3	As (V)	6.113	2.832	16.325	2.800
Elm As 4	DMAA	6.113	2.832	16.325	2.800
Elm As 5	As (III)/As (V)	5.969	2.833	16.225	2.800
Elm As 6	As (III)/DMAA	6.401	2.830	16.397	2.799
Elm As 7	As (V)/DMAA	6.184	2.831	16.397	2.801
Elm As 8	As (III)/As (V)/DMAA	6.285	2.830	16.397	2.799

Table 6 Molecular parameters of cellulose, Mn—average molecular mass, Mw—average weight mass, Mw/Mn—degree of polydispersion, DP—degree of polymerisation

Sample	System	Mn (g/mol)	Mw (g/mol)	Mw/Mn	DP
Elm 1—control	Control	35,360	229,233	6.5	1415
Elm As 2	As (III)	35,150	210,380	6.0	1299
Elm As 3	As (V)	36,567	220,660	6.2	1362
Elm As 4	DMAA	36,373	230,247	6.3	1421
Elm As 5	As (III)/As (V)	33,287	175,853	5.3	1086
Elm As 6	As (III)/DMAA	34,555	221,152	6.4	1420
Elm As 7	As (V)/DMAA	33,977	214,610	6.3	1325
Elm As 8	As (III)/As (V)/DMAA	35,727	235,747	6.6	1455

Table 7 Percentage contents of fractions in cellulose samples, M—molecular mass

Sample	System	M < 20,000	20,000 < M < 200,000	M > 200,000
Elm 1—control	Control	15.19	53.47	31.34
Elm As 2	As (III)	13.84	55.99	30.17
Elm As 3	As (V)	15.04	53.91	31.05
Elm As 4	DMAA	14.65	54.56	30.97
Elm As 5	As (III)/As (V)	15.85	57.94	26.21
Elm As 6	As (III)/DMAA	15.05	53.07	31.88
Elm As 7	As (V)/DMAA	15.34	53.97	30.69
Elm As 8	As (III)/As (V)/DMAA	14.87	53.08	32.05

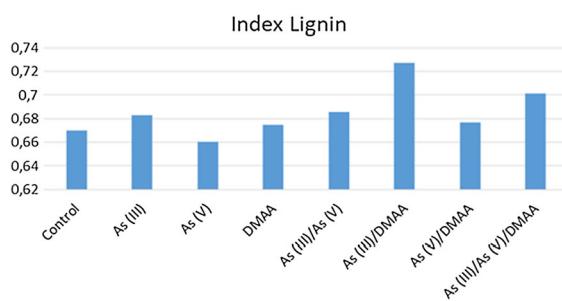


Fig. 2 Condensation index of lignin

by plants (Drzwięcka et al. 2018). These compounds are precursors of lignin (Kumar and Pruthi 2014) constituting a mechanical barrier against various heavy metals. Observed changes in this polymer are the response to oxidative stress caused by external factors (Elobeid et al. 2012).

Results of the spectral analysis concerning the lignin condensation index are presented in Fig. 2. For most applied systems an increase was recorded for the lignin condensation index with the greatest changes for the As III/DMAA (elm As 6) and As III/AsV/DMAA systems (elm As 8).

Conclusions

World literature includes reports on phytoremediation, the effect of environmental pollution with heavy metals on plant growth and contents of various elements in plants. However, there are few reports on changes in contents of individual wood components and their structure under the influence of pollutants. Various forms and compositions of arsenic compounds selected for the experiment made it possible to determine the effect of organic and inorganic arsenic on growth of young elm seedlings and thus—on changes in contents of cellulose, lignin and hemicellulose. Addition of arsenic compounds to the substrate also caused changes in the structure of these basic wood components. Knowledge obtained from this experiment made it possible to obtain elm seedlings for potential plantings in the substrate containing hazardous arsenic and for plantings in degraded areas, and thus to improve the condition of the natural environment.

Results of this study confirm literature reports on the effect of the polluted environment on changes in the chemical composition and structure of basic wood components.

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