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# INFLUENCE OF AIR POLLUTION AND EXTREME FROST ON WOOD CELL PARAMETERS AT MOUNTAIN SPRUCE STANDS (*PICEA ABIES* (L.) KARST.) IN THE ORE MOUNTAINS

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## ABSTRACT

The aim of the research was to evaluate the potential of wood anatomy parameters as stress indicators on base of changing cell characteristics and proportion of latewood in Picea abies stands damaged by extreme climatic conditions in combination with high air pollution load during the winter 1995/96. The research was carried out in the Ore Mountains (Czech Republic), where sites were located along the gradient of forest damage. Preliminary analyses showed the decrease of lumen width, cell wall thickness and the number of tracheid in the tree rings of spruce at heavily damaged site. Significant difference was shown between sites with different damage level. Moreover the difference in reaction dynamics of earlywood and latewood parameters was recorded. The length of stands regeneration was shown to be around 3 years depending on the assessed parameter and the damage rate.

KEYWORDS: Radial lumen width, latewood proportion, cell-wall thickness, air pollution, *Picea abies*, Ore Mountains.

## INTRODUCTION

Radial growth of the tree is a result of a combined influence of tree genetics and environmental factors (Fritts 1976). Cook (1985) defines radial growth as a function of climate, disturbances (external and internal) and biological variability. Though this model was primarily constructed by Cook (1985) for ring-width characteristic, it is applicable for cell growth analysis as well, as changes in tree growth are determined by modifications in cell structure (Vaganov et al. 2006, Wimmer 2002). The study of the environmental influence on wood anatomy is usually defined as "ecological wood anatomy" (Wimmer 2002). Ecological wood anatomy is focused on two main points: 1) the description of direct changes in wood structure as a response to one or several stress factors and 2) the evaluation of adaptation strategies of the tree on base of correlation values between environmental events and individual cell parameters (Wimmer 2002). Different cell features (both continuous and discontinuous) such as cell diameter, tissue proportions, wall thickness, lumen area, latewood proportion, resin ducts, density fluctuations etc. can provide useful information about the growth environment (Ziaco et al. 2014, Olano et al. 2012, Wimmer 2002, Wimmer and Grabner 1997). All these factors react on stress events in a different way thus make them a good proxy for dendroecological studies.

In Central Europe one of the most significant abiotic stress factors influencing forest growth during the last 150 years was continuous transboundary air pollution. One of the most polluted areas in Europe is considered to be known as "Black triangle" – the area that covers northern Bohemia, southern Saxony and part of lower Silesia. This area received its name due to the high emissions of sulphur dioxide, nitrogen oxides and dust, coming from anthropogenic activities (mainly power plants burning brown coal, petrochemical and heavy industry, Renner 2002, Blažková 1996). Emission of main pollutants SO2, NOx culminated in 1980´s. While in the beginning of 90's situation has significantly changed with the changing of the political and economic situation in Europe.

Generally it is assumed, that conifer trees are more susceptible to pollution load than deciduous trees (Vacek et al. 2013). In Europe the most significant influence was detected at high mountain regions covered with Norway spruce (*Picea abies* L. Karst) (Godek et al. 2015, Staszewski et al. 2012, Fleischer et al. 2005, Herman et al. 2001, Bytnerowicz et al. 2003), one of the most common tree species in Europe with a high economic value. Among the Central European countries the forests in Czech Republic are considered to be the most influenced ones (Kolář et al. 2015, Lomský et al. 2012, Šrámek et al. 2008, Bridgman et al. 2002, Blažková 1996).

In this case study we will present the first results of air pollution influence on the wood anatomy structure of spruce stands in the Ore Mountains, where the positive development of forest health stay after 1990 was interrupted by extreme winter 1995/96 (Lomský et al. 2013, Lomský and Šrámek 2004). Extreme winter 1995/1996 is characterized by a sudden temperature decrease in November 1995, which was followed by heavy frosts and long-term inversion, resulted in extreme frosts deposits. It also created very good conditions for air pollutants accumulation ($SO_2$ and F) (Lomský et al. 2012, Bridgman et al. 2002). The most affected area within the Czech Republic was an eastern part of the Ore Mountains where air pollution load was the highest. Here about 12 500 ha of spruce stands were heavily damaged, 1 300 ha of them have completely died (Lomský et al. 2013, Lomský and Šrámek 2004). The rest gradually regenerated in the following years.

Our aim was to see: 1) how pollution and climatic conditions during the winter of 1995/1996 influenced the main cell anatomy parameters (radial lumen width, cell wall thickness, proportion of latewood and tracheid number); 2) if there is a difference in stress reaction between the earlywood and latewood and 3) if there is a lag in tree growth reaction on stress event.

## MATERIAL AND METHODS

### Study sites

The Ore Mountains are situated on the border between Czech Republic and German Saxony. It is a typical mountain region with the area of 114 357 ha. Mother rock is formed by gneiss, granite, phyllite and mica schist. Climate of the Ore Mts. is moderately cold with mean July temperatures around 12 - 15°C and mean annual temperature 5.4°C. On top parts of the range mean July temperatures can decrease under 10°C. Border parts of the mountains are warmer with higher amount of precipitations. Average precipitation rate is equals to 750 mm, with around 450 mm during the vegetation period that lasts for 112 days (Plíva and Žlábek 1986).

For our research purposes 7 permanent plots along the main ridge were established. Plots were located along the gradient of forest damage after the winter 1995/1996 in similar site conditions to exclude the microsite influence. All the stands were chosen to be pure spruce stands with 50-60 year old trees. The sites were divided into three groups according to the defoliation rate: Slight damage (defoliation rate under 40 %), medium damage (40 – 60 %) and heavy damage (above 60 %). In this preliminary paper the results from 3 sites, Cínovec (CIN, heavy damaged site), Kálek (KAL, medium damage site) and Loučná (LOC, slight damage site), will be presented (Fig. 1, Tab. 1). While plot CIN in the eastern part of the Ore Mountains represents the most damaged stands with the mean defoliation rate of 77.4 %, plot KAL in the middle part of the mountain ridge is moderately damaged with mean defoliation of 46 %. Plot LOC (mean defoliation rate of 33.3 %) was selected in less affected stands in the western part of the Ore Mountains.

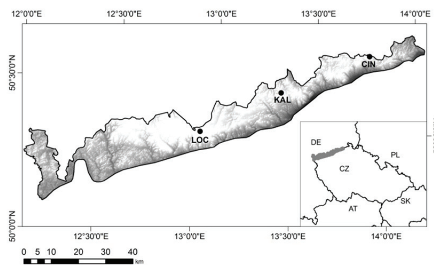


*Fig. 1: Map of sampled sites.*

*Tab. 1: Description of studied sites.*

| Plot | Coordinates JTSK | | Altitude, m a.s.l. | Exposition | Age class* | Forest type | Demage level 1995/96 |
|---|---|---|---|---|---|---|---|
| | X | Y | | | | | |
| LOC: Loučná | 993239,23 | 839595,89 | 990 | NW | 5 | Acidic Beech–Spruce | slight |
| KAL: Kálek | 979101,47 | 809840,91 | 815 | SW | 6 | Acidic Beech–Spruce | medium |
| CIN: Cínovec | 777321,85 | 965822,91 | 820 | NW | 6 | Acidic Beech–Spruce | heavy |

*Age class 5 - 40 – 50 years old stands, age class 6: 50 – 60 years old stands.

## Dendrochronology

For tree-ring analysis the increment cores were sampled from at least 20 dominant or co-dominant trees per each plot in spring 2014. Two cores per tree were taken at breast height (1.3 m above ground). Ring widths were measured to an accuracy of 0.01 mm, using time Tab. 2, and were subsequently visually cross-dated and statistically verified using the COFECHA program (Holmes 1983). The basic statistics of ring-width chronologies such as the mean sensitivity (a measure of the annual variability in tree rings), the average correlation with master chronology and the first-order autocorrelation in the series (a measure of the association between growth in the previous year and that in the current year) were computed.

The ring-width series were standardised to eliminate the age trend using the ARSTAN program (Cook and Holmes 1996). The trend was approximated by the Hugershoff function (Warren 1980), which effectively reflects the exponential decrease of annual increments in young trees. The resulting chronologies were aggregated in stand-level chronology by calculating bi-weight robust mean.

## Wood anatomy

For wood anatomy analyses were sampled 3 trees per site, always two cores per tree. The 1991 – 2001 period was analysed. Cores were cut using the GSL-1 microtome (Gärtner et al. 2014). The microsections were sliced into 15 µm and non-Newtonian fluid was used to avoid the breakage of cell walls (Schneider and Gärtner 2013). The microsections were double-stained using Astrablue and Safranin, dehydrated with alcohol and embedded in Canada balsam (Gärtner and Schweingruber 2013). The permanent slides were then photographed at a magnification of 20x with Nicon Eclipse 80i microscope.

The microscopic images were analysed using NIS-Elements software. As conifers have quite a homogeneous structure, within the each tree ring from 3 to 5 files were measured depending on the ring width (Axelson et al. 2014, DeSoto et al. 2011). It was assumed that wood anatomy characteristics will change differently for earlywood (EW) and latewood (LW) part of the tree. Thus chosen anatomical parameters were measured separately for both EW and LW: Tracheid number (NT), radial lumen width (RLW), cell-wall thickness (CWT) and proportion of LW (PrLW). EW and LW were distinguished on base of the Mork index (Mork 1928, Denne 1988). A latewood tracheid was defined as one in which the twice cell wall width between two adjacent tracheids was equal to or greater than the radial width of the lumen. The non-parametric Kruskal-Wallis test (Quinn and Keough 2002) was employed to test the differences in anatomical properties of wood during the period prior to stressful winter 1995/1996 (1993 – 1995), three years after the stress event (1996 – 1998) and the period of recovered growth (1999 – 2001).

# RESULTS AND DISCUSSION

## Dendrochronology

The lowest average ring width was recorded for the most damaged plot CIN. In the same time the highest annual variability in tree-ring widths expressed in the mean sensitivity value was observed for this plot (Tab. 2). Wider tree rings but lower annual variability was proved for tree-ring series of less affected trees at the plots KAL and LOC. The growth in the previous year strongly affected the growth in the current year primarily on slightly damaged plot LOC.

*Tab. 2: Properties of ring-width chronologies.*

| Plot | Number of radii | Master chronology | Missing rings (%) | Avg ring width (mm) | Std dev | Mean sensitivity | Corr with master (r) | Autocorr of 1st order |
|---|---|---|---|---|---|---|---|---|
| CIN Cínovec | 42 | 1963-2013 | 0.7 | 2.21 | 1.038 | 0.319 | 0.652 | 0.640 |
| KAL Kálek | 42 | 1961-2013 | - | 2.91 | 1.046 | 0.250 | 0.641 | 0.627 |
| LOC Loučná | 42 | 1978-2013 | - | 3.63 | 1.048 | 0.167 | 0.582 | 0.708 |

An abrupt growth decrease in 1996 was observed at plots CIN and KAL, but no growth reduction was obvious for LOC (Fig. 2). There was a difference in growth regeneration between CIN and KAL. While at the first plot the growth declined even in 1997 and growth depression lasted till 1999, at the second plot the ring widths had increased sharply already in 1997 and growth rate fully recovered in 1998.

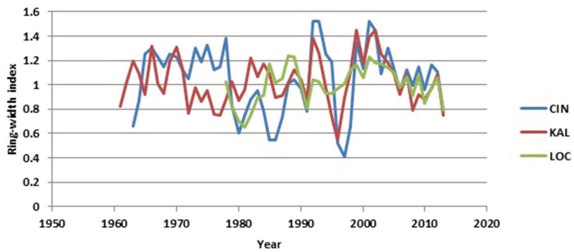


*Fig. 2: Standard ring-width chronologies.*

Radial growth showed a clear reaction to the stress event in the winter of 1995/96, which manifested as an abrupt decrease in the values recorded. The intensity of growth reduction was dependent on forest damage. On the heavily damaged plots CIN the mean defoliation in 1996 and 1997 was 77.4 and 60 % respectively. In 1996 most of the first and the second year needles reddened and subsequently fell as a consequence of direct impact of $SO_2$ (Fabiánek 1997). The increment responds sensitively as the youngest, most photosynthetically active needle sets are impaired (Straw et al. 2002). In tree-ring series of heavily damaged trees the missing rings were detected in the period 1996-1998 with maximum in 1997. In the case of foliage loss over 60 % the radial growth is significantly reduced not only in the year of stress event but also in 2 or 3 years following it (Armour et al. 2003, Kurkela et al. 2005). It also coincides with the results of Lomský et al. 2013 when the recovery of tree growth after 1995/1996 stress years continued for around 3 years.

## Wood anatomy

Altogether for wood anatomy purposes 18 cores from 9 trees sampled at 3 sites were analysed. According to the aims of the research we conducted more detailed analyses of the period prior to stress event (1993-1995) and two periods after the stress event (1996 - 1998 and 1999 - 2001). The main descriptive characteristics for all three periods are introduced in Tab. 3. At CIN and LOC sites was observed the decrease in mean values of all wood anatomy characteristics after the stress event. Similar trend was shown at KAL site with the exception of CWT parameter. All characteristics returned to their near pre-stress values or even exceeded it in a period 1999 - 2001.

*Tab. 3: Mean values of radial lumen width (RLW), radial cell diameter (RCD), cell wall thickness (CWT) and tracheid number (NT) at three chosen sites before stress event and after it for the whole tree ring (TR), earlywood (EW) and latewood (LW).*

| Plot | | RLW (µm) | | | RCD (µm) | | | CWT (µm) | | | NT | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 1993 – 1995 | 1996 – 1998 | 1999 – 2001 | 1993 – 1995 | 1996 – 1998 | 1999 – 2001 | 1993 – 1995 | 1996 – 1998 | 1999 – 2001 | 1993 – 1995 | 1996 – 1998 | 1999 – 2001 |
| CIN | TR | 18.42 | 15.88 | 19.65 | 24.81 | 21.09 | 26.18 | 3.20 | 2.71 | 3.27 | 39.98 | 16.84 | 32.61 |
| | EW | 28.20 | 21.76 | 30.30 | 31.41 | 23.83 | 33.32 | 2.35 | 2.33 | 2.55 | 59.28 | 25.89 | 55.83 |
| | LW | 10.22 | 9.27 | 10.02 | 18.22 | 15.70 | 18.15 | 4.01 | 3.25 | 3.99 | 13 | 8.5 | 16.83 |
| KAL | TR | 18.03 | 17.95 | 18.94 | 24.87 | 23.88 | 25.74 | 3.43 | 3.72 | 3.41 | 51.60 | 45.70 | 64.00 |
| | EW | 26.02 | 25.50 | 27.27 | 31.53 | 31.08 | 32.61 | 2.75 | 2.92 | 2.67 | 67.94 | 72.28 | 108.89 |
| | LW | 10.43 | 10.41 | 10.61 | 18.76 | 18.85 | 18.87 | 4.19 | 4.25 | 4.14 | 22.83 | 19.17 | 19.11 |
| LOC | TR | 20.67 | 20.06 | 20.56 | 27.14 | 26.1 | 27.18 | 3.25 | 3.04 | 3.32 | 67.51 | 60.39 | 59.53 |
| | EW | 27.73 | 28.16 | 29.33 | 32.54 | 33.08 | 34.16 | 2.41 | 2.47 | 2.41 | 113.56 | 108.78 | 96.06 |
| | LW | 12.63 | 9.31 | 9.45 | 20.98 | 15.42 | 17.21 | 4.30 | 3.15 | 3.90 | 17.72 | 12.67 | 24.06 |

The initiation of cambial activity in trees growing in the polluted areas is usually delayed in comparison with trees of normal conditions. The cease of vegetation period at polluted sites also starts earlier than at unstressed sites. The difference is shown in structure and arrangement of xylem derivatives between affected and unstressed sites as well, which leads to the decrease of radial cell diameter and number of tracheids (Rajput et al. 2008).

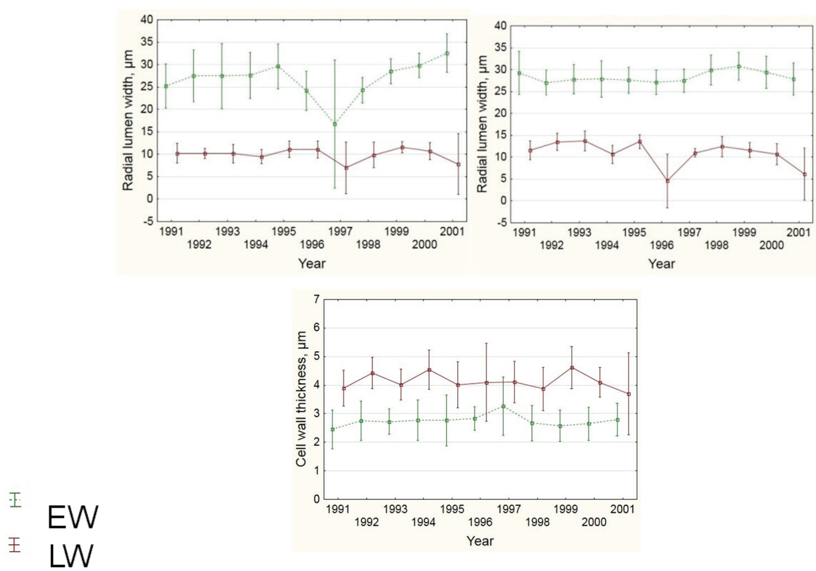


*Fig. 3: Radial lumen width of cells for earlywood and late wood at three-level damaged sites (a – Cínovec, b – Loučná, c – Kálek).*

At CIN site the missing ring of 1997 year was registered in 2 cores. No missing rings were found at KAL and LOC sites. The lowest values for lumen width, cell wall thickness, proportion of LW and number of tracheids at CIN site were reached in 1997 year. The growth at LOC site as the least stressed one showed the slight decrease of cell wall thickness, proportion of LW and tracheid number in 1996, the significant decrease in tracheid number at KAL site was also registered in 1996 (Figs. 3, 4, 5).

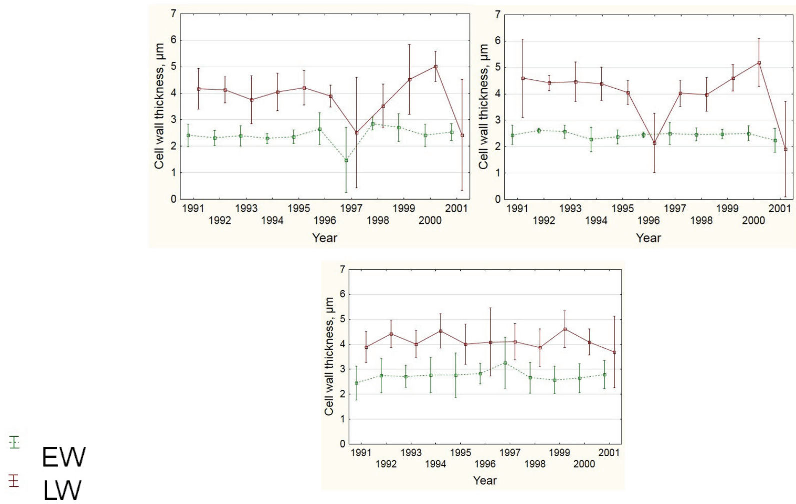


*Fig. 4: Cell wall thickness of cells for earlywood and latewood at three-level damaged sites (a – Cínovec, b – Loučná, c – Kálek).*

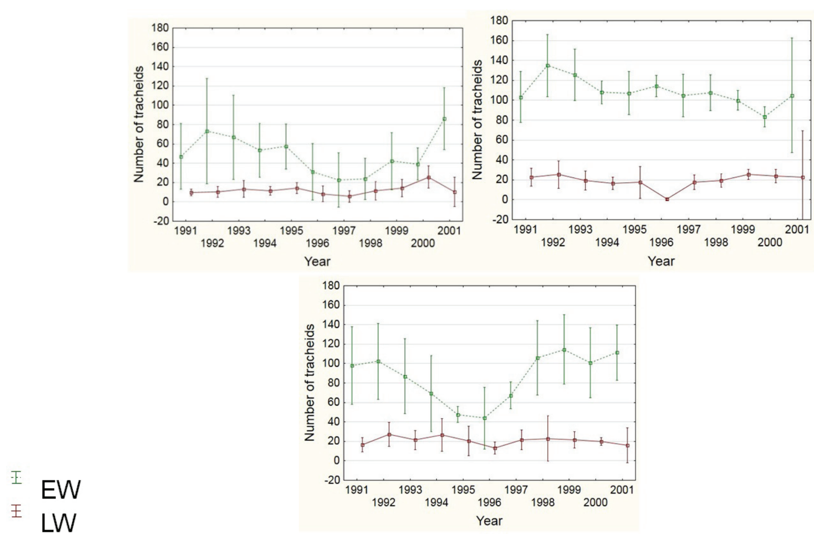


*Fig. 5: Number of tracheids in tree rings for earlywood and latewood at three-level damaged sites (a – Cínovec, b – Loučná, c – Kálek).*

At CIN site the period of decreased lumen width and tracheid number lasted from 1996 to 1998, from 1999 was observed the recovery of growth (Fig. 3, 5). For cell wall thickness the period of decline lasted from 1997 to 1998, thus this parameter reacted on stress event with the one year lag (Fig. 4). At less damaged KAL and LOC sites the decline period of tracheid number lasted from 1996 to 1997, thus the recovery hear started earlier than at heavy damaged CIN site (Fig. 5). The time-lag of the growth reaction to environmental stress was also observed by Axelson et al. (2014). In our study, however, EW part reacted more intensively to the stress event which is in contrast to the results of Axelson et al. (2014). This is however directly connected to the type of stress event suppressing the tree growth. In the Ore Mts. the stress event occurred during the winter time causing serious damage to the foliage and disrupting the EW formation. Strong air pollution influences the cambium and differentiation of cambial derivatives, thus influencing the time of recovery. Its influence on xylogenesis however needs further investigations (Kurczynska et al. 1997).

At CIN site the decrease of all cell parameters in after-stress period was characterized for EW, for LW the decrease of only cell wall thickness was registered, the same was observed at LOC site. At KAL and LOC site the results were not so conclusive. The decrease of tracheid number has been clearly seen only at earlywood zone at KAL site (Figs. 3, 4, 5).

The analyses implied the different sensitivity of EW and LW of different parameters to stress event. The difference between pre-stress and after-stress periods at KAL and LOC sites were not significant. At CIN site the decrease of lumen width and tracheid number for EW and cell wall thickness for LW was found to be significant in comparison to prior to stress period. The different sensitivity of EW and LW parameters was also shown by the other research (Ziaco et al. 2014, Olano et al. 2012, Park and Spiecker 2005). Wimmer and Halbwachs (1992) observed the changing wood-anatomical features of Norway spruce under the pollution on German side of the Ore Mountains. They pointed out the decrease thickness of the latewood tracheids and decrease of tracheid length. A reduction of earlywood tracheid cross-area was also observed.

Besides the effects of stressful 1995/1996 period on tree growth and wood anatomy, a decrease of LW cell wall thickness was observed at CIN and LOC sites in 2001, which also corresponds with the decrease of TRW in the same year. The reasons for this event are still unclear However the weakened of temperature – growth relationship at this period was also observed at Krušne Hory by Kolář et al. (2015).

The results of wood anatomy analyses correspond well with the dendrochronology results. Our analyses showed, that the most responsive anatomy parameter to pollution was the number of tracheids. Minimum values of tracheid number were reached in 1996 or 1997 depending on the pollution load at the site. Both EW and LW amount of cells within the tree ring decreased in the years following the stress event.

Sites with higher defoliation level were also characterised by smaller proportion of LW in the years following the stress event due to the pollution influence on the cambial activity through changing the differentiation of cambial derivatives in xylem side (Kurczyńska et al. 1997). The same impact of pollution on conifer growth was also observed by Kurczyńska et al. (1997) in Scots pine and by Wimmer (2002) in Norway spruce. Other anatomical parameters as radial lumen width and cell wall thickness showed clear response to the pollution stress in decreasing values only at heavy damaged site. Similar impact of pollution was observed by Maranho et al. (2009) on the example of *Podocarpus lambertii* Klotzsch ex Endl. (*Podocarpaceae*) in Southern Brazil. They showed that the trees that were exposed to petroleum pollution had smaller cell diameter and cell wall thickness.

In comparison to heavy polluted CIN site, only a slight decrease of anatomy values was observed at control sites KAL and LOC which highlight the role of pollution intensity in activating the changes in wood anatomy. Genetics, age of the stand (younger forest stands at LOC site) and local site specifics can further modify the reaction of tree growth on pollution stress. Wimmer (2002) highlights that different wood anatomy parameters in the tree are under the genetic control to some degree. The genetic influence can change with time thus causing the changing of environmental signal strength in wood anatomy parameters. Lomský and Šrámek (1999) and Sperry et al. (2006) pointed out, that younger trees are more sustained to pollution and are capable of faster regeneration.

## CONCLUSIONS

The research was conducted on the Norway spruce stands in the Ore Mountains that were intensively suffered from industrial pollution during the 20th century. A special focus was put on extremely harsh winter of 1995/1996, when the fog and atmospheric inversion created favorable conditions for drastic emission accumulation. Our preliminary research showed that pollution caused the abrupt growth reduction in 1996-1997 in affected sites. The decrease in values of the main cell anatomy parameters as radial lumen width, cell wall thickness, number of tracheids and the proportion of LW was identified. Number of tracheids was defined as the most responsive parameter to the stress event, which was registered at all sites with different damage level. The difference in sensitivity of LW/EW cells was shown. Further research in this field however is needed to identify which EW and LW parameters are more sensitive to stress and how fast they are able to recover after it. In general, longer regeneration period was observed at heavily polluted site in comparison to control site.

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