

## Decay and leaching resistance of spruce and larch woods treated with preservatives following different incising pretreatments

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### Abstract:

The objective of this study was to determine the extent to which different incising pretreatments can increase decay and leaching resistance of *Picea orientalis* (oriental spruce) and *Larix decidua* (european larch) woods. To this purpose, mechanical, biological, and laser incision treatments were applied to sapwood samples of these refractory wood species to enhance permeability. After the incision treatments, cross sections of the samples were sealed with a polyurethane-based paint commonly used in marine applications to minimize excessive uptake of impregnation chemicals. Subsequently, the treated samples were impregnated using a vacuum method with an alkaline copper quat (ACQ) solution (Celcure C<sub>4</sub>), a copper (Cu)-based preservative.

The impregnated spruce and larch samples with and without different incision treatments were then subjected to leaching. Non-pretreated and non-impregnated samples, pretreated and non-impregnated samples, non-pretreated and impregnated samples (leached and non-leached), and pretreated and impregnated samples (leached and non-leached) spruce and larch were exposed to brown rot (*Tyromyces palustris*) and white rot (*Trametes versicolor*) fungi for 16 weeks.

The highest Cu content (ppm) in spruce before and after leaching was generally observed in samples treated with laser incision; in larch, the highest values were obtained with biological incision. Moreover, incising treatments did not increase Cu leaching in larch, while only biological incision increased leaching in spruce.

Decay resistance against both fungal species generally improved in blocks impregnated with Cu compounds following mechanical and laser incising treatments, both before and after leaching. In addition, leaching reduced decay resistance in the control and in laser incised samples exposed to brown rot fungus.

**Keywords:** Biological incising, decay resistance, laser incising, leaching, mechanical incising, preservative.

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## Introduction

Improved durability of less durable wood depends on the ability of wood preservatives to sufficiently penetrate the wood or increase its permeability, which is the wood's most important property; therefore, in terms of economy, environment, and forest existence, it is crucial that materials obtained from wood species with low natural durability and difficult impregnation (such as spruce and fir) can be used in areas with high-hazard class, where durability or mechanical resistance is important. Adequate penetration of preservatives into the wood would ensure its long service life. This can be achieved by choosing the most appropriate incision treatment (e.g., fungal incision, bacterial incision, laser incision, extraction treatment, microwave treatment, and mechanical incision) to increase permeability. This may vary depending on the wood species and wood parts, and by strictly following the protocols to be considered for these incision treatments, especially where the wood material would be used (Bakir 2022). Thus, the performances of some wood species that are not preferred in the sector can be improved and the usage areas of these species can be increased by carefully selecting the appropriate incision processes for difficult-to-impregnate wood species (Bakir 2022).

Several recent studies have examined improving permeability using different incision treatments (Chang *et al.* 2020, Terziev *et al.* 2020, Tajrishy *et al.* 2021, Bakir *et al.* 2022, Bakir 2022, Rezaei *et al.* 2022, Bakir *et al.* 2023, Qi *et al.* 2023, Winandy *et al.* 2023, Bakir 2023, Tajrishy *et al.* 2023, Liang *et al.* 2024); however, most of these studies have focused

only on either the effectiveness of the incision treatment in increasing permeability or, in part, on its effects on the anatomical and chemical properties of the wood and ignored complementary procedures, such as other mechanical tests, leaching tests, and biological resistance tests. The absence of one or more of these tests in previous studies involving different incision treatments raises doubts about their overall effectiveness and reliability, which in turn hampers their practical application in the forest industry. It is very important that leaching not increase in incised wood (Bakir *et al.* 2022) This is generally desirable, of course, but if a large increase in preservative uptake is achieved, a small increase in leaching may be considered acceptable. Furthermore, incision treatments should not reduce the uptake of preservatives and adversely affect physical properties and mechanical strength (Schwarze *et al.* 2006, Thaler *et al.* 2012, Fuhr *et al.* 2013). In addition, treatments should increase the service life of the wood material against biotic and abiotic factors that damage to the wood structure; in particular, decay resistance resulting from the increased uptake of preservatives as a result of improved permeability (Schubert *et al.* 2011).

The present study determined net copper (Cu) contents (ppm) in wood powders obtained from samples oriental spruce (*Picea orientalis* (L.) Peterm) and european larch (*Larix decidua* Mill.) subjected to different incision treatments before and after the leaching tests, to determine which incision treatment was more effective. Spruce and larch samples with different incision treatments, were leached and subjected to biological resistance tests. For this purpose, the samples were exposed to brown rot and white rot fungi to determine the possible effects of different incision treatments and leaching on the decay resistance of different wood species. Because there are no studies in the literature that have compared the decay resistance of impregnated wood of various wood species subjected to different incision treatments.

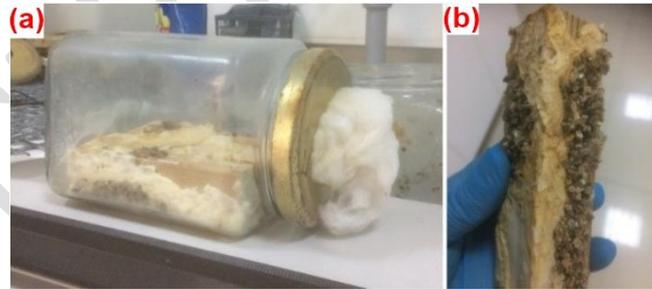
## **Materials and methods**

### **Test materials**

Samples of oriental spruce (*Picea orientalis* (L.) Peterm), an indigenous tree species from Artvin province Türkiye and european larch (*Larix decidua* Mill.), an exotic tree species from Karabula in the Krasnoyarskiy Kray region of Russia were selected for the study. The wood was cut into defect-free 120 mm × 30 mm × 30 mm (length × radial × tangential) samples, that were grouped into 48 samples each for mechanical, biological, and laser incision treatments. There were 192 samples used in the present study, comprising the control samples and those to which incision treatments were applied (Table 1). The samples were selected to coincide with their annual rings along the stems to avoid any effects on the results from the natural variability within the wood itself. All samples used in the study were cut in radial and tangential directions.

### **Biological incision treatment**

The fungus *Physisporinus vitreus* (Pers.) P. Karst. (FP 103669-T), which has the potential to cause both white rot type I and II, was used. Glass jars 170 mm × 100 mm × 100 mm (length × width × height) were used and their metal lids were pierced in a circle using a hole punch. The forty-eight samples were then placed into the glass jars containing 4 % malt-agar (MEA) nutrient medium that had previously been inoculated with the fungus and incubated at 20 °C and 65 % relative humidity (RH) for 2 weeks. The holes in the jar lids were plugged with tinsmith cotton to allow air exchange and prevent contamination. Air-dried spruce and larch samples were exposed *Physisporinus vitreus* (Pers.) P. Karst. for 8 weeks at 26 °C and 75 % RH to achieve a mass loss of approximately 10 %, as described in the European Standard EN 113-1 (2020). A controlled decay procedure that is achieved at a desired level depends on all conditions (Figure 1).



**Figure 1:** Example of (a) decay chamber and (b) uniformly colonized sample.

Mass losses (%) as a result of biological incision were calculated using Equation 1.

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

$WL$ : Mass loss of the sample (%),

$W_0$ : Kiln dry mass of the sample before incision (g),

$W_1$ : Kiln dry mass of the sample after incision (g).

### Mechanical incision treatment

Forty-eight samples were conditioned at 20 °C and 65 % RH for 2 weeks before being subjected to mechanical incision by a drilling 2 mm diameter by 10 mm deep holes at a density of 10000 incisions/m<sup>2</sup> (Figure 2) on both the radial and tangential surfaces. Figure 2 provides the sample sizes and the distances between the drilled holes.

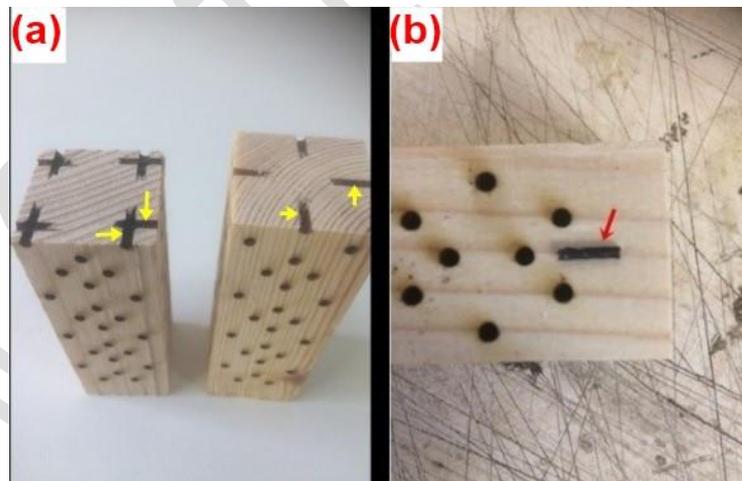


**Figure 2:** Distances between holes opened in the lateral and longitudinal directions on the radial and tangential surfaces of samples of both species.

Mass losses as a result of mechanical incision were calculated using Equation 1.

## Laser incision treatment

Laser incision treatment was conducted using a CO<sub>2</sub> laser (VLS6.60, Universal Laser Systems, Scottsdale, AZ, USA) on the radial and tangential surfaces of samples of both species with a power of 60 W irradiation time, speed setting of 4,0 and height of 30 mm. The drilling density was designed to produce 10000 incisions/m<sup>2</sup> (Figure 2). All burn holes were drilled to 10 mm deep and 2 mm in diameter. The charred portions (cores) (Figure 3b, red arrow) of the laser-burned holes (Figure 3a, yellow arrows) were removed using fine-tipped tweezers and collected for further studies (Figure 3) (Kamke and Peralta 1990, Goodel *et al.* 1991, Islam *et al.* 2008).



**Figure 3:** The laser incised samples. (a) Burning of wood samples using a CO<sub>2</sub> laser and appearance of the burned cavities (yellow arrows), (b) Core-central core portions (red arrow) of the laser burned wood extracted in a burned or charred state.

Mass losses (%) as a result of laser incision were calculated using Equation 1

## Impregnation processes

Some spruce and larch samples were not exposed to incision treatment so that they could be used as controls. To determine and compare the permeability of the samples, the incised and control samples were treated with preservative containing 3 % alkaline Cu, benzalkonium chloride, and cyproconazole to a direct contact with the ground or above ground use (EN 335 (2013) in hazard classes 3 and 4). Vacuum methods (40 min, 40 mbar) were applied for impregnation according to the EN 113-1 (2020) standards. Except for the non-impregnated samples, the cross sections of impregnated samples were covered with polyurethane-based paint to compare the preservative solution uptake in radial and transverse directions and to prevent excessive preservative uptake in directions parallel to the fibers. The samples treated with preservatives were then kept at 20 °C for 2 weeks to allow on fixation reaction to occur. Table 1 shows the test samples and the procedures followed in the present study.

**Table 1:** Test samples and procedures followed in the study.

			Fungi species			
			T. versicolor		T. palustris	
Species	Pretreatment	Treatment	Leaching	Non-leaching	Leaching	Non-leaching
Spruce	Bioincised	Impregnated	4	4	4	4
Spruce	Mechanically incised	Impregnated	4	4	4	4
Spruce	Laser incised	Impregnated	4	4	4	4
Spruce	None	Impregnated	4	4	4	4
Larch	Bioincised	Impregnated	4	4	4	4
Larch	Mechanically incised	Impregnated	4	4	4	4
Larch	Laser incised	Impregnated	4	4	4	4
Larch	None	Impregnated	4	4	4	4
Spruce	Bioincised	Non-impregnated	4		4	
Spruce	Mechanically incised	Non-impregnated	4		4	
Spruce	Laser incised	Non-impregnated	4		4	
Spruce	None	None	4		4	
Larch	Bioincised	Non-impregnated	4		4	
Larch	Mechanically incised	Non-impregnated	4		4	
Larch	Laser incised	Non-impregnated	4		4	
Larch	None	None	4		4	

**Determination of Cu content (ppm) in treated and non-pretreated samples before and after leaching tests**

The impregnated samples were air-dried for 2 weeks before the leaching tests. The leaching tests were conducted according to the American Wood Protection Association - AWWA E11-16 (2022). Four samples from each treatment group were placed in separate plastic containers and soaked in 1800-mL deionized water which was changed at 6-h intervals for 1, 2, 4, 6, 8, 10, 12, and 14 d. The content of Cu (ppm) retained in the wood was examined

using inductively coupled plasma atomic emission spectroscopy (ICP - OES PerkinElmer optima 8000, Waltham, MA, USA) before and after the 14-d leaching test. The zones 0-5 and 5-10 mm from the surface of each wood sample were cut a to obtain strips that represented the inner and outer parts of the impregnated wood sticks. The strips were ground to wood powder (0,5 mm) using a Willey mill. The digested material was analyzed for Cu by inductively coupled plasma optical emission spectroscopy analysis. The samples were digested according to the AWWA A7-19 (2022). A total of 32 Cu contents were determined for different incising potentials on spruce sapwood and 32 Cu contents on larch sapwood before and after the leaching tests.

### **Decay resistance**

The samples were kept at 20 °C and 65 % relative humidity for 2 weeks. The same glass jars, which have a larger volume than the Kolle culture bottles used in the biological incision processes above within the scope of the EN 113-1 (2020) standards, were again preferred for the decay resistance tests after appropriately cleaning and sterilizing them. The sterilised samples were placed directly into glass jars containing 4 % malt extract agar we nutrient media that were previously inoculated with brown rot (*Tyromyces palustris* TYP-6137) and white rot (*Trametes versicolor* COV 1010) fungi. The holes in the jar lids were plugged with tinsmith cotton and sealed with parafilm to maintain oxygen and humidity requirements of the fungi. All samples were incubated for 16 weeks at 27 °C and 70 % relative humidity. Pretreated and non-impregnated, and non-pretreated and non-impregnated control spruce

and larch samples were directly exposed to wood decay fungi without leaching tests (Table 1). After 12 weeks of incubation, the fungal mycelia were removed from the samples with a brush and the samples were weighed again after they were oven dried. Percent mass loss of the samples after the decay tests was then calculated using Equation 1.

### **Statistical analyses**

The data were evaluated using the JMP 5,0 (2020) (SAS Institute, Cary, NC, USA) software program. Multivariate analysis was used to investigate the factors affecting the copper content and decay resistance. In addition, Tukey's test was used to determine the differences between the means, at 0,05 level of significance. According to the results, pretreatment ( $p \leq 0,05$ ), leaching ( $p \leq 0,05$ ), and the interaction between these variables were found to be significant. The R-square value indicating model accuracy was found to be 0,96. This value being close to 1 is important for the accuracy of the model.

### **Results and discussion**

#### **Copper content in the pretreated and non-pretreated wood samples before and after the leaching test**

Table 2 compares the differences in the Cu content in the spruce and larch samples before and after different incision treatments, and the samples subjected to leaching tests were compared with non-leached samples in Table 2 and Table 3 to determine the effects of leaching on different incision treatments and decay resistance.

**Table 2:** Copper content in spruce and larch samples with and without incised before and after the leaching test.

Wood species	Pretreatment	Leaching test	Copper content in the wood (ppm)
Oriental spruce	None (Control)	Non-leaching	2530,0 (583,3)d
		Leaching	2447,0 (351,8)de
	Bioincised	Non-leaching	4350,0 (310,9)c
		Leaching	1983,0 (177,7)def
	Mechanical incised	Non-leaching	4889,0 (552,8)bc
		Leaching	4778,0 (695,2)bc
	Laser incised	Non-leaching	6316,0 (615,2)a
		Leaching	5841,0 (698,8)ab
European larch	None (Control)	Non-leaching	560,0 (139,5)gh
		Leaching	218,0 (67,0)h
	Bioincised	Non-leaching	2655,0 (466,2)d
		Leaching	1983,0 (542,3)def
	Mechanical incised	Non-leaching	1423,0 (192,6)efg
		Leaching	474,0 (105,1)gh
	Laser incised	Non-leaching	952,0 (286,8)fgh
		Leaching	983,0 (69,2)fgh

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 ( $p \leq 0,05$ ).

Laser and mechanical incision of spruce increased the Cu content (i.e., permeability) in the wood in both the leached and non-leached samples, while biological incision increased Cu content only in non-leached samples (Table 2). However, the Cu content in larch increased only in the biologically incised samples ( $p \leq 0,05$ ) (Table 2). Only biological incision

significantly increased permeability in the larch; however, nearly all incision treatments increased permeability in the spruce, with laser incision increasing permeability the most (Table 2).

The results indicate that *Physisporinus vitreus* fungal activity was higher in the larch. These results suggest that the type and amount of some extractive substances contained in the larches wood may have increased the activity of *Physisporinus vitreus*. On the other hand, it's also possible that the absence of some extractives that inhibit fungal activity or growth in spruce could lead to this result. In short, types and amounts of extractive substances in wood play a role in fungal activities. For example, Brischke and Alfredsen (2023) with reference to Celimene *et al.* (1999) reported that the fungistatic activity of different stilbenes (i.e., pinosylvin, pinosylvin monomethyl ether, and pinosylvin dimethyl) extracted from jack pine (*Pinus banksiana* Lamb.) and red pine (*Pinus resinosa* Aiton) cones was investigated. They reported that these three stilbenes inhibited the growth of white rot fungi (*Trametes versicolor* and *Phanerochaete chrysosporium*) but stimulated and slightly increased the activity of brown rot fungi (i.e., *Neolentinus lepideus*, *Gloeophyllum trabeum*, and *Postia placenta*) on agar media.

In short, larch sapwood has a higher resin/extractive material content than spruce sapwood (Lüxford 1953, Wu and Hu 1997, Wagner 2010). High resin or extractive content, may have increased *Physisporinus vitreus* activity as evidenced by extensive mycelial growth of hyphae (Figure 1); whereas mechanical and laser incising only affected parts of the wood tissue where incising was conducted (Figure 2 and Figure 3). Laser and mechanical incising of difficult-to-impregnate wood tissues resulted in local and more superficial/shallow holes; however, with biological incision processes, fungal hyphae penetrated much deeper.

The reason that laser incision in spruce increased permeability more than in other incision treatments applied in spruce and in laser incision in larch could be that the average wood

density value of european larch (515-560 kg/m<sup>3</sup>) (Karlman *et al.* 2005) is higher than that of oriental spruce (401-425 kg/m<sup>3</sup>) (Bozkurt *et al.* 1993). Similarly, Bakir (2022) found that the average wood density value of european larch (620-640 kg/m<sup>3</sup>) was higher than that of oriental spruce (400-420 kg/m<sup>3</sup>). Wood anatomy and density impact the depth, diameter, and circularity of the incised hole, which in turn, affects intracellular flow paths and may play a role in increasing incision efficiency and subsequent preservative uptakes (Nath *et al.* 2022). Considering the wood species used in the present study, when both non-leached and leached samples of spruce and larch wood with different incision treatments were compared, there was a difference in Cu content among the samples in the control group and those to which different incision treatments were applied (except for leached and biologically incised spruce and larch samples) ( $p \leq 0,05$ ). In other words, the Cu content in spruce was much higher than that in larch in all sample groups. Nath *et al.* (2020a) reported that the anatomical structure and density of wood had a high impact on the incision treatments performed at equal CO<sub>2</sub> laser power.

It is obvious that this will have a similar effect on mechanical incision treatments. In short, denser wood species are generally more difficult to incise or cut (Fukuta *et al.* 2016, Nath *et al.* 2020b); therefore, as a result of laser and mechanical incision, it was possible that the Cu content in wood species with lower density, such as spruce, would be higher than that in larch (Table 2). According to Nath *et al.* (2020b), the loss of building material from the surface of the wood as a result of surface melting or erosion (ablation) was higher in the low-density earlywood region of pine incised using a CO<sub>2</sub>-transverse excitation atmospheric laser than in the latewood region. This suggests that the proportion of the low-density earlywood region may be higher in the spruce wood used in the study; however, the proportion of high-density latewood region may be higher in the larch wood.

When considering the Cu content before and after leaching, no incision treatment applied to larch wood statistically increased leaching; however, in spruce wood, only biological incision increased leaching (Table 2). The present study revealed that leaching, which was expected to be in parallel with the increased amount of preservative uptake because of improved permeability from different incision treatments, did not generally follow the same rate and parallelism as stated in previous studies (Schubert *et al.* 2011, Wang and Kamdem 2012).

On the other hand, it is known that lignin functional groups are the targeted adsorption sites for transition metal ions in wood after impregnation, regardless of the carrier solvent (Petrič *et al.* 2000, Stirling *et al.* 2008); therefore, *Physisporinus vitreus*, which can cause selective white rot (type I) as well as simultaneous white rot (type II), that is selective delignification (Lehringer *et al.* 2010, Schubert and Schwarze 2011, Fuhr *et al.* 2013, Schubert *et al.* 2013, Gilani and Schwarze 2014) degraded lignin-containing wood cell elements much more in spruce than in larch, which may have increased leaching in spruce samples.

### **Decay resistance before and after leaching test**

Table 3 compares the results obtained for the mass losses of impregnated and non-impregnated samples subjected to different incision treatments before and after the leaching tests as a result of exposure to wood decaying fungi.

**Table 3:** Comparison of the results obtained for the % mass loss of impregnated and non-impregnated wood samples subjected to different incision treatments and exposed to wood decay fungi before and after leaching tests.

Wood species	Leaching tests	Fungi species	Mass loss (%)								
			Impregnated samples				Nonimpregnated samples				
			**Control-2	Bioincised	Mechanical incised	Laser incised	*Control-1	Bioincised	Mechanical incised	Laser incised	
Oriental spruce	Non-leaching	T. palustris	16,9 (2,7)c	15,0 (4,0)cd	10,2 (1,6)d	11,4 (2,3)d	39,3 (7,8)a	44,9 (5,8)a	42,3 (8,2)a	27,0 (3,5)b	
		T. versicolor	16,8 (5,3)c	13,8 (4,1)cd	14,0 (5,8)cd	11,4 (2,3)d	29,3 (0,5)b	37,7 (8,4)a	40,0 (7,4)a	26,9 (8,8)b	
	Leaching	T. palustris	18,7 (2,1)c	20,6 (3,2)c	11,5 (6,8)d	17,4 (7,2)cd	39,3 (7,8)a	44,9 (5,8)a	42,3 (8,2)a	27,0 (3,5)b	
		T. versicolor	18,2 (1,2)c	14,6 (3,3)cd	15,0 (5,3)cd	12,8 (0,8)d	29,3 (0,5)b	37,7 (8,4)a	40,0 (7,4)a	26,9 (8,9)b	
	European larch	Non-leaching	T. palustris	14,8 (2,8)c	16,2 (5,7)bc	10,0 (3,1)cd	9,0 (4,2)d	30,3 (9,0)a	29,1 (11,0)a	29,0 (8,0)a	19,2 (4,5)b
			T. versicolor	20,7 (5,5)c	12,5 (3,4)d	10,4 (3,7)d	10,0 (2,0)d	30,0 (9,6)b	35,8 (8,4)a	25,8 (4,1)bc	19,0 (4,9)c
Leaching		T. palustris	18,3 (4,3)b	12,5 (4,7)c	9,7 (2,7)c	11,3 (2,8)c	30,3 (9,0)a	29,1 (11,0)a	29,0 (8,0)a	19,2 (4,5)b	
		T. versicolor	14,4 (5,1)c	11,9 (4,0)cd	9,5 (3,6)d	9,1 (2,6)d	30,0 (9,6)b	35,8 (8,4)a	25,8 (4,1)bc	19,0 (4,9)c	

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 ( $p \leq 0,05$ ).

\*Control-1: Non-impregnated and non-pretreated

\*\*Control-2: Impregnated and non-pretreated

### Mass loss of non-pretreated and non-impregnated, and pretreated and non-impregnated samples

None of the different incision treatments applied to the non-impregnated spruce and larch samples increased mass loss from exposure to *Tyromyces palustris*; therefore, the decay resistance of the samples was not reduced, which was also desirable. In addition, laser incision actually decreased mass loss after exposure to the brown rot fungus ( $p \leq 0,05$ ) (Table 3).

Biological incision increased mass loss of non-pretreated and non-impregnated, and pretreated and non-impregnated spruce and larch samples when exposed to *Trametes versicolor* ( $p \leq 0,05$ ), an undesirable outcome. In addition, although mechanical incision of spruce wood also increased mass loss from *Trametes versicolor* activity ( $p \leq 0,05$ ), laser incision did not cause any change ( $p \geq 0,05$ ). In the larch, laser incision decreased *Trametes versicolor* activity compared with that in the control samples ( $p \leq 0,05$ ). It was observed that laser incision decreased the activities of both *Tyromyces palustris* and *Trametes versicolor* fungi in non-impregnated larch samples but only that of *Tyromyces palustris* fungi in spruce ( $p \leq 0,05$ ) (Table 3).

Laser incising of wood tissue can occur at high temperatures, which results in some parts of the tissue being burned and charred (Bakir 2023). Many studies have reported that heat treatment can increase the resistance of wood to decay from fungal attack (Metsä-Kortelainen and Viitanen 2009, Metsä-Kortelainen and Viitanen 2010, Bazyar 2012, Lekounougou and Kocaefe 2012, Lekounougou and Kocaefe 2014a, Lekounougou and Kocaefe 2014b, Metsä-Kortelainen and Viitanen 2017, Cantera *et al.* 2022). Based on the literature, there are four reasons that the resistance of wood against fungal attack can be increased by heat treatment as follows: 1) increased hydrophobic character, 2) production of fungicidal extractives, 3) modified wood polymers, and 4) degraded hemicelluloses (Metsä-Kortelainen and Viitanen 2009, Lekounougou and Kocaefe 2012).

Biological incision increased mass loss in both the spruce and larch samples exposed to *Trametes versicolor* ( $p \leq 0,05$ ); however, it is noteworthy that in the present study, the sensitivity of non-impregnated spruce and larch wood to *Tyromyces palustris* did not increase after pretreatment with *Physisporinus vitreus*. The cellulose-degrading enzymes of a white rot fungi, such as *Trametes versicolor*, can more easily penetrate the cellulose and hemicellulose layers of biologically incised wood as a result of a slight delignification of the

cell wall, especially in the S<sub>2</sub> layer, during biological incision and can simultaneously degrade all wood polymers (lignin, hemicellulose, and cellulose).

The mass loss caused by *Trametes versicolor* in biologically incised wood was higher than that in the control samples ( $p \leq 0,05$ ), which was most likely related to the increased presence of readily available carbohydrates (e.g., hemicellulose and cellulose) resulting from the selective delignification of the *Physisporinus vitreus* used for biological incision. In addition, the higher moisture content in the wood required for *Physisporinus vitreus* fungal activity and the sensitization of wood resulting from cell wall modifications characteristic of *Physisporinus vitreus* in particular may have increased the activity of the fungi (Schubert *et al.* 2011).

Besides the reason that mechanical incision treatment increased mass loss in the spruce samples exposed to *Trametes versicolor* ( $p \leq 0,05$ ) but not in larch wood may be related to the lower density of spruce wood, which provided for deeper and wider holes created by mechanical incision (Bakir 2023) and allowed *Trametes versicolor* hyphae to easily reach much deeper, resulting in more severe degradation.

### **The mass loss of non-pretreated and pretreated samples impregnated with Celcure C<sub>4</sub> before and after leaching**

As shown in Table 3, when non-pretreated and pretreated spruce and larch samples impregnated with Celcure C<sub>4</sub> were exposed to *Tyromyces palustris* before and after leaching, mass loss was reduced in spruce after mechanical incision and in larch after laser incision ( $p \leq 0,05$ ). Moreover, it was determined that laser incision before leaching also reduced mass

loss in the spruce, and biological and mechanical incisions after leaching reduced mass loss in the larch ( $p \leq 0,05$ ).

On the other hand, mass losses of non-pretreated and pretreated spruce and larch samples impregnated with Celcure C<sub>4</sub> and exposed to *Trametes versicolor* before and after leaching were reduced by laser incision in both wood species ( $p \leq 0,05$ ). Moreover, in the spruce, other incision treatments before and after leaching did not change mass losses from white rot fungus ( $p \geq 0,05$ ). Similarly, in the larch, mechanical incision before and after leaching reduced mass losses. It was also observed that mass losses in the larch decreased as a result of biological incision after leaching ( $p \geq 0,05$ ) (Table 3).

According to the data in Table 2, the Cu content of spruce before and after leaching was higher after mechanical and laser incision than in the control and biological incising ( $p \leq 0,05$ ); therefore, it was expected that mass loss in the spruce samples exposed to *Tyromyces palustris* and *Trametes versicolor* before and after leaching would decrease based on the amount of impregnation material that increased much more from mechanical and laser incision. Similarly, the data in Table 2 revealed that among the different incision treatments applied before and after leaching in the larch, Cu content after biological incision treatment was much higher ( $p \leq 0,05$ ) than that in the control; therefore, it was an expected result that mass loss in the larch samples exposed to *Tyromyces palustris* after leaching and *Trametes versicolor* before leaching decreased ( $p \leq 0,05$ ) based on the amount of impregnation material that increased much more as a result of biological incision.

However, the decrease in mass loss in the larch samples exposed to *Tyromyces palustris* and *Trametes versicolor* before and after leaching as a result of mechanical incision treatments may be because Cu content after mechanical and laser incision treatments was slightly higher ( $p \geq 0,05$ ) than that in the larch control samples and, moreover, leaching did not increase after any incision treatment (Table 2).

## **Comparison of non-pretreated and pretreated samples, both non-impregnated and impregnated with Celcure C<sub>4</sub>**

It was determined that mass loss decreased in non-leached and leached samples when the non-pretreated and non-impregnated spruce and larch samples were exposed to *Tyromyces palustris* after impregnation. This decrease after exposure to brown rot fungus was also observed after biological, mechanical, and laser incision treatments ( $p \leq 0,05$ ) (Table 3).

Similarly, mass loss after exposure to *Trametes versicolor* after impregnation of non-pretreated and non-impregnated spruce and larch samples decreased in the non-leached and leached samples. This decrease in mass loss after exposure to white rot fungus was also observed after biological, mechanical, and laser incision treatments ( $p \leq 0,05$ ) (Table 3).

These results indicated that mass loss of non-pretreated and impregnated spruce and larch samples and impregnated spruce and larch samples after different incision treatments decreased significantly after decay tests compared to that in non-pretreated and non-impregnated spruce and larch samples and non-impregnated samples after different incision treatments.

In addition, tests indicated that the amount of preservative leached generally did not significantly increase. The results obtained for the determination of Cu content in the samples indicated that different incision treatments applied to the spruce (except for the biological incision treatment) and larch samples did not increase leaching (Table 2).

## Comparison of the leached and non-leached samples

Based on Table 3, the mass loss of control and laser-incised impregnated larch wood exposed to *Tyromyces palustris* increased after leaching ( $p \leq 0,05$ ); however, leaching had no effect on mass loss after decay ( $p \geq 0,05$ ) in the biologically and mechanically incised samples. In the spruce, leaching had no effect on mass loss after decay in both the control samples and samples to which different incision treatments were applied ( $p \geq 0,05$ ) (Table 3).

On the other hand, the mass losses in the spruce and larch samples exposed to *Trametes versicolor* and the control, spruce, and larch samples subjected to different incision treatments did not change after leaching ( $p \geq 0,05$ ) (Table 3). The mass losses in control and laser incised larch samples exposed to *Tyromyces palustris* changed after leaching, which indicates that, as a result of the different incision treatments, leaching is expected to be directly proportional to the increasing amount of preservative uptake because of the partially improved permeability at the same rate and parallelism in the larch wood, as stated in previous studies (Schubert *et al.* 2011, Wang and Kamdem 2012).

These and similar results obtained within the scope of the present study showed that whether the increased preservative uptake from improved permeability after incision and the amount of leached preservative agent are parallel to each other may vary depending on the wood species and the type of incision treatments applied.

## Conclusions

Laser incision was the most effective incision process by which to increase Cu content in the spruce samples followed by mechanical incision. Biological incision was the most effective incision process by which to increase Cu content in the larch samples.

In the spruce and larch samples before impregnation, laser incision generally reduced the mass loss from exposure to both brown and white rot fungi; however, biological incision, specifically, decreased decay resistance against white rot fungus. In addition, mechanical incision decreased the decay resistance against white rot fungus in spruce samples.

In the spruce and larch samples after impregnation, laser incision generally reduced mass loss caused by both brown rot and white rot fungi more than that in non-pretreated and impregnated samples. Mechanical incision reduced mass loss caused by brown rot fungus in both leached and non-leached spruce samples. In the larch samples, mechanical incision generally reduced mass loss caused by both brown rot and white rot fungi before and after leaching.

Based on Cu contents detected in the wood dust of pretreated and impregnated spruce samples, biological incision was the only treatment that increased leaching. In the larch samples, none of the different incision treatments applied affected leaching. Based on these results, leaching did not change the decay resistance in the samples subjected to different incision treatments in the spruce exposed to brown and white rot fungi. In addition, leaching

decreased decay resistance both in the control and laser incised larch samples exposed to brown rot fungus.

The mass losses in the non-pretreated and impregnated samples exposed to wood decay fungi before and after leaching were significantly reduced as a result of incision treatments applied before impregnation. This was more evident in the larch samples than in the spruce samples. In other words, incision treatments applied before impregnation increased decay resistance in larch samples much more than in spruce samples. Complementary studies should be conducted to determine the effects of incision treatment or treatments applied in incision studies to increase the permeability of the wood on leaching, physical and mechanical properties, and decay resistance to interpret the results correctly obtained.

#### **Authorship contributions**

D.B.: Analysis, literature review, investigation, writing - review & editing, validation, supervision.

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#### **Declaration of interest**

The authors declare no conflict of interest.

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