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THE RELATIONS BETWEEN NON-STRUCTURAL SUBSTANCES, ANNUAL RINGS WIDTH AND LATEWOOD SHARE IN *PINUS SYLVESTRIS* L. STEM

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ABSTRACT

Extractives and ash contents, share of latewood and annual rings width were analyzed in wood at different heights and different zones of the *Pinus sylvestris* L. stem cross-section. Additionally, the high performance liquid chromatography (HPLC) with a conductometric detector was applied to determine the sulphate (SO_4^{2-}) and hydrogen phosphate (HPO_4^{2-}) anions concentrations in wood and bark of *Pinus sylvestris* L. stem. In this paper, based on the results it was observed, that generally extractives content increased in the direction from sapwood perimeter to middle heartwood and pith adjacent heartwood zone. Moreover, the results showed that the greater share of latewood in annual rings the lower ash and extractives contents in the wood, but the higher sulphate (SO_4^{2-}) and hydrogen phosphate (HPO_4^{2-}) anions concentration. The sulphate (SO_4^{2-}) and hydrogen phosphate (HPO_4^{2-}) anions concentrations in the bark of the *Pinus sylvestris* L. stem were higher at the top than at the butt-end part.

KEYWORDS: Pine wood, bark, extractives, ash, sulphur, phosphorus, cross-section, stem.

INTRODUCTION

During the process of tree growth, mineral salts naturally accumulate in the tree. Water-soluble salts are deposited in the resin ducts and pith rays. In the tracheids, mineral substances accumulate in the primary and secondary walls and are also deposited as needles or prismatic crystals in the spaces of parenchyma, as well as in the middle lamina (Fengel and Wegener 1984). The mineral substances content of wood growing in temperate climates ranges from 0.3 to 1.2%, based on its dry weight.

The relatively low content of mineral substances fulfil an important function in the physiological process of wood tissue formation (Mansilla et al. 1991). The content of these substances in the wood and bark depends on the tree species, its age, the growth habitat, as well as the sampling location on the cross-section and longitudinal of the trunk (Pereira 1982, 1988, Rademacher et al. 1986, 1988, Krutul 1995, Watmough and Hutchinson 1999, Krutul et al. 2012, 2014, 2018a,b). Data from Denne and Dodd (1980), Pereira (1982, 1988), Fengel and Wegener (1984), Rademacher et al. (1986), Marques and Pereira (1987), Loto and Fakankun (1989) and Adamopoulos et al. (2005) show that in the wood of the same species, the content of mineral substances varies over a wide range. Significant differences in mineral substances content were stated in *Pinus sylvestris* L., *Quercus petraea* Liebl. and *Quercus robur* L. wood (approx. 100-year-old) between wood from the top section and the butt-end section. Mineral substances content is higher in wood from the top section but is lower in bark from the top section (Krutul 1998). Mineral substances content in wood and bark is usually determined based on the ash amount obtained from the burning and ashing of a sample.

Extractives in wood are very diverse concerning chemistry and their distribution on the cross-section and longitudinal of the trunk in wood and bark depends on their function. The decrease of extractives content in the direction from the pith to the perimeter takes place on the cross-section apart from the tree habitat (Krutul 1998, Krutul et al. 2012). Extractives content in the bark is much higher than in wood. Ohara and Hemingway (1989) noted that the content of substances extracted with an acetone-water (1:1, v/v) mixture equals 10.7% in internal bark, 14.8% in middle bark and 5.5% in outer bark.

Phosphorus (P) is an element with a key role in transfer reactions and energy accumulation and phosphorylation. It is taken up by plants in the form of $H_2PO_4^-$ (dihydrogen phosphate) and HPO_4^{2-} (hydrogen phosphate) anions. It actively incorporates a variety of compounds in its oxidized form to form phosphoric acid (V) esters (Kopcewicz and Lewak 1998). Regardless of the degree of environmental contamination by the nitrogen industry and the height of the cross-sections taken of the stem of *Pinus sylvestris* L., the sapwood zone is characterized by a two times to thirteen times higher hydrogen phosphate anions content relative to their content in pith adjacent heartwood zone (Krutul et al. 2015). The bark is characterized by a nine times lower hydrogen phosphate anion content compared to their content in the sapwood zone from the cross-section at 0.1 m (butt-end) of the stem, fivefold lower from the cross-section at 0.5 m and 1.1 m and more than threefold lower from the cross-section at 2.2 m. Whereas, in relation to the pith adjacent heartwood zone, the bark is characterized by one and a half to fourteen times higher hydrogen phosphate anion content, and in relation to the heartwood zone from one and a half to two times higher this anion content (Krutul et al. 2015).

Sulphur (S) is the element necessary for proper plant development. Plants take up the sulphur by the roots system from the ground in the form of sulphate (SO_4^{2-}) anions or in small amounts as sulphur dioxide from the atmosphere. It is transported by the xylem and appears in many different compounds. Hu et al. (2013) reported that nitrogen and sulphur influence the enzymes activity in a boreal mixed wood forest in western Canada. According to Krutul et al. (1999), sulphur content in the sapwood of 110-year-old *Pinus sylvestris* L. in the butt-end part equals 220 mg/kg, in sapwood adjacent heartwood – 165 mg/kg and in heartwood – 134 mg/kg. In the stem of *Pinus sylvestris* L. obtained from different environmental pollution, the sulphur content in the sapwood zone is higher than in pith adjacent heartwood and in heartwood (Krutul et al. 2015). Sulphur content in the bark from the *Pinus sylvestris* L. in the butt-end stem section is six times higher compared to wood (Krutul et al. 1999).

According to Fengel and Wegener (1984), there is a relationship between the extractives and the mineral substances contents in a wood. The wood characterized by high extractives content is also characterized by increased mineral substances content. However, this relationship not always was found in the works of other researchers (Krutul et al. 1999, Lachowicz et al. 2019). Therefore, in this case, verification and additional tests are necessary.

In the studies of the mechanical properties of wood, it was found that there is a relationship between the share of latewood on the cross-section and longitudinal in the trunk and changes in mechanical properties. Changes in the width of annual rings on the cross-section and longitudinal in the trunk, on the other hand, play an indirect role. According to the Polish standard PN-D-94021 (2013), coniferous structural timber with the best strength properties has a maximum permitted annual rings width of 4 mm. Krzysik (1984) also stated that coniferous wood of the width equals from 2 to 3 mm has the best mechanical properties. There is little information on this subject in the available literature. Therefore the proposed research seems to be interesting and important from a scientific and practical point of view. Also, in the available literature there is little or even no data on the content of phosphorus and sulphur in the wood and bark of *Pinus sylvestris* L. growing in an unpolluted environment. These are essential elements for trees because they are needed for their proper development. Hence, the results presented in this work are valuable and fill a gap in the literature.

The aim of this paper is the fill gap in the literature and determination of the concentration of hydrogen phosphate and sulphate anions on the cross-section at different heights of the *Pinus sylvestris* L. stem. Additionally, the relationship between extractives and ash contents, width of annual rings, and share of latewood on the cross-section at different heights of *Pinus sylvestris* L. stem were examined.

MATERIAL AND METHODS

Samples for the studies were gained from about 100-year-old stem of *Pinus sylvestris* L. The tree grew in Poland, Mazovia-Podlaski forest region and was cut in December. The height of the tree was about 24 m, including a crown of 9.2 m, and the length of the stem to the first branch was 14 m. Wood samples for studies were taken from eight disks, collected at the heights of 0.2, 4, 8.5, 14, 19, 21, 22.5 and 23.5 m from the ground, which were harvested so that the spacing between disks was 10 meristems. In each disc, wood was collected from individual zones: sapwood perimeter (SP), middle sapwood (MS), sapwood adjacent heartwood (SAH), heartwood adjacent sapwood (HAS), middle heartwood (MH) and pith adjacent heartwood (PAH). Bark samples were taken from three disks, collected at the heights of 0.2, 14 and 23.5 m from the ground. Tab. 1 shows the characteristics of the cross-section at different heights of the examined disks.

Wood and bark samples were disintegrated in a laboratory mill and fractioned using sieves. Fractions of wood samples with dimensions of 1.2 mm - 0.49 mm and <0.15 mm were taken for analysis of extractives content. The Soxhlet apparatus was used for extractives content determination with the ethanol-benzene mixture (1:1)v/v (Krutul 2002). The solvents were of analytical grade and purchased from Chempur (Piekary Śląskie, Poland). The mineral substances content in the form of ash was analyzed on a dusty fraction of wood (fraction <0.15 mm) in a muffle oven at 600°C (Sluiter et al. 2008). The high performance liquid chromatography (HPLC, LC-20AD, Shimadzu, Kyoto, Japan) with a conductometric detector was applied to determine the concentration of sulphate (SO_4^{2-}) and hydrogen phosphate (HPO_4^{2-}) anions in wood and bark of *Pinus sylvestris* L. stem according to PN-A-79011-7 (1998). In this analysis fraction of wood and bark <0.15 mm was used. Determinations of percentages of latewood and width of annual rings were carried out following with PN-55/D-04110 (1955). All of the determinations were done in triplicate and standard deviations were calculated.

Tab. 1: The characteristics of the cross-section at different heights of the examined disks.

Height of section (m)	The pith location	Number of annual rings		Annual increments around the pith	Diameter (mm)	Distinction of the zone in the disk
		Heartwood	Sapwood			
0.2	centre	61	40	regular	442	SP, MS, SAH, HAS, MH, PAH, B
4.0	centre	55	45	regular	400	SP, MS, SAH, HAS, MH, PAH
8.5	centre	49	38	regular	388	SP, MS, SAH, HAS, MH, PAH
14.0	eccentric	41	35	not regular	253	S, SAH, HAS, H, B
19.0	eccentric	37	34	not regular	235	S, H
21.0	eccentric	27	26	not regular	184	S, H
22.5	eccentric	17	18	not regular	110	S, H
23.5	centre	-	17	regular	54	S, H, B

Sapwood perimeter (SP), middle sapwood (MS), sapwood adjacent heartwood (SAH), heartwood adjacent sapwood (HAS), middle heartwood (MH) and pith adjacent heartwood (PAH), bark (B), sapwood (S), heartwood (H).

RESULTS AND DISCUSSION

Non-structural substances content

The content of extractives decreased in the direction from the pith adjacent heartwood to the sapwood perimeter zone irrespective of the sample fraction (1.2 mm - 0.49 mm and <0.15 mm) on which the determination was carried out (Fig. 1).

From the data shown in Fig. 1, it can be seen that the extractives content was slightly higher in the samples with a fraction of <0.15 mm in relation to samples with a fraction of 1.2 mm - 0.49 mm. In both samples with a fraction of 1.2 mm - 0.49 mm and <0.15 mm, the extractives content in pith adjacent heartwood and middle heartwood was about three times higher than in the sapwood perimeter zone at the heights of 0.2, 4 and 8.5 m of the stem from the ground surface. Whereas at the heights of 14 and 19 m of the stem, the extractives content in pith adjacent heartwood and middle heartwood zone was from 40 to 45% higher compared to the sapwood perimeter zone, with only 15% higher at the heights of 21 and 22.5 m. It should be noted that with the increasing height of the *Pinus sylvestris* L. stem, the differences in extractives content between sapwood perimeter and middle heartwood zones decreased. In the stems of 80- 90- and 160-year-old *Pinus sylvestris* L. extractives content on the cross-section decreased from pith adjacent heartwood to sapwood zone at the butt-end part (Krutul 1998). According to Krutul et al. (2014), the extractives content in the *Quercus robur* L. trunk on the cross-section increased in the direction from the outer wood to the pith.

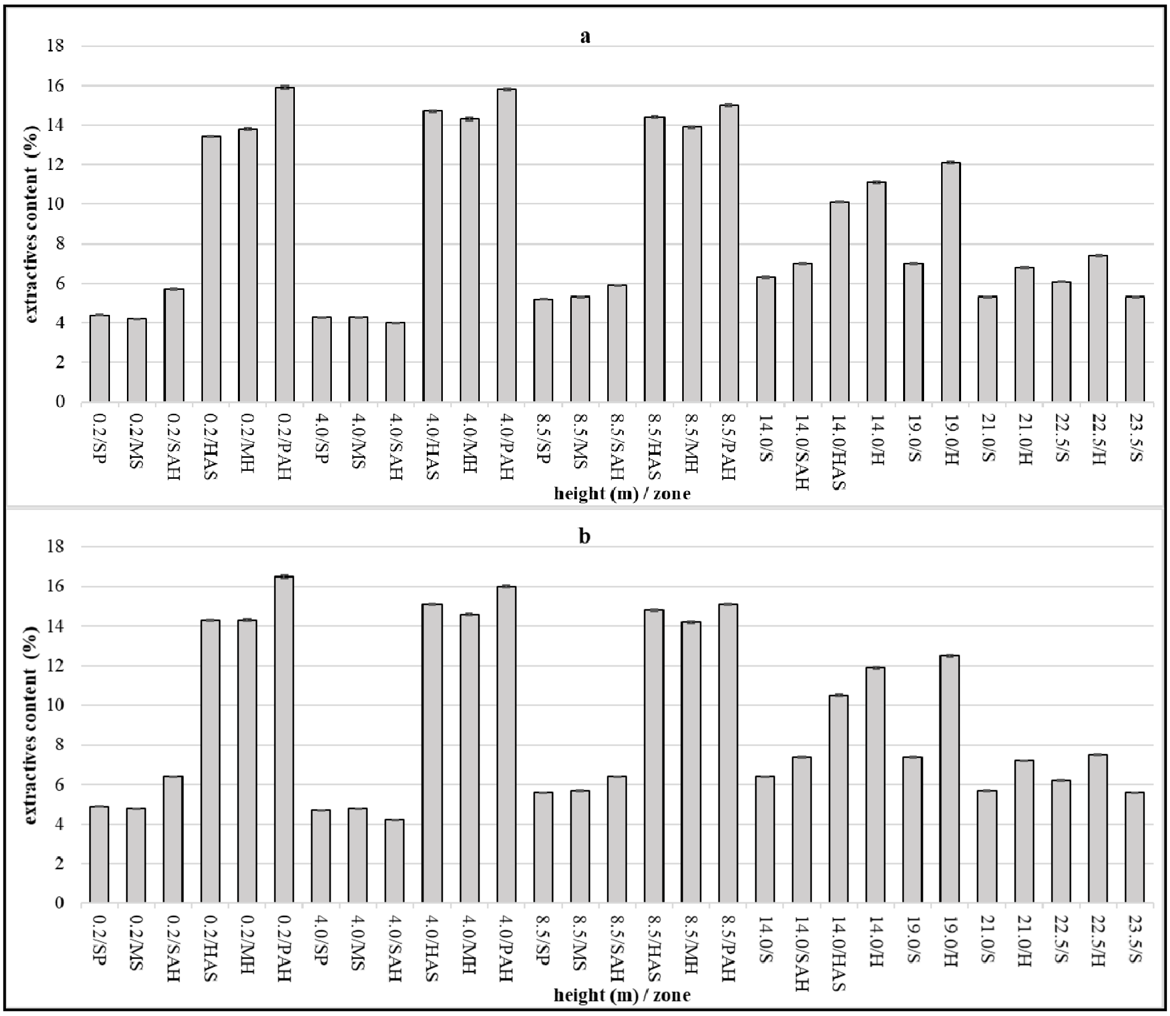

Fig. 1: Extractives content in wood on cross-section at different heights of the Pinus sylvestris L. stem: a) fraction 1.2 mm - 0.49 mm, b) fraction <0.15 mm.

Ash content (which constitute metal oxides) on the cross-section of the stem *Pinus sylvestris* L. in pith adjacent heartwood and middle heartwood was higher compared to sapwood perimeter, sapwood adjacent heartwood and heartwood adjacent sapwood regardless of the height of the cross-section of the *Pinus sylvestris* L. stem (Fig. 2). The ash content in the stem of *Pinus sylvestris* L. at the butt-end part and at the heights of 4 and 8.5 m in pith adjacent heartwood and middle heartwood zone was about 30% higher than in sapwood perimeter, and at the heights of 14, 19, 21 and 22.5 m was about 40 to 50% higher.

Regardless of tree growth habitat, 80-, 90- and 100-year-old *Pinus sylvestris* L. sapwood perimeter zone at the top part of the stem was characterized by higher mineral substances content compared to sapwood at the middle and butt-end part (Krutul 1998). According to Fengel and Wegener (1984), there is a correlation between the extractives content of wood and its mineral substances content. Wood characterized by high extractives content is also characterized by increased mineral substances content.

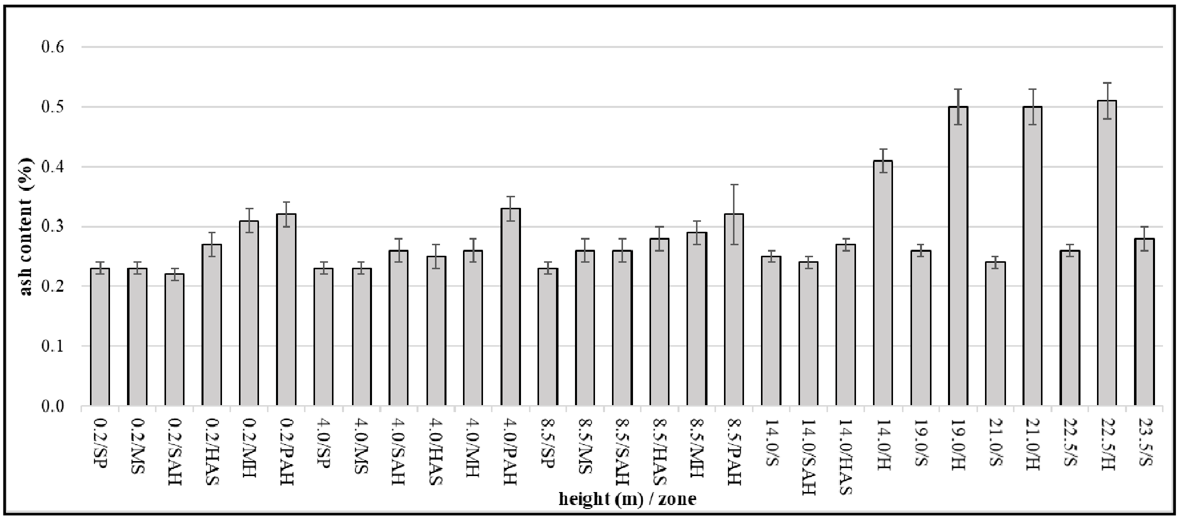

Fig. 2: Ash content in wood on cross-section at different heights of the Pinus sylvestris L. stem.

From the data shown in Figs. 1 and 2, it can be concluded that at the heights of 0.2, 4, 8.5 and 19 m of the *Pinus sylvestris* L. stem on the cross-section pith adjacent heartwood was characterized by higher extractives content and higher ash content in relation to sapwood perimeter which is in accordance with the statement of Fengel and Wegener (1984). Meanwhile, at the heights of 21 and 22.5 m of *Pinus sylvestris* L. stem, the extractives and the ash contents in the middle heartwood zone were higher (20% and 50% resp.) in relation to the sapwood perimeter zone. At the top of the stem, ash content in the middle heartwood zone was about 65% higher compared to their content in the middle heartwood zone at 0.2 m height of the stem, while extractives content was over two times less. According to Krutul et al. (2014) in the trunk of *Quercus robur* L. extractives content in pith adjacent heartwood was higher in relation to the sapwood zone. In the pith adjacent heartwood zone extractives content at the butt-end was 80%, at the middle part - two times and at the top of the trunk it was 50% higher compared to the sapwood zone. On the contrary mineral substances content was higher in sapwood in relation to the pith adjacent heartwood zone longitudinal of the trunk. So, our results showed that extractives and ash contents in wood on the cross-section at different heights of the stem were variable and depended on different factors such as wood species, age and tree habitat.

Annual ring width

Fig. 3 shows data on the annual ring width on the cross-section at different heights of the *Pinus sylvestris* L. stem. From the presented data it can be concluded that, regardless of the height of the cross-section of the stem, the pith adjacent heartwood and middle heartwood zones were characterized by higher annual ring width in relation to the sapwood perimeter zone. On the cross-section at the stem heights of 0.2, 4 and 8.5 m annual rings width in pith adjacent heartwood was about 65% higher in relation to the sapwood perimeter zone (Fig. 3).

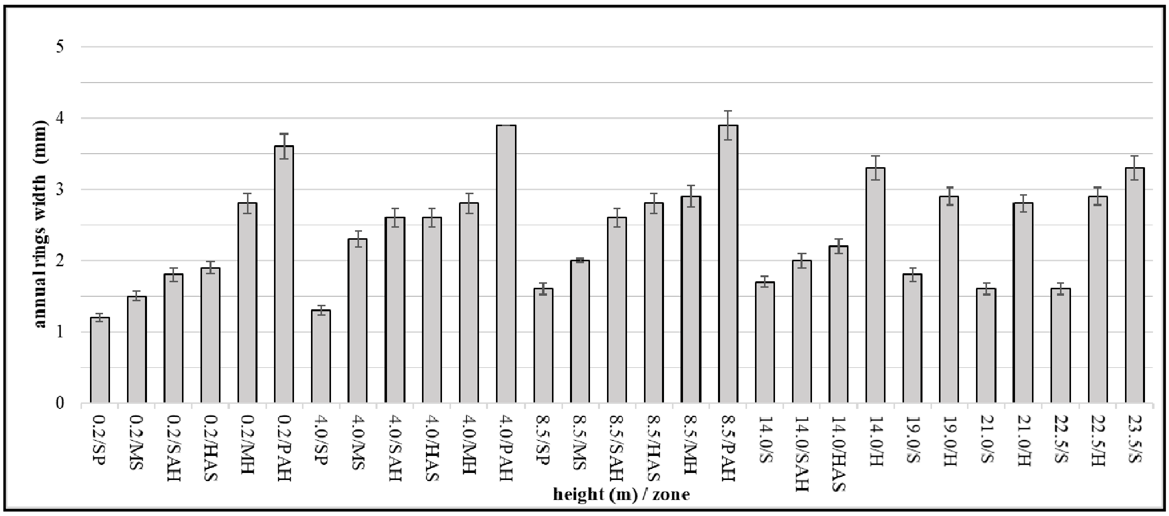

Fig. 3: Annual rings width on cross-section at different heights of the Pinus sylvestris L. stem.

On the cross-section of the stem annual rings width increased regularly from sapwood perimeter to middle heartwood and pith adjacent heartwood zone (Fig. 4). According to Krutul et al. (1999) at the heights of 2, 6 and 10 m from the ground of the stem of about 110-year-old *Pinus sylvestris* L. annual rings width was about three times higher in the pith adjacent heartwood than in the sapwood perimeter zone. From the data presented in Figs. 2 and 4, it can be concluded that greater annual ring width in pith adjacent heartwood and middle heartwood zone influenced increased content of ash in relation to the sapwood perimeter zone.

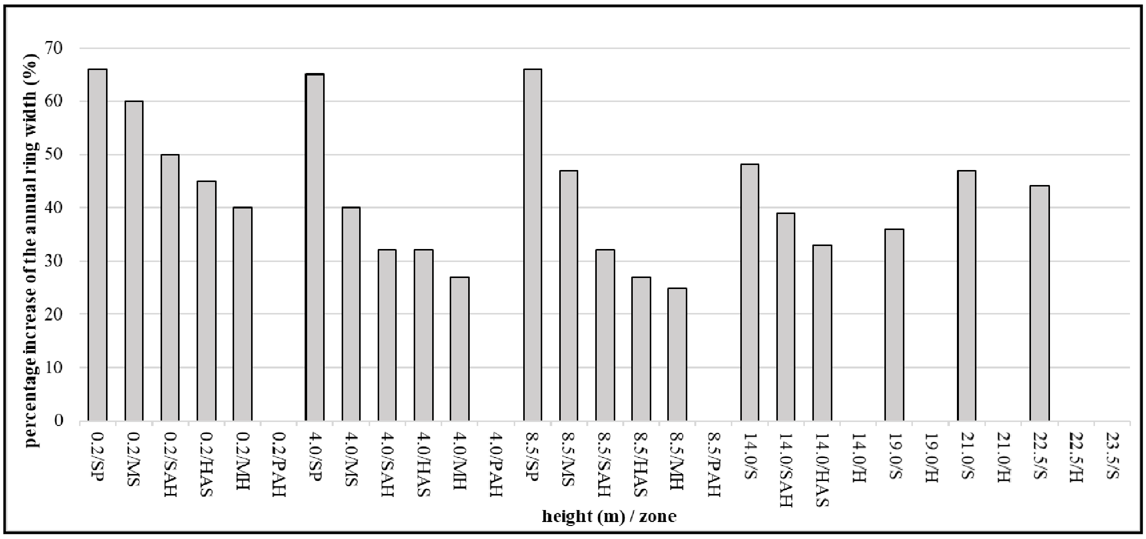

Fig. 4: Percentage increase of the annual ring width from sapwood to pith adjacent heartwood and middle heartwood zone on the cross-section at different heights of the Pinus sylvestris L. stem.

Share of latewood

Share of latewood on the cross-section in annual rings of the *Pinus sylvestris* L. stem showed that sapwood perimeter zone was characterized from 40% to about three times higher share of latewood in relation to pith adjacent heartwood and middle heartwood zone (Fig. 5). At different heights of the stem share of latewood in annual rings was similar. The greatest differences in the share of latewood were from pith adjacent heartwood and middle heartwood at heights of 4 and 8.5 m and they amounted 14.8 and 14.5% respectively. On the other hand, at the heights of 0.2, 14, 19, 21 and 22.5 m - from 18.8 to 21.4%.

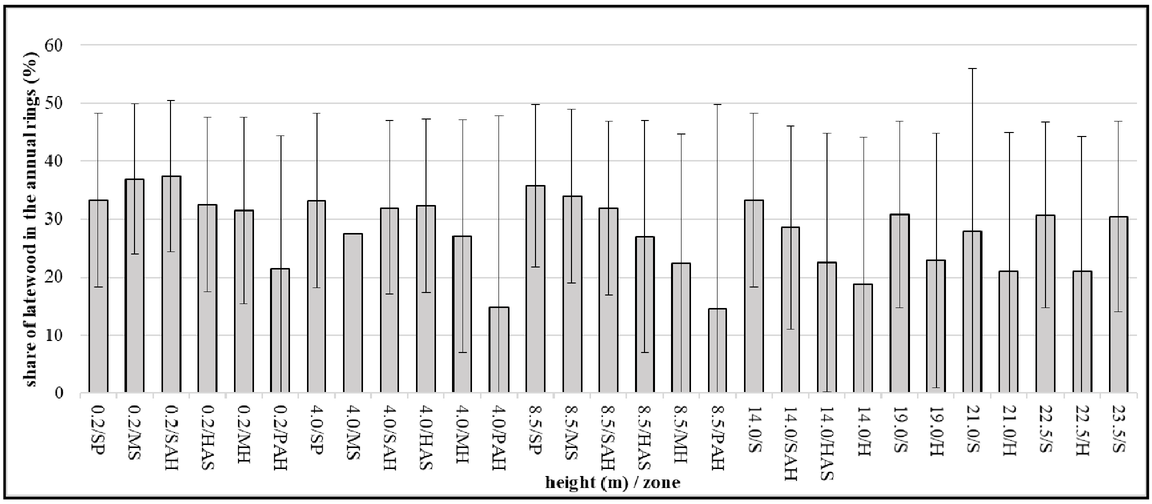

Fig. 5: Share of latewood in the annual rings on cross-section at different heights of the Pinus sylvestris L. stem.

From the data shown in Figs. 1 and 5, it can be concluded that the greater share of latewood in annual rings, the lower the extractives content. The sapwood perimeter zone of the *Pinus sylvestris* L. stem was characterized by a higher share of latewood in relation to pith adjacent heartwood and middle heartwood zone and extractives content was between three and 10 times lower than middle heartwood zone. Depending on the share of latewood in the investigated zones of the wood, the ash content on the cross-section at different heights of the *Pinus sylvestris* L. stem was similar to extractives content (Figs. 1 and 2). On the cross-section at the heights of the stem 0.2, 4 and 8.5 m share of latewood was 30% lower in pith adjacent heartwood compared to the sapwood perimeter zone. At the heights of the stem 14, 19, 21 and 22.5 m from the ground was about 50% lower in middle heartwood in relation to the sapwood perimeter zone (Fig. 5).

Hydrogen phosphate anions concentration

Ash is composed of metals oxides, while non-metals oxides are gases oxides and therefore phosphorus and sulphur are not included in total content of ash. Both phosphorus and sulphur are essential elements for proper plant development and are classified as macroelements. It can be seen from the data presented in Fig. 6 that the hydrogen phosphate anions concentration on the cross-section of *Pinus sylvestris* L. stem from sapwood perimeter was about 8 times higher in relation to pith adjacent heartwood at the heights of the stem 0.2, 4 and 8.5 m from the ground.

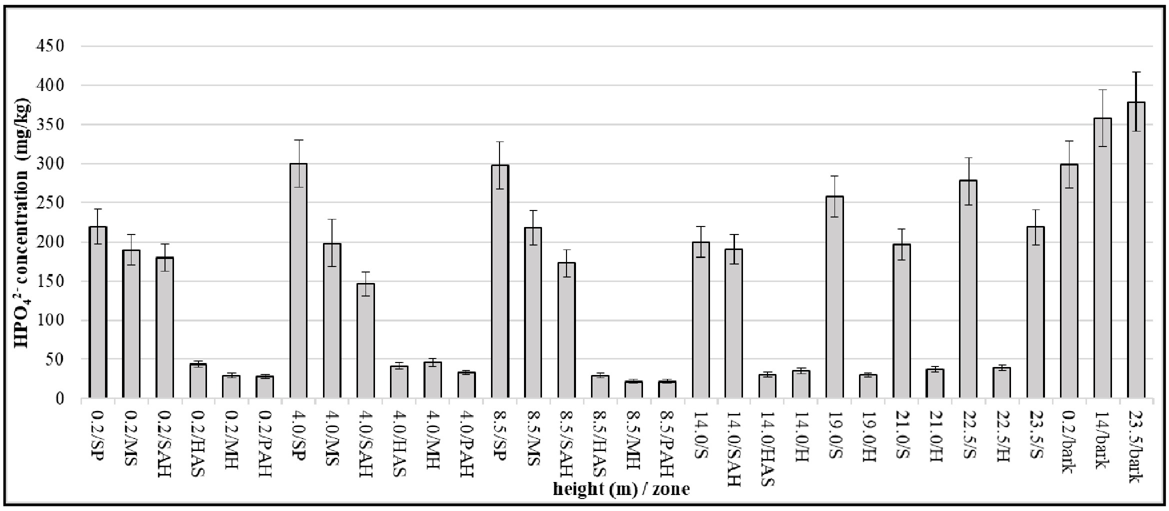

Fig. 6: Hydrogen phosphate anions (HPO_4^{2-}) concentration in examined wood zones and bark of the Pinus sylvestris L. stem.

At the heights of the stem 14, 19, 21 and 22 m from the ground hydrogen phosphate anions concentration was from two to six times higher in sapwood perimeter in comparison to the middle heartwood zone. According to Krutul et al. (2014) apart from environmental pollution, sapwood is compatible with heartwood. These results are compatible with the data of Krutul et al. (1999) which showed the same relationship in 110-year-old *Pinus sylvestris* L. According to Passialis et al. (2008) phosphorus content in *Robinia pseudoacacia* L. in sapwood was sevenfold higher in comparison to juvenile and mature wood obtained from different habitats (Greece, Bulgaria and Hungary). Regardless of the height of the cross-section of the trunk of *Morus alba* L., a higher content of hydrogen phosphate anions (HPO_4^{2-}) was characterized in the sapwood zone in relation to their content in pith adjacent heartwood and heartwood zone (Krutul et al. 2018a).

The data presented in Figs. 1, 2 and 6 show that the distribution of hydrogen phosphate anions (HPO_4^{2-}) on the cross-section of the *Pinus sylvetsris* L. stem was different from the distribution of extractives and mineral substances. The sapwood perimeter zone of the stem was characterized by lower annual rings width and a higher share of latewood in comparison to pith adjacent heartwood and middle heartwood. The hydrogen phosphate anions concentration in the sapwood perimeter zone was several times higher in comparison to pith adjacent heartwood and middle heartwood (Figs. 4 and 6). The hydrogen phosphate anions concentration in the bark of the *Pinus sylvestris* L. stem equalled 299 mg/kg at the butt-end (0.2 m), 358 mg/kg at a height of 14 m and 319 mg/kg at the top part (23.5 m) (Fig. 6). In the bark of the pine stem hydrogen phosphate anions concentration at the top part and 14 m height in comparison to the butt-end was 16 and 21% higher, respectively.

The hydrogen phosphate anions concentration in the bark of the trunk of *Morus alba* L. varied from 82 to 130 mg/kg. On the cross-section at the heights of 0.1 and 0.5 m from the ground the hydrogen phosphate anions concentration was from 40 to 60% lower in comparison to heights of 1.1 and 2.2 m. The bark had nine times lower hydrogen phosphate anions concentration in comparison to their content in the sapwood perimeter zone from the cross-section at the height of 0.1 m and five times lower from the cross-section at the heights of 0.5 and 1.1 m and more than three times lower from the cross-section at the height of 2.2 m from the ground. Whereas hydrogen phosphate anions concentration in the trunk of *Quercus petraea* Liebl. at the height of 5 m was eight times less in comparison to the sapwood zone, but one and a half times higher than the heartwood zone (Krutul et al. 2007). Bark from the stem of *Pinus sylvestris* L. obtained from environments with varying degrees of contamination was characterized by one and a half to ten times lower hydrogen phosphate anions concentration relative to their content in sapwood zone, but one and a half to four times higher relative to their content in pith adjacent heartwood (Krutul et al. 2015).

Sulphate anions concentration

The data presented in Fig. 7 show that regardless of the height of the cross-section of the *Pinus sylvestris* L. stem, the sapwood perimeter zone had a higher concentration of sulphate anions (SO_4^{2-}) in comparison to the concentration in pith adjacent heartwood and middle heartwood zone.

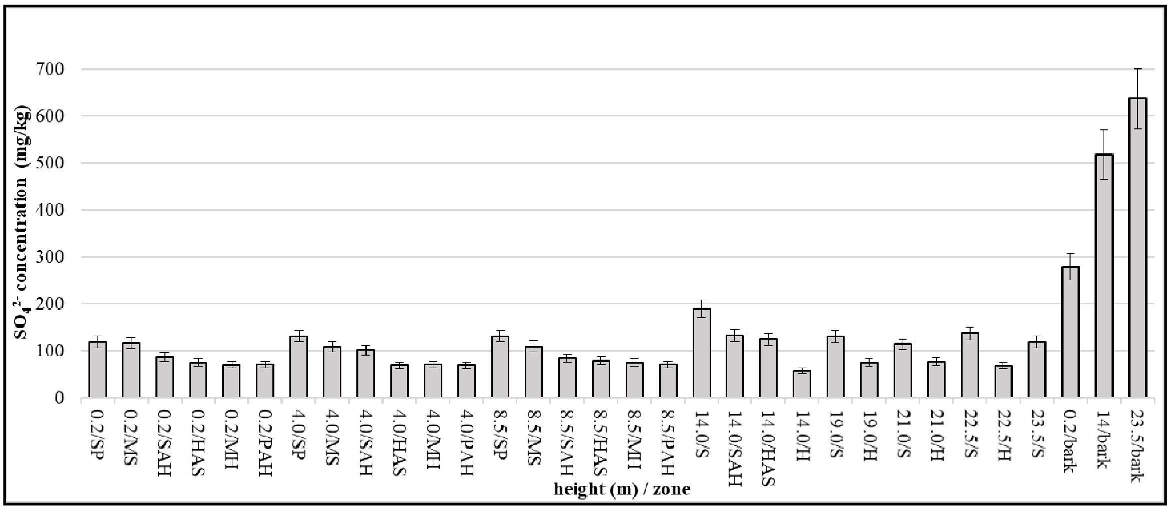

Fig. 7: Sulphate anions (SO_4^{2-}) concentration in examined wood zone and bark of the Pinus sylvestris L. stem.

In the bark from trunk of the *Quercus petraea* Liebl. at the height of 5 m from the ground sulphate anions (SO_4^{2-}) concentration equalled 647 mg/kg. On the other hand, in the bark of the *Morus alba* L. trunk concentration of these anions was from 278 mg/kg to 627 mg/kg. At different heights of the trunk sulphate anions concentration increased from the butt-end to the height of 2.2 m (Krutul et al. 2018a). Based on the data presented in Figs. 1, 2 and 7 it can be stated that sulphate anions distribution on the cross-section of the *Pinus sylvestris* L. stem was different than extractives and ash distribution. Sulphate anions distribution on the cross-section was similar to hydrogen phosphates anions (HPO_4^{2-}) distribution (Figs. 6 and 7). Sulphate anions distribution on the cross-section at different heights of the *Pinus sylvestris* L. stem depended on the annual rings width and the share of latewood in annual rings (Figs. 4 and 7).

CONCLUSIONS

On the base of the results following conclusions can be drawn: *(1)* Extractives content on the cross-section of the *Pinus sylvestris* L. stem regardless of the fraction (1.2 mm - 0.49 mm and <0.15 mm) of analyzed samples increased in the direction from sapwood perimeter to middle heartwood and pith adjacent heartwood zone. *(2)* Annual rings width was higher in pith adjacent heartwood and middle heartwood in relation to sapwood perimeter zone and influenced higher ash content in these zones relation to sapwood perimeter zone. *(3)* The sapwood perimeter zone was characterized from 40% to three times higher share of latewood on the cross-section in annual rings of the *Pinus sylvestris* L. stem compared to pith adjacent heartwood and middle heartwood zone. *(4)* The greater the share of latewood in annuals rings the lower extractives and ash contents. *(5)* Hydrogen phosphate anions (HPO_4^{2-}) concentration in sapwood perimeter zone was several times higher compared to pith adjacent heartwood and middle heartwood zone and the distribution of this anion on the cross-section at different heights of the stem was similar with the share of latewood in annual rings. *(6)* Sulphate anions (SO_4^{2-}) distribution on the cross-section of the *Pinus sylvestris* L. stem was different from the extractives and ash distribution, but it is similar to hydrogen phosphate anions (HPO_4^{2-}) distribution. *(7)* Sulphate anions (SO_4^{2-}) concentration on the cross-section of the stem depended on the annual rings width and the share of latewood in annual rings.

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