Anatomical Structure and Copper Microdistribution in Mechanical, Biological, and Laser Incised Spruce and Larch Refractory Woods

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The anatomical structure of wood and the application of three different incision pretreatments affect the distribution of preservatives in refractory woods. This study focused on *Picea orientalis* (L.) Peterm.) and *Larix decidua* Mill. and investigated the distribution of copper-based preservatives in the wood microstructure. Different incision pretreatments were applied before impregnation to increase the permeability of spruce and larch sapwood samples. After the incision pretreatments, transverse cross-sectional surfaces of the samples were sealed with polyurethane-based paint to prevent excessive preservative uptake into open ends of longitudinal tracheid lumens. The samples were then impregnated with Celcure C_4, by applying a vacuum method. The structure of wood samples with preservatives before and after the incision pretreatments were observed. Copper microdistribution was observed to increase significantly in refractory wood species after different incision pretreatments, especially in larch wood. The degradation of pits caused by biological incision effectively increased the microdistribution of copper. The difference in the microdistribution of increased copper with the laser and mechanical incision pretreatments – following the same incision model – was attributed to the different anatomical structure and density of spruce and larch wood species and the fact that the hole depth and geometry were different in the laser and mechanical incision processes.

DOI: 10.15376/biores.18.1.1368-1383

Keywords: Laser incision; Mechanical incision; Biological incision; Refractory wood species; Anatomical structure; Copper microdistribution

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INTRODUCTION

Incision pretreatment is a technique used to improve fluid flow in wood with low permeability during wood impregnation. It is very important to protect the wood by impregnation in areas of use where longer service life is required, such as poles, fences, wood sidings used outdoors, and wooden structural elements in buildings and bridges. The effectiveness of the protective treatment is determined by considering the penetration and retention values governed by the permeability of the wood (Wang and DeGroot 1996; Watanabe *et al*. 1998; Lehringer *et al*. 2010; Ahmed *et al*. 2012; Panigrahi *et al*. 2018). The permeability is low in refractory woods, and parts of the wood (*e.g*., heartwood) affect preservative penetration, retention, and distribution. Permeability affects the uptake of wood preservatives and other industrial processes such as pulp and wood drying (Voulgaridis *et al*. 2015). Wood permeability affects the processing time and product quality, which are important factors affecting price (Poonia *et al*. 2016). Therefore, the wood protection industry must have appropriate strategies and techniques to ensure that the impregnation agents penetrate the wood sufficiently and effectively (Nath *et al*. 2020a). Wood species with poor impregnation, and hence short service life, can be pretreated and used as an alternative to expensive tropical wood species with high natural durability or permeability, where a long service life is required.

The impregnability of tree species with low permeability, such as spruce and larch species, is very difficult due to the presence of heartwood and the aspiration of the bordered pits on the radial walls of the longitudinal tracheids in the sapwood part of the wood (Matsumura *et al*. 1999; Messner *et al*. 2003; Pánek and Reinprecht 2011; Yıldız *et al*. 2012; Durmaz and Yıldız 2016; Panigrahi *et al*. 2018). This problem has not been solved optimally. Incision pretreatments can be physical or biological. Physical incision techniques use teeth, knives, drills, needles, lasers or high-pressure water jets to create flow paths in wood to the desired depth of penetration, requiring a uniform operation. In biological incision techniques, enzymes, bacteria, and fungi are used to increase permeability (Winandy *et al*. 2022). In practice, various mechanical or laser incision technologies are generally used to increase the liquid uptake from the outer surfaces of the wood. Mechanical incision pretreatments used today significantly reduce the aesthetic and mechanical properties of wood products (Pánek and Reinprecht 2011).

Terziev *et al*. (2020) examined the effect of two industrial frequency microwave treatments on the microstructure and ultrastructure of *Picea abies* (L.) Karst. and *Pinus radiata* D. Don. Improvement in the permeability of the wood could be explained by the decrease in mechanical performance. Dale *et al*. (2019) stated that mechanical incision pretreatment can be used to improve preservative penetration, but it cannot be equivalent to a typical impregnated wood since it changes the appearance of the wood while reducing its mechanical properties. However, laser incision technology has potential to improve liquid impregnation (Islam *et al*. 2007, 2008). The laser incision technique has been applied on wood surfaces and is promising for improving the permeability of refractory wood. Various process parameters (such as incision type, depth, layout and geometry, as well as the unique physical properties of tooth, needle, drill, laser, or water jet incisions and biologically-derived fluid flow paths) affect the machinability and strength of wood (Winandy *et al*. 2022). However, there is still considerable speculation and debate about the relative effectiveness and future of each of these parameters on the physical, mechanical, chemical, and anatomical properties of wood. Numerous studies have been carried out to increase the permeability of wood species that are difficult to impregnate using various techniques; however, there are limited studies on the comparative effects of these different incision pretreatments (Tajrishi *et al*. 2021; Bakir 2022a,b).

This study examined the effects of laser, mechanical, and biological incision pretreatments on the anatomical properties of two different refractory wood species. The copper distribution values in the wood microstructure were analyzed.

EXPERIMENTAL

Test Materials

Kiln-dried sapwood samples that did not contain any defects were collected from Oriental spruce (*Picea orientalis* (L.) Peterm) and European larch (*Larix decidua* Mill.) grown in Karabula in the Krasnoyarskiy Kray region of Russia. They represent native and exotic wood species that are difficult to impregnate. All wood samples were cut into dimensions of 120 mm × 30 mm × 30 mm (length × width × height). The samples were grouped to apply different incision pretreatments. A total of 80 samples were prepared, including 10 spruce sapwood samples with impregnation non-pretreated, 10 larch sapwood samples with impregnation non-pretreated, 10 spruce sapwood samples with impregnation biological incised, and 10 spruce sapwood samples with impregnation biological incised were prepared along with larch sapwood samples including 10 spruce sapwood samples with impregnation mechanical incised, 10 larch sapwood samples with impregnation mechanical incised, 10 spruce sapwood samples with impregnation laser incised, and 10 larch sapwood samples with impregnation laser incised. The specimens were chosen to coincide with the same annual rings throughout the trunk to minimize any effect of the natural variability of the wood within itself. All samples were cut based on radial and tangential directions.

Biological Incision Pretreatment

Physisporinus vitreus (Pers.) P. Karst. (FP 103669-T) white rot fungus was used. The cultures were procured from USDA Forest Service Forest Products Laboratory, Madison, WI, USA. The wood samples were kept in a climatic chamber at 20 °C and 65% relative humidity for two weeks. Glass jars with dimensions of 170 mm × 100 mm × 100 mm (length × width × height) were used, as a larger volume was needed than the Kolle culture flasks used within the scope of the BS EN 113-1 (2020) standard for biological incision pretreatment. The metal lids of the jars used in the *P. vitreus* incubation processes were punched into a circular shape with a punch tool. The hole was clogged with cotton wool to meet the air and humidity needs of the fungi. Wood samples were placed directly into glass jars containing 4% malt-agar (MEA) nutrient medium pre-inoculated with fungus. Sterilized wet vermiculite was added to keep the humidity of the environment in the jar in suitable conditions for fungal growth. The air-dried sapwood samples were exposed to *P. vitreus* FP 103669-T white rot fungus with selective delignification for 8 weeks at 26 °C and 75% relative humidity to achieve a weight loss of approximately 10%. The resistance reductions for weight losses of less than 10% that occur in wood as a result of the biological incision are insignificant (Schwarze *et al*. 2006; Humar *et al*. 2012; Fuhr *et al*. 2013). Ensuring the effectiveness of incubation periods and proper development of *P. vitreus* fungus depends on the appropriateness of all conditions (nutrient, temperature, water activity, oxygen, and pH). A homogeneous biological incision process depends on the complete coating of the sample surfaces by the mycelia formed (Fig. 1).

Fig. 1. Images for: a) heterogeneous and b) homogeneous colonization of wood samples by *P. vitreus* fungus in glass jars

Kiln-dried samples were weighed before and after the biological incision, and the percentage weight losses were calculated by Eq. 1,

$$WL\ (\%) = \{(W_0 - W_1)|\ W_0\} \times 100 \quad (1)$$

where WL is the weight loss in the sample (%), W_0 is the kiln-dried weight of the sample before pretreatment (g), and W_1 is the kiln-dry weight of the sample after pretreatment (g).

Mechanical Incision Pretreatment

Some of the Oriental spruce and European larch wood samples were mechanically drilled to an incision depth of 10 mm and a diameter of 2 mm on the radial and tangential surfaces of the samples (Fig. 2) based on the same incision density model (10,000 incisions/m^2) (Fig. 3). Before the mechanical incision pretreatments, the wood samples were kept in a climatic chamber at 20 °C and 65% relative humidity for 2 weeks. The kiln-dried samples were weighed before and after the mechanical incision, and the percentage weight losses were calculated according to Eq. 1.

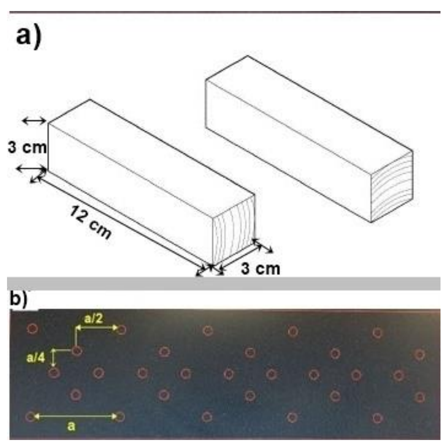


Fig. 2. Image of (a) the radial and tangential surfaces in the mechanical and laser incision model (b) the distances between the holes drilled in the lateral and longitudinal directions

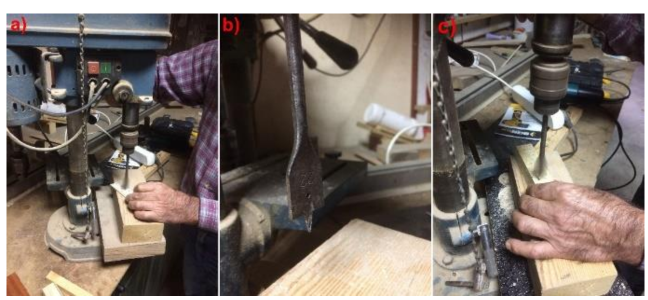


Fig. 3. (a) Preparation of a wood surface mold (10,000 incisions/m^2) for all mechanical incision operations (b) drill bit depth of 10 mm (c) incision of wood samples with drill bit

Laser Incision Pretreatment

Holes were drilled with a CO_2 laser (VLS6.60, Universal Laser Systems, Scottsdale, AZ, USA) on the radial and tangential surfaces of wood samples, at a laser power of 60 W, a speed setting of 4.0 and a 30 mm drill depth. The same incision pattern was used for all samples, as shown in Fig. 4. The incision density was designed to be the same (10,000 incisions/m^2) for both mechanical and laser incision pretreatments (Fig. 2b). All holes were drilled to a 10 mm depth and a 2 mm diameter. The parts (incision cores) that were carbonized as a result of the laser burning inside the holes were removed by removing them with fine-tipped tweezers (Fig. 4) (Islam *et al*. 2008). Kiln-dried samples were weighed before and after the laser incision, and the percentage weight losses were calculated according to Eq. 1.

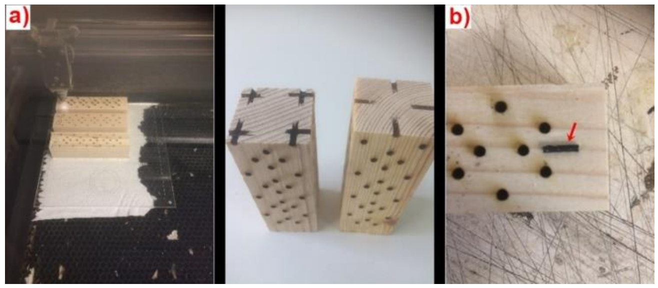


Fig. 4. (a) CO_2 laser incision of wood samples and (b) A view of drilled cavities in the laser-drilled wood shown in a burnt or charred state (arrow)

Impregnation Treatments

Spruce and larch sapwood samples that were not treated with any incising process were the controls. All wood samples were treated with Celcure C_4 wood preservative solution to compare their permeability. Celcure C_4 is a water-based wood preservative that contains an alkaline copper quaternary system and two organic co-biocides (benzalkonium chloride and cyproconazole). The concentration of Celcure C_4 solution for impregnation processes was 3%, and vacuum was applied for 40 min at 40 mbar according to BS EN 113-1 (2020). According to the instructions for use on the marine coatings, some samples' end-grain surfaces (transverse surfaces of the specimens) were sealed with a polyurethane coating (Moravia – MORAGEL polyurethane finish, Trieste, Italy) by brushing before treatment to compare the uptake in the transverse directions of preservative solutions into these surfaces. The preservative-treated samples were stored at 20 °C for 2 weeks to allow fixation of the preservatives.

Macroscopic and Microscopic Evaluations

Macroscopic examinations were made with the naked eye, loop, and a stereomicroscope on the transverse, radial, and tangential surfaces of the spruce and larch wood samples. By considering the lengths measured on the planks obtained from the trunks from a height of at least 30 cm from the ground contact, the trunk was classified as the bottom, middle, and top parts of the trunk. All wood samples (3 spruce and 3 larch sapwood samples) were selected from the bottom, middle, or top of the trunk to coincide with the same annual rings in it in order to minimize any effect of the natural variability of the wood. All samples were cut based on the radial and tangential directions. Before microscopic examinations, a total of 6 sticks ($10 \times 2 \times 2$ cm^3, length × radial × tangential) were obtained from each of the larch (3) and spruce (3) tree species, corresponding to the bottom, middle, and top parts of the timber. Each stick was then cut into three samples for light microscopy studies, yielding a total of 18 cubes, with each side 2 cm long (Fig. 5).

The changes that occurred as a result of laser, mechanical, and biological incision pretreatments were examined by light microscopy. Before the microscopic examinations, test samples (untreated spruce and larch sapwood samples) were kept in water at room temperature to soften them, and then 20 μm-thick sections were taken with the help of a sliding microtome (SM 2010 R, Leica, Wetzlar, Germany). To evaluate the samples under the light microscope, safranin was used to stain the lignin component red and to create a contrast between the cell walls. The control group samples and the laser and mechanically incised samples were stained only with safranin, while the biologically incised samples were stained additionally with picro-aniline blue to make the fungal mycelium visible (Wilcox 1964). Microscopic sections were observed under an Olympus BX51 Light Microscope (Olympus, Tokyo, Japan). Images were taken digitally using a DP 71 Digital Camera (Olympus, Tokyo, Japan) in the microscope. The microscope micrographs and ultramicrographs shown here are selected from the most severely damaged specimens during the fungal, laser beam and mechanical incision operations, to observe the visible effects of the *P. vitreus* and laser beams.

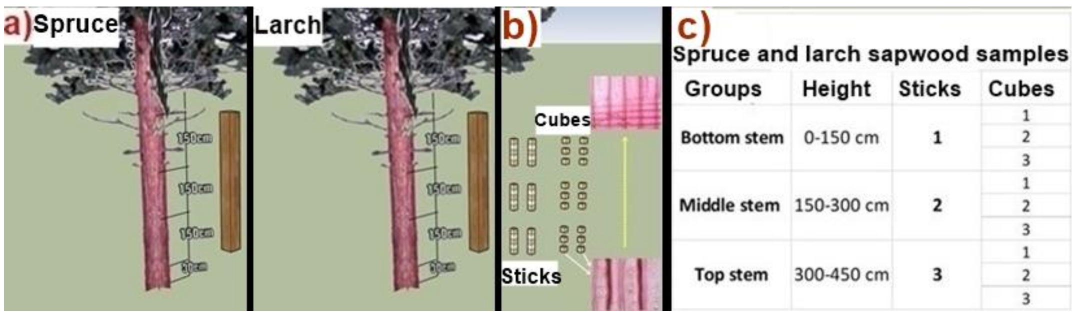


Fig. 5. Wood samples (a to b) and sample groups (c) were used in the anatomical studies of Oriental spruce and European larch woods

Ultramicroscopic Evaluations

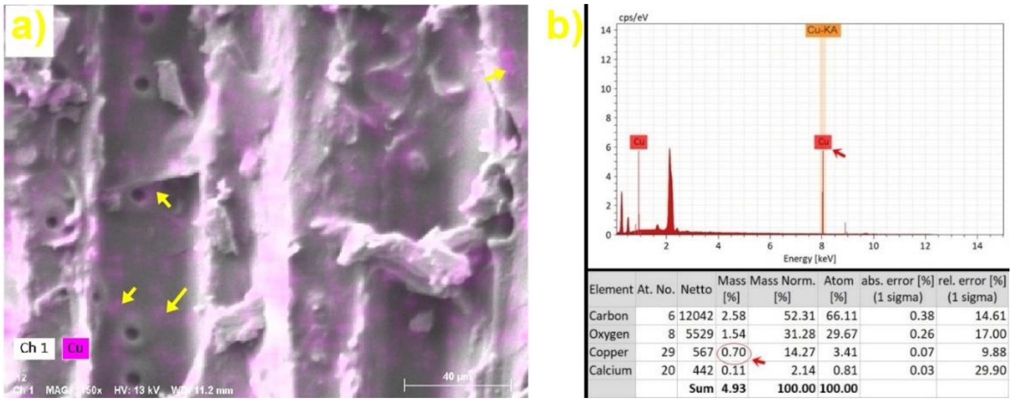
Ultramicroscopic examinations were performed on the same samples used for light microscopy. The samples were cut into $5 \times 5 \times 10\ mm^3$ pieces using a sliding microtome to obtain smooth surfaces. Ultramicroscopic evaluations were performed using scanning electron microscopy (SEM) (Carl Zeiss EVO LS10- Bruker EDS, Jena, Germany) under low vacuum conditions at the Artvin Coruh University Science-Technology Application and Research Center in Turkey. To improve the conductivity and quality of the SEM images, mature cypselas were placed on stubs using double-sided adhesive tape. The surfaces of the samples were coated with a thin layer of gold in a coating apparatus (Cressington Sputter Coater 108auto, TED PELLA INC.). A total of 200 micrographs and 40 ultramicrographs were examined.

Detection of Copper Microdistribution

For copper distribution, $5 \times 5 \times 10$ mm test samples were cut from the spruce and larch sapwoods. To improve the conductivity and quality of the SEM images, mature cypselas were placed on stubs using double-sided adhesive tape. The samples were coated with a thin layer of gold (Fig. 6). A Carl Zeiss EVO LS10-Bruker EDS (Weimar, Germany) SEM-EDX was used to detect the copper microdistribution in the wood structure (Fig. 6).

Statistical Analysis

Analysis of variance (ANOVA) was adopted to compare various observation groups in statistical evaluations. The Tukey test was used to determine the differences between the mean values of the properties examined in the applications, and a 95% confidence level was taken as the basis. ANOVA and Tukey tests were performed using JMP 5.0 statistical software (SAS Institute, Cary, NC, USA).



Element	At. No.	Netto	Mass [%]	Mass Norm. [%]	Atom [%]	abs. error [%] (1 sigma)	rel. error [%] (1 sigma)
Carbon	6	12042	2.58	52.31	66.11	0.38	14.61
Oxygen	8	5529	1.54	31.28	29.67	0.26	17.00
Copper	29	567	0.70	14.27	3.41	0.07	9.88
Calcium	20	442	0.11	2.14	0.81	0.03	29.90
		Sum	4.93	100.00	100.00		

Fig. 6. Copper (Cu) distribution (yellow arrows) (a) and copper microdistribution percentage (red arrows) (b) in the wood microstructure after different incising pretreatments (Radial section)

RESULTS AND DISCUSSION

Effects of Biological Incision Pretreatment on Wood Anatomy

The effects of biological incision pretreatment on structures of spruce wood are shown in Figs. 7 and 8. As a result of biological incision, *P. vitreus* fungus caused ruptures in the longitudinal tracheid cell walls (white arrows) and splits (yellow arrows) in the middle parts of the lamella (Figs. 7b to c and 8b to c).

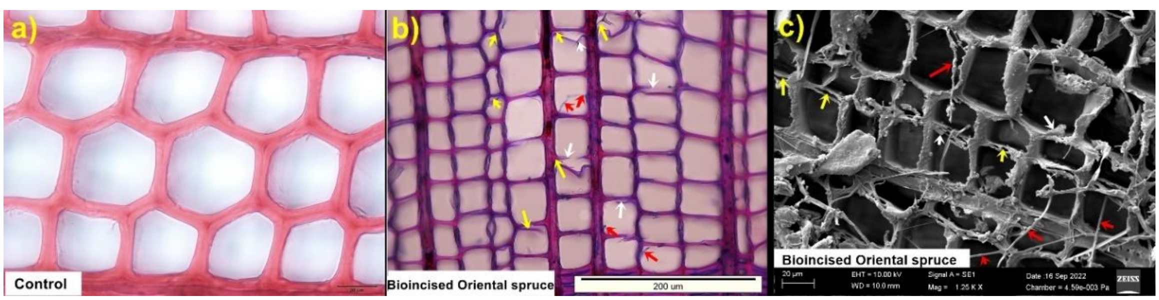


Fig. 7. a) Degradations caused by *P. vitreus* fungus in the sapwood microstructure of Oriental spruce Control and b to c) Bioincised Oriental spruce with splits in compound middle lamella (yellow arrows), ruptures on cell wall (white arrows) and fungus mycelia observed in longitudinal tracheid cell lumens (red arrows); transverse sections

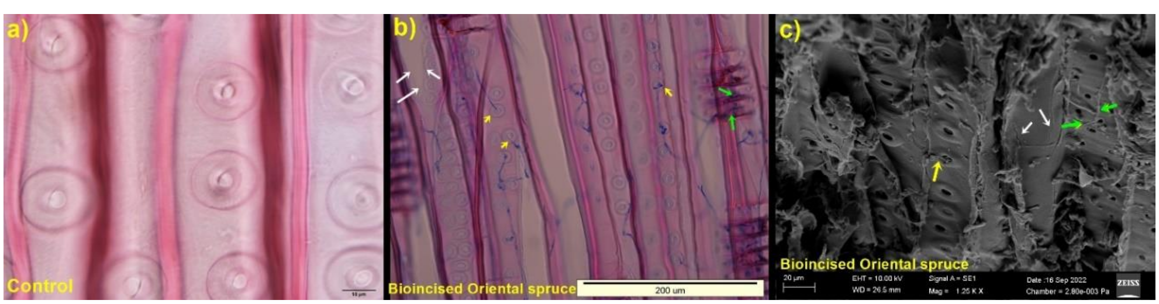


Fig. 8. a) Degradations caused by *P. vitreus* fungus in the sapwood microstructure of Oriental spruce Control: Intact bordered pits and b to c) Bioincised Oriental spruce: Degraded bordered pits (yellow arrows), ruptures and cracks on cell wall (white arrows) and degraded piceoid-type pits (green arrows); (radial sections)

The effects of biological incision pretreatment on microscopic and ultra-microscopic structures of larch wood are given in Figs. 9 to 10. Similar to the decompositions of Oriental spruce, *P. vitreus* fungus resulted in ruptures and cracks in the longitudinal tracheid cell walls (Figs. 9b to c and 10b to c, white arrows) and splits in the central parts of the lamella (Fig. 9b to c, yellow arrows) as a result of the biological incision.

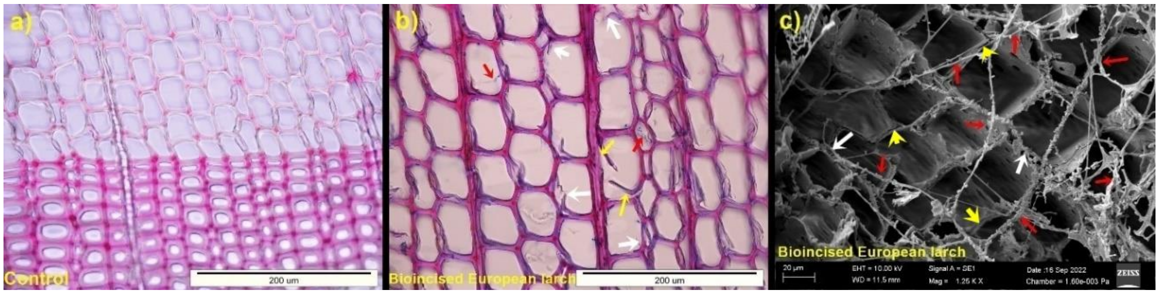


Fig. 9. a) Degradations caused by *P. vitreus* fungus in the microstructure of European larch sapwood Control and b to c) Bioincised European larch, splits in compound middle lamella (yellow arrows), rupture on cell wall (white arrows), and fungus mycelia observed in longitudinal tracheid cell lumens (red arrows); transverse sections

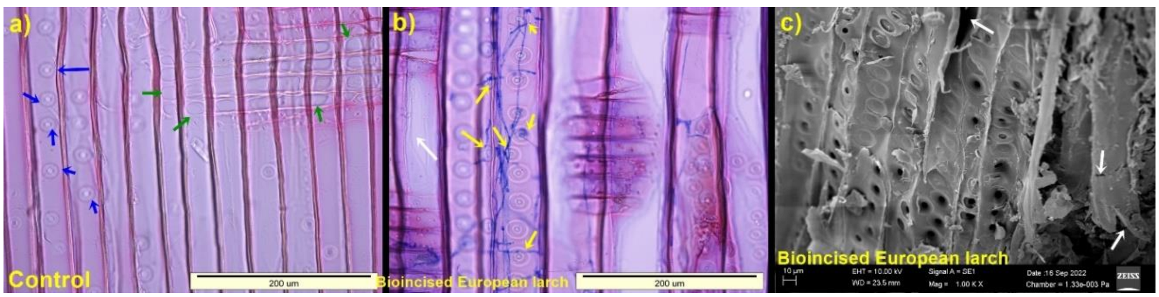


Fig. 10. a) Degradations caused by *P. vitreus* fungus in the microstructure of European larch sapwood Control: Intact bordered pits (blue arrows), intact window-type pits (green arrows) and b to c) Bioincised European larch: Degraded bordered pits (yellow arrows), ruptures and cracks on cell wall (white arrows); radial sections

Evaluations of samples of Oriental spruce and European larch sapwood after biological incision pretreatment showed that degradation, such as the splits and cracks indicated by yellow arrows (Figs. 7b to c and 9b to c) shown in spruce and larch sapwood, occurred at almost the same intensity in the samples. It can be argued that the intensities of degradation, such as the degraded bordered pits (yellow arrows) on the radial walls of the longitudinal tracheids of spruce and larch sapwood samples, and the cracks observed on the walls shown by white arrows, were similar in the two different wood types (Figs. 8b to c and 10b to c). In other words, it was understood that *P. vitreus* fungal activity is similar in the spruce and larch sapwood samples.

Lehringer *et al*. (2010) reported that *P. vitreus*, a basidiomycetes class fungus, causes both selective delignification and soft rot of types I and II in tracheid cell walls, which have a high heterogeneity during wood colonization. Decay patterns produced by this fungus resulted in selective degradation of the pit membranes in the bordered and semi-bordered pits. Various studies (Schmidt *et al*. 1997; Schwarze and Landmesser 2000; Schwarze 2007; Lehringer *et al*. 2009) suggested that *P. vitreus* fungus primarily targets pectin-containing bordered pits, and simultaneously or immediately after, it targets lignin and hemicellulose in cell walls. The literature also suggested that the types and amounts of wood cell elements and wall chemical components, which the aforementioned fungus degrades in spruce and larch sapwood samples in this current study, may be similar. To better understand this case, extensive wood chemistry analyses are needed for the type and amount of wood chemical components degraded after *P. vitreus* fungal activity. Although many studies have been conducted on the effects of *P. vitreus* fungus on the permeability, physical, mechanical, and anatomical properties of wood (Lehringer *et al*. 2010; Lehringer 2011; Lehringer *et al*. 2011; Fuhr *et al*. 2012; Gilani *et al*. 2014; Emaminasab *et al*. 2016; Bakir *et al*. 2021; Tajrishi *et al*. 2021; Bakir *et al*. 2022a,b, 2022a,b), studies on wood chemical components seem to be limited.

Effects of Mechanical Incision Pretreatment on Wood Anatomy

The effects of mechanical incision pretreatment on the structures of spruce and larch wood are given in Figs. 11 and 12, respectively.

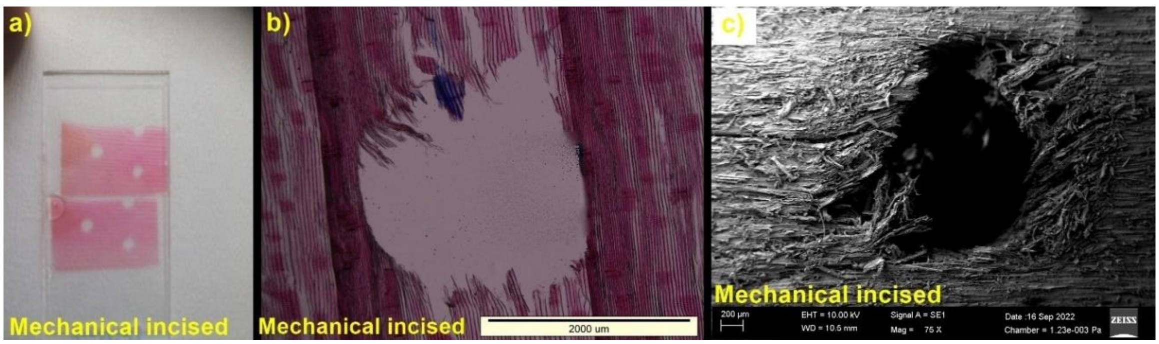


Fig. 11. (a) As a result of the mechanical incision pretreatment in spruce, cavities appear to be perfectly round macroscopically during preparation but (b to c) not fully rounded when viewed microscopically; radial sections

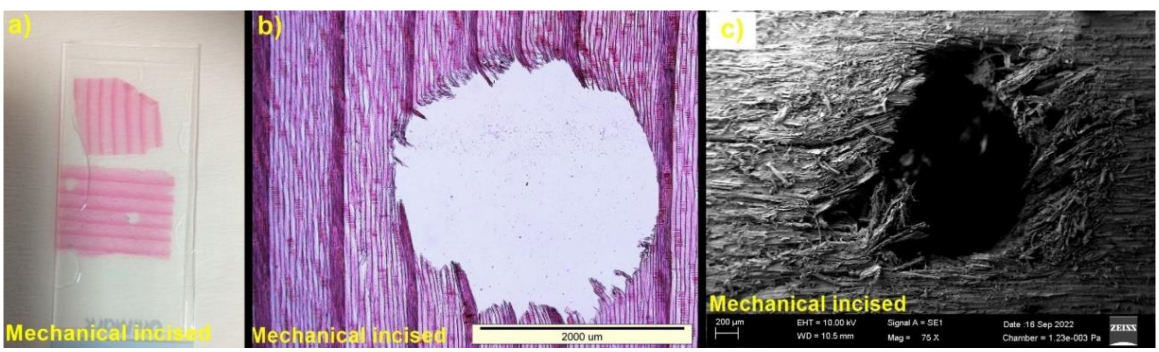


Fig. 12. (a) As a result of the mechanical incision pretreatment in the larch, cavities that appear to be perfectly round macroscopically during preparation but (b to c) not completely rounded when viewed microscopically; radial sections

Mechanical incision tends to create millimeter-scale holes in the wood – up to several millimeters deep – for impregnation penetration. While mechanical incisions are effective in improving protective treatment processes, they are limited by the fact that small holes or complex incision geometries are difficult or impossible to achieve (Nath *et al*. 2020a; Figs. 11 and 12). Mechanical incision pretreatment technologies used today, such as cutting and hollowing, significantly reduce the aesthetic and mechanical properties of wood products (Pánek and Reinprecht 2011; Dale *et al*. 2019). In observations of specimens with mechanical incision pretreatment, the circular nature of the incisions was reduced (Figs. 11 and 12). Such a situation was not detected in larch and spruce samples with applied laser incision (Figs. 13 and 14). This may also affect the depth, diameter, and quality of the drilled cavities (Nath *et al*. 2020a) and thus, albeit partially, may adversely affect copper uptake and microdistribution in wood subjected to mechanical incision. The results obtained in Table 1 somewhat support this conclusion.

Effects of Laser Incision Pretreatment on Microscopic and Wood Anatomy

The effects of laser incision pretreatment on the structures of spruce and larch wood are given in Figs. 13 and 14, respectively.

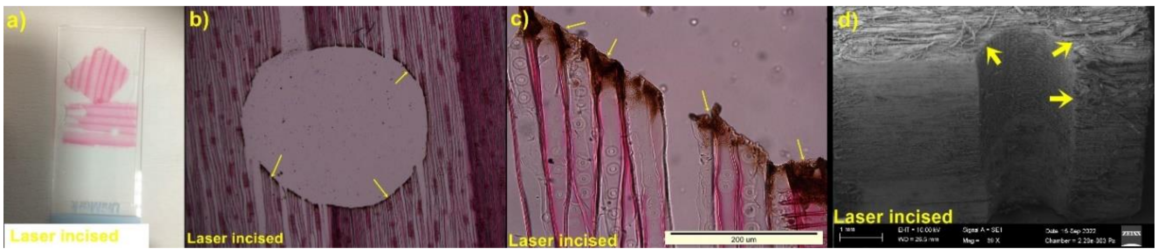


Fig. 13. (a) As a result of the laser incision pretreatment in the spruce, the holes macroscopically appeared to be perfectly round during preparation and (b to c to d) microscopically appeared to be perfectly round, and the areas burned by the effect of the laser beam (yellow arrows); radial sections

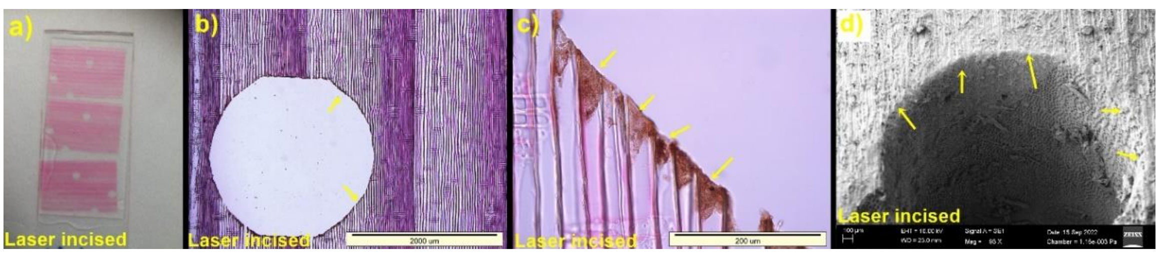


Fig. 14. (a) As a result of the laser incision pretreatment in the larch, it macroscopically appeared to be perfectly round during preparation and (b-c-d) microscopically appeared to be perfectly round, and the areas burned by the effect of the laser beam (yellow arrows); radial sections

Nath *et al*. (2020a) performed a CO_2 laser incision pretreatment on four different wood species (Southern yellow pine, radiata pine, European redwood, and beech), each with different densities. The researchers stated that laser-engraved hole shapes and depths were uniform, but circularity was significantly affected by the presence of spring wood and summer wood layers. The maximum hole diameter was determined in the radiata pine at ~1.3 mm, whereas the minimum diameter was determined in the beechwood species to be ~0.7 mm. In a similar vein, as a result of equal laser powers applied to the radial surfaces of the samples and the duration of the CO_2 laser pulse, the depth of the laser-engraved holes was determined to be a maximum in the European redwood wood species at ~33 mm, while it was a minimum in the beechwood species at ~25 mm. The researchers reported that wood anatomy and density had a significant effect on the efficiency of the CO_2 laser incision pretreatment performed at the same laser power. In other words, it is much more difficult to cut a piece or to perform incision pretreatments in denser wood (Fukuta *et al*. 2016; Nath *et al*. 2020b).

The average wood density in European larch trees (515 to 560 kg/m^3) (Karlman *et al*. 2005) is higher than that in Oriental spruce trees (401 to 425 kg/ m^3) (Bozkurt *et al.*

1993). In addition, the previous study has demonstrated that the average density (D_0: 0.62 g/cm^3; D_{12}: 0.64 g/cm^3) of larch sapwood is higher than the average density (D_0: 0.40 g/cm^3; D_{12}: 0.42 g/cm^3) of spruce sapwood (Bakir 2022b). Nath *et al*. (2020a) reported that the presence and proportion of earlywood and latewood layers in one annual ring had a significant effect on the incision properties during CO_2 laser incision. Also, laser incision pretreatments resulted in shallower holes on denser latewood layers (Nath *et al*. 2022). These results suggest that the diameter and depth of the holes carved in the laser-incised samples (larch and spruce) may be different in this present study, depending on the tree species and the presence and ratio of the early–latewood layers. Briefly, it can be argued that the hollowed hole diameter values and depths, which are thought to differ depending on the presence of the low-density earlywood layer and the high-density latewood layer contained in the annual rings, will depend on the number of cavities that will occur in these layers in the samples during the incision pretreatments (Nath *et al*. 2020b), and affect the copper uptake and microdistribution. Based on these considerations, the results obtained in Table 1 can be interpreted accurately and reliably. Therefore, comprehensive studies are needed to reveal the microstructural changes that may occur in the wood after mechanical and laser incisions, and to reveal the parametric effects of incision pretreatments on the geometric shape, size and quality of the incisions.

Copper Microdistribution

The comparison of the percentage copper microdistribution values determined in the anatomical structure of spruce and larch wood samples subjected to different incision pretreatments is given in Table 1. In spruce, no significant differences were found between the control samples and the samples with different incision pretreatments. In larch wood samples, although there were no significant differences between the control and the samples with mechanical and biological incision pretreatment, there was a significant difference between the samples with laser incision pretreatment and the samples of both the control, and the mechanical and biological incision methods (Table 1).

Table 1. Copper Microdistribution in the Wood Samples Before and After Different Incising Pretreatments

Wood Species	Number of Wood Samples	Pretreatment	Copper Microdistribution in the Samples (%)
Oriental spruce	10	None (Control)	0.433 (0.136)d
	10	Bioincised	0.907 (0.076)cd
	10	Mechanical incised	0.683 (0.096)cd
	10	Laser incised	0.727 (0.055)cd
European larch	10	None (Control)	2.600 (0.376)bc
	10	Bioincised	4.980 (1.862)ab
	10	Mechanical incised	4.050 (0.589)ab
	10	Laser incised	6.040 (1.253)a

Values in parentheses are standard deviations. The same letters in each column indicate that there is no statistical difference between the samples, according to Tukey's test ($p \leq 0.05$).

Although there does not seem to have been a significant difference between the groups in both spruce and larch wood samples as a result of the different incision pretreatments generally applied here, the copper microdistribution values increased partially after different incision pretreatments (Table 1). In particular, mechanical incision pretreatment was less effective relative to copper microdistribution values in spruce and larch wood samples compared to laser and biological incision pretreatments (Table 1). Although the same incision pattern (10,000 incisions/m^2) was used in the laser and mechanical incision pretreatments, it was thought that the reason why the copper microdistribution values detected in the spruce and larch wood samples were much lower in the mechanical incision pretreatments were because the drilled cavities did not have a perfectly circular shape (Figs. 11, 12, 13, and 14). Also, the hollowness of the holes may vary depending on the fact that the anatomical structure and densities of spruce and larch wood species are different from each other, and that each annual ring may have different amounts of early–latewood layers both within the same tree species and between various tree species. In such cases, the different microstructural features may cause the copper distribution values to differ from each other in the mechanical and laser incision pretreatments (Nath *et al*. 2020a).

Considering all the micrographs (Figs. 7, 8, 9, and 10), it can be argued that the *P. vitreus* fungus is equally active in both spruce and larches because copper microdistribution values increased approximately two-fold in both larch and spruce control wood samples after biological incision (Table 1). Applying laser incision pretreatment in larch wood samples was more advantageous than other incision pretreatments, so it was determined that the use of laser pre-incision is more suitable for larch wood samples than for spruce wood samples. However, the copper microdistribution values detected in larch wood before and after different pre-incision processes were much higher than in spruce (Table 1). Larch sapwood has a higher resin/extractive substance content than spruce sapwood (Lüxford 1953; Wu and Hu 1997; Wagner 2010). The copper uptake and microdistribution values were much higher in the larch before and after the pretreatments, suggesting that the high resin/extractive substance content in the wood does not have a negative effect on the pretreatments. The type and amount of resins or certain extractive substances in larch sapwood may increase *P. vitreus* fungal activity. To better understand this outcome, future chemical studies are needed to determine the type and amount of interactions of *P. vitreus* fungus with laser beams and resin/extractive material.

CONCLUSIONS

1. The micrographs and ultramicrographs obtained from Oriental spruce and European larch sapwood samples after biological incision pretreatment showed that *P. vitreus* fungal activity intensity was similar in spruce and larch sapwood samples.
2. In the spruce and larch wood samples with different density values, the cavities, as a result of the macroscopic observations made on the sections obtained from the samples with mechanical incision pretreatment, appeared to be completely circular, whereas the circularity of the incisions decreased as a result of further examination of the completely circular cavities, as could be seen under the microscope and scanning electron microscopy (SEM). However, this was not the case in larch and spruce wood samples subject to laser incision; the non-circularity appeared to adversely affect the copper uptake and microdistribution in the wood microstructure.

3. It is very important to determine the effects of wood anatomy and density on the effectiveness of various pre-incision processes; however, comprehensive studies are needed to reveal parametric effects of applied abiotic/physical incision pretreatments on the geometric shape, size, and quality of the incisions.
4. Depending on the different incision pretreatments applied, the types and amounts of chemical components and substances in the wood tissue may vary in their degradation in both the same and different tree species. Comprehensive anatomical and chemical studies are needed on various refractory wood species subjected to different incision pretreatments to explain and interpret all these results accurately and reliably.

ACKNOWLEDGMENTS

This study was financially supported by The Coordination Unit for Scientific Research Projects (BAP), Artvin Çoruh University (Project No: 2021.F11.02.01). The author would like to thank Professor Doctor A. Dilek Dogu, Professor Doctor S. Nami Kartal, and Assoc. Prof. Dr. Evren Terzi for their expert technical assistance. The author would also like to thank Mrs. Rita Rentmeester of USDA Forest Service Forest Products Laboratory, Madison, WI, USA, for preparing the *P. vitreus* fungal strains. A part of this study was presented at the 4th International Congress on Engineering Sciences and Multidisciplinary Approaches on 03-04-05 November 2022 in Istanbul, Turkey.

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Article submitted: November 11, 2022; Peer review completed: December 21, 2022; Revised version received and accepted: December 25, 2022; Published: January 3, 2023.
DOI: 10.15376/biores.18.1.1368-1383