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THE EFFECTS OF BIOINCISING BY *Physisporinus vitreus* ON CuO RETENTION AND COPPER ELEMENT LEACHING IN ORIENTAL SPRUCE WOOD

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ABSTRACT

Since the treatability of Oriental spruce wood (*Picea orientalis*) with preservative solutions is difficult and considered as a refractory wood species, this study was intended to bring its treatability class by a bioincising process to the level of sapwood of Scots pine (*Pinus sylvestris*), a desirable wood species for the forest products industry. Bioincising process by *Physisporinus vitreus* fungus was applied to wood samples from sapwood and heartwood portions of spruce wood. The samples with two different weight loss groups (5-10 % and 10-15 %) in the bioincising process were used to detect changes in treatability with wood preservative solutions caused by the fungus. The bioincised and unincised control samples were treated with either micronized copper quat (MCQ) or alkaline copper quat type D (ACQ-D) wood preservative solutions by either dipping or vacuum methods. Following impregnation with the preservative solutions, the effects of the bioincising process on CuO (copper oxide) retention, and the leaching of Cu (copper) element were determined. The results showed that CuO retention levels increased after the bioincising process. Moreover, there was greater CuO retention in the spruce heartwood samples compared to the spruce and Scots pine sapwood samples. Amount of Cu element released from the Scots pine sapwood samples was found to be lower than that from the spruce sapwood and heartwood samples after the bioincising. process. The results suggest that the bioincising process by *P. vitreus* in refractory wood species might improve the treatability of wood by Cu-based wood preservatives.

Keywords: Bioincising, copper-based preservative, *Picea orientalis,* refractory wood species, treatability.

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INTRODUCTION

The demand for wood materials has increased in recent years; however, the rapid destruction of forests has made it imperative that the service life of wood products increases by using specific processes to treat the wood with wood preservatives. In order to treat the wood effectively, the penetrating fluid must be taken into the wood to a specific depth; however, the degree of success of preservatives depends on the type of preservative, depth of penetration (Wang and DeGroot 1996, Watanabe *et al.* 1998, Tripathi and Poonia 2015, Panigrahi *et al.* 2018, Dale *et al.* 2019, Messaoudi *et al.* 2020). Generally, the sapwood of tree species can be successfully impregnated (with some notable exceptions, such as the spruce species). In contrast, the heartwood is more challenging or even impossible to treat by conventional methods (Wang and DeGroot 1996).

Apart from sapwood and heartwood differentiation, Kartal and Lebow (2002) have stated that while some wood species are easily treated with wood preservatives, others are classified as refractory. For example, sapwood and heartwood of Oriental spruce wood (*Picea orientalis*) are classified as "Class 3 - difficult to treat" and "Class 4 - extremely difficult to treat" according to the BS EN 350-2 standard (2016). Even though refractory wood species are readily available, the poor treatability of such species may limit their use in exterior applications due to low retention and inadequate penetration of wood preservatives (Kumar and Morrell 1989).

One solution to improve the treatability of both refractory species and heartwood portions of wood is the use of mechanical incising. During mechanical incising processes, the wood is incised by means of toothed rollers resulting in small slits parallel to the grain. Studies regarding the mechanical incising and treatability of refractory wood species have generally focused on penetration and retention levels of the treated wood (Perrin 1978, Ruddick 1991, Winandy *et al.* 1995, Morris 1995). However, larger wood surface area after incising and greater preservative loadings can cause an elevated release of preservative components from the treated wood in service.

Mechanical incising and biotechnological procedures are also available for bioincising of refractory wood species. Enzymes (Durmaz *et al.* 2015), bacteria (Kobayashi *et al.* 1998, Hansmann *et al.* 2002, Yıldız *et al.* 2012), and blue-stain fungi (Lehringer *et al.* 2010, Danihelová *et al.* 2018) have been applied to improve the permeability of wood. For years, wood-decaying fungi in biotechnological applications have been studied for their effects on increasing wood permeability in the forest products industry. Although some of these studies have discussed hyphal growth rate, fungal hyphae penetration velocity-capacity, and effects of *Physisporinus vitreus* in wood using different technological systems or models (Schubert *et al.* 2009, Lehringer *et al.* 2010, Schubert and Schwarze 2011, Lehringer *et al.* 2011, Schwarze and Schubert 2011, Fuhr *et al.* 2012a, Fuhr *et al.* 2012b, Fuhr *et al.* 2013, Schubert *et al.* 2013, Gilani *et al.* 2014, Schubert *et al.* 2014, Gilani and Schwarze 2014), some studies (Schwarze *et al.* 2006, Lehringer *et al.* 2009, Lehringer *et al.* 2010, Volkmer *et al.* 2010, Schubert *et al.* 2011, Humar *et al.* 2012, Emaminasab *et al.* 2016, Chang *et al.* 2020) also focused on increasing the permeability in wood portions (sapwood/heartwood) of refractory tree species.

Lehringer *et al.* (2010) have revealed that improvement in the permeability and activity of *P. vitreus* was higher in the sapwood of Norway spruce (*Picea abies*); however, a notable effect was also recorded in the heartwood. In contrast, Schwarze *et al.* (2006) have reported that *P. vitreus* is capable of colonizing sapwood and heartwood and can improve their permeabilities. Additional studies on bioincised wood by Schwarze *et al.* (2006) and Schubert *et al.* (2011) have indicated improved permeability from *P. vitreus* in the heartwood of *P. abies* and *Abies alba*. Emaminasab *et al.* (2016) have reported improved permeability from *P. vitreus* and *Xylaria longipes* (a soft-rot fungus) in Douglas-fir sapwood (*Pseudotsuga menziesii*) containing compression wood. In addition, to the best of our knowledge, it appears that studies that have evaluated the effect of bioincising on the new generation Cu (copper) preservatives leaching in the wood are limited. Volkmer *et al.* (2010) have reported that pretreating Norway spruce and Scots pine (*Pinus sylvestris*) woods with *P. vitreus* for 4 weeks resulted in an increased uptake of the biocide 3-iodo-2-propynyl butyl carbamate (IPBC). Subsequent weathering tests showed that *P. vitreus* caused significant leaching in Norway spruce and Scots pine woods after the samples were exposed to outdoor for 6 months.

Due to its potential to increase treatability, a bioincising process by *P. vitreus* was applied to the sapwood and heartwood portions of Oriental spruce wood (*Picea orientalis*) in the recent study. The primary purpose of this study was to discuss the potential of biotechnological methods to enhance the treatability of spruce wood largely distributed in the Eastern Black Sea Region of Turkey. We also aimed to reveal the effects of bioincising process on CuO (copper oxide) retention in micronized copper quat (MCQ) and alkaline copper quat type D (ACQ-D) preservative treated spruce wood as well as the amount of Cu leached from the treated wood as a result of bioincising.

MATERIALS AND METHODS

Bioincising process

The defect-free and kiln-dried sapwood and heartwood samples used in the study were obtained from Oriental spruce wood (*Picea orientalis* (L.) Link) and Scots pine (*Pinus sylvestris L.*) trees grown in Artvin province of Turkey. All wood samples measuring 100 mm x 25 mm x 25 mm (longitudinal x radial x tangential) (160 samples spruce sapwood and heartwood with impregnation without the bioincising treatment, 80 samples spruce sapwood and heartwood with impregnation with bioincising treatment, and 80 samples Scots pine sapwood with impregnation without the bioincising treatment) were taken in a longitudinal direction and corresponding as much as possible to the same growth rings to minimize any influence of natural variability. The radial and tangential orientation of the growth rings were always strictly maintained. Before bioincising processes, the wood samples were conditioned for 2 weeks in a climate chamber at 20 °C and 65 % relative humidity (RH).

Glass jars (150 mm x 85 mm x 85 mm; length x width x height) were used in the bioincising process as they provided larger usage volume for wood samples rather than Kolle flasks required in the BS EN 113-1 standard (2020). The wood samples were directly placed into glass jars containing 4 % malt extract agar (MEA) nutrient medium previously inoculated with the fungal strain, where wet vermiculite was also added under sterile conditions. The sapwood and heartwood samples (air-dried) exposed to *P. vitreus* FP 90121 white rot fungus to induce different weight loss ranges (i.e., 5-10 % and 10-15 %) for 4, 6, and 8 weeks at 26 °C and 75 % RH. From the literature, we consider a weight loss of 10 % from insignificant strength losses of < 10 % in the wood by bioincising. The samples with two different weight loss ranges (5-10 % to 10-15 %) were briefly used in the bioincising process to understand better the importance of < 10 % weight loss and detect changes in the wood microstructure resulting from the weight loss caused by fungus. The wood decay fungi used in biotechnological approaches such as *P. vitreus* fungus either increase or decrease their activities (i.e., penetration velocity, penetration work, and penetration capacity) in response to changing environmental conditions. Therefore, weight losses that occurred in the samples were evaluated according to the necessity of ensuring the homogeneous penetration of fungal mycelium into the wood structure. Besides, the efficiency of the incubation periods and proper growth of *P. vitreus* depend on all conditions (i.e., nutrient, temperature, water activity, oxygen, and pH) being favorable, while homogeneous bio-incising depends on the complete coverage of the wood surface (Figure 1).

Figure 1: (a) Heterogeneous and (b) homogeneous colonization of wood samples by *P. vitreus* fungus in glass jars, and (c) the removal of the surface mycelium after infection with the fungus.

Before and after bioincising processes, the kiln-dried weights of the samples were measured and the percentage weight loss of each sample after bioincising was then calculated according to the following Equation 1:

$$WL(\%) = \left(\frac{W_0 - W_1}{W_0}\right) x100 \qquad (1)$$

Where;

W_o is the oven-dried weight of the sample before exposure to the fungus (g), and

W_1 is the oven-dried weight of samples after exposure to the fungus (g).

Table 1 shows test samples and procedures followed in the study. Considering the mycelial growth speed and homogeneity of fungal activity, weight loss groups that occurred in the samples were classified as 5-10 % and 10-15 % (Table 1).

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

Bioincising	Wood species	Sapwood / Heartwood	Wood preservative	Treatment	Number of wood samples
NO	Oriental spruce	Sapwood	MCQ	Vacuum	20
NO	Oriental spruce	Sapwood	MCQ	Dipping	20
NO	Oriental spruce	Sapwood	ACQ-D	Vacuum	20
NO	Oriental spruce	Sapwood	ACQ-D	Dipping	20
NO	Oriental spruce	Heartwood	MCQ	Vacuum	20
NO	Oriental spruce	Heartwood	MCQ	Dipping	20
NO	Oriental spruce	Heartwood	ACQ-D	Vacuum	20
NO	Oriental spruce	Heartwood	ACQ-D	Dipping	20
NO	Scots pine	Sapwood	MCQ	Vacuum	20
NO	Scots pine	Sapwood	MCQ	Dipping	20
NO	Scots pine	Sapwood	ACQ-D	Vacuum	20
NO	Scots pine	Sapwood	ACQ-D	Dipping	20
YES	Oriental spruce	Sapwood (5-10 % weight loss)	MCQ	Vacuum	10
YES	Oriental spruce	Sapwood (5-10 % weight loss)	MCQ	Dipping	10
YES	Oriental spruce	Sapwood (5-10 % weight loss)	ACQ-D	Vacuum	10
YES	Oriental spruce	Sapwood (5-10 % weight loss)	ACQ-D	Dipping	10
YES	Oriental spruce	Sapwood (10-15 % weight loss)	MCQ	Vacuum	10
YES	Oriental spruce	Sapwood (10-15 % weight loss)	MCQ	Dipping	10
YES	Oriental spruce	Sapwood (10-15 % weight loss)	ACQ-D	Vacuum	10
YES	Oriental spruce	Sapwood (10-15 % weight loss)	ACQ-D	Dipping	10
YES	Oriental spruce	Heartwood (5-10 % weight loss)	MCQ	Vacuum	10
YES	Oriental spruce	Heartwood (5-10 % weight loss)	MCQ	Dipping	10
YES	Oriental spruce	Heartwood (5-10 % weight loss)	ACQ-D	Vacuum	10
YES	Oriental spruce	Heartwood (5-10 % weight loss)	ACQ-D	Dipping	10
YES	Oriental spruce	Heartwood (10-15 % weight loss)	MCQ	Vacuum	10
YES	Oriental spruce	Heartwood (10-15 % weight loss)	MCQ	Dipping	10
YES	Oriental spruce	Heartwood (10-15 % weight loss)	ACQ-D	Vacuum	10
YES	Oriental spruce	Heartwood (10-15 % weight loss)	ACQ-D	Dipping	10

Bioincised and unincised samples served as controls were subsequently treated with either MCQ (micronized copper quat) or ACQ-D (alkaline copper quat type D) wood preservative solutions (Table 2):

Table 2: Wood preservatives used in the study.

Wood preservative	Commercial name	Composition	Form of copper
MCQ	Osmose Micro Pro (Celcure MC), Osmose UK Protim Solignum Ltd., UK	Quat (benzalkonium chloride (10 %); Micronized copper carbonate hydroxide (17,39 %); Boric acid (5,23 %)	Micronized
ACQ-D	Osmose Celcure AC-500 (Osmose Naturewood), Osmose UK Protim Solignum Ltd., UK	Quat (benzalkonium chloride) (4,8 %); Copper carbonate hydroxide (16,53 %); Boric acid (5 %)	Soluble

In addition, Scots pine samples were impregnated with MCQ and ACQ-D wood preservatives to detect and compare the permeability of bioincised spruce samples. Both dipping (10 min) and vacuum methods (20 min, 40 mbar) were applied in the impregnation processes according to the BS EN 113-1 standard (2020). The samples' end-grain surfaces were sealed with a polyurethane coating before treatment to limit the penetration of preservative solutions into these surfaces. The preservative treated samples were then stored for a good fixation of the preservatives at 20 °C for 2 weeks.

Determination of CuO (Copper oxide) retention by uptake

CuO retention levels in the samples were calculated as the difference between the wet weight of the wood samples after impregnation and the air-dried initial weight before impregnation considering percentage CuO concentration in treating solutions in MCQ and ACQ-D wood preservatives used according to the BS EN 113-1 standard (2020).

Leaching of Cu element from bioincised samples

The samples treated with MCQ and ACQ-D wood preservative solutions were air-dried for 2 weeks before leaching tests. Leaching tests on the bioincised and control samples treated with the wood preservatives were conducted according to the AWPA E11-16 standard (AWPA 2016). Ten samples from each treatment group were separately placed in individual plastic containers and submerged in 4,500 mL of deionized water. Twelve replicates were used for each treatment group and all the leachates were removed and replaced with an equal amount of deionized water at intervals of 6 h and then after 1, 2, 4, 6, 8, 10, 12, and 14 days. Copper content in leached and unleached wood samples was analyzed by Inductively Coupled Plasma Atomic Emission Spectroscopy (ICP – OES PerkinElmer optima 8000, Waltham, MA, USA) following the standard protocols listed in the AWPA A21-16 standard (2016). Percent Cu leaching from treated wood samples after the 14 day-leaching-course was calculated based on the Cu content (ppm) in the samples before and after the leaching procedure.

Statistical analysis

Data were statistically analyzed by one-way analysis of variance (ANOVA) to determine the effects of the various applications on studied properties. The Tukey's test was used to identify statistically significant differences at 0,05 probabilities among the mean values of the studied properties within the applications. Both the ANOVA and least significant difference tests were conducted using JMP 5,0 (JMP 2020). Four repetitions were used for each group in the leaching tests.

RESULTS AND DISCUSSION

Tables 3 and 4 show CuO retention levels (kg/m^3) in the samples before and after bioincising considering the weight loss groups by the fungus. In the present study, Scots pine sapwood samples served as the main control samples in order to compare the effects of fungal activity on the permeability of spruce sapwood and heartwood. As the weight losses by the fungus increased in the sapwood and heartwood, CuO retention levels by MCQ and ACQ-D treatments increased generally in the vacuum treatment; however, no statistically significant change in the dipping treatment was observed. Furthermore, due to the bioincising process, the CuO retention was much higher after the vacuum method than after the dipping method in both sapwood and heartwood samples. Similar results were also determined for the impregnation of the Scots pine sapwood samples.

Table 3: CuO retention (kg/m^3) in treated Spruce and Scots pine sapwood samples before and after bioincising process based on the weight loss groups occurred by *P. vitreus*.

Wood species		MCQ		ACQ-D	
		Dipping	Vacuum	Dipping	Vacuum
Scots Pine Sapwood	Control (Unincised)	0,11	0,73	0,09	0,54
		(0,02)a	(0,15)a	(0,01)a	(0,14)a
Spruce Sapwood	Control (Unincised)	0,04	0,24	0,04	0,26
		(0,02)b	(0,08)b	(0,01)b	(0,06)b
	5-10 % WL	0,06	0,25	0,06	0,51
		(0,03)b	(0,11)b	(0,01)ab	(0,21)a
	10-15 % WL	0,06	0,73	0,06	0,39
		(0,03)b	(0,14)a	(0,02)ab	(0,16)ab

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 ($p \leq 0,05$). WL: Weight loss.

The results for sapwood samples showed that in the dipping methods, although CuO retention in MCQ treatments of Scots pine samples was higher than that in control and bioincised spruce samples, ACQ-D treatments resulted in more CuO retention in Scots pine than controls. However, no or only negligible differences were determined for incised spruce samples. In the vacuum methods of sapwood samples, CuO retention in MCQ treatments of Scots pine samples was higher than that in control and spruce samples in the 5-10 % weight loss group. However, there was no significant difference between Scots pine samples and the samples from the 10-15 % weight loss group. In ACQ-D treatments of Scots pine samples, CuO retention was found to be higher than that in controls. However, there was no significant difference among Scots pine samples and wood samples from the two weight loss groups.

Table 4: CuO retention (kg/m^3) in treated Spruce heartwood and Scots pine sapwood samples before and after bioincising process based on the weight loss groups occurred by *P. vitreus*.

Wood species		MCQ		ACQ-D	
		Dipping	Vacuum	Dipping	Vacuum
Scots Pine Sapwood	Control (Unincised)	0,11	0,73	0,09	0,54
		(0,02)a	(0,15)a	(0,01)a	(0,14)c
Spruce Heartwood	Control (Unincised)	0,04	0,10	0,04	0,12
		(0,01)c	(0,02)b	(0,01)b	(0,02)d
	5-10 % WL	0,07	0,73	0,07	0,98
		(0,02)b	(0,16)a	(0,02)a	(0,20)b
	10-15 % WL	0,08	0,87	0,07	1,36
		(0,02)b	(0,43)a	(0,02)a	(0,17)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 ($p \leq 0,05$). WL: Weight loss.

In heartwood samples, as weight loss increased, CuO retention levels increased in both treatment methods; however, there was no significant difference between the wood samples from the 5-10 % and 10-15 % weight loss groups except for ACQ-D treatments by vacuum (Table 4). In the dipping methods, CuO retention in MCQ treatments in Scots pine samples was higher than that in controls and bioincised spruce heartwood samples, while ACQ-D treatments resulted in higher CuO retention in Scots pine samples in comparison with controls. However, no or only negligible differences were observed for bioincised spruce heartwood samples. In the vacuum methods, as the weight loss increased in heartwood samples, higher CuO retentions in MCQ treatments of Scots pine samples were obtained when compared to controls; however, there was no significant difference between Scots pine and bioincised spruce heartwood samples. In ACQ-D treatments, CuO retention in Scots pine samples was relatively higher than that in controls, but much lower than that in the bioincised spruce heartwood samples. In addition, CuO retention levels were much higher in the vacuum method than those in the dipping methods in both sapwood and heartwood samples after the bioincising process.

As shown in Table 3 and Table 4, as a result of the bioincising process, surprisingly, much more wood preservative was absorbed in the heartwood samples than in the sapwood samples. It is known that *P. vitreus* used in the bioincising process selectively delignifies the wood, prefers mainly bordered pits primarily containing pectin in the wood (Schwarze 2007, Lehringer *et al.* 2009, Lehringer *et al.* 2010, Schubert and Schwarze 2011, Fuhr *et al.* 2013, Schubert *et al.* 2013, Gilani and Schwarze 2014). It is likely that *P. vitreus* decomposes the aspirated pits in heartwood more than in sapwood. The fungus opens the closed pit membranes of heartwood that have become thickened by incrustations of compounds such as lignans and can remove the extractive substances and phenolic components that cause the adhesion of the aspirated pit membranes with the porus (Matsumura *et al.* 1996, Messner *et al.* 2003, Durmaz *et al.* 2015). Generally, we found that CuO retention levels in the MCQ and ACQ-D treatments increased when the weight losses increased, but there was no significant difference among the wood samples in the 5-10 % and 10-15 % weight loss groups (Table 3 and Table 4). Therefore, it is more appropriate to aim a weight loss less than 10 % in the application of bioincising process. On the other hand, the overall retention levels suggest that MCQ wood preservative may not ensure a much higher CuO retention in Oriental spruce wood after bioincising process.

In this study, *P. vitreus* was found to more active in heartwood than in sapwood (Table 3 and Table 4). These results are consistent with our previous microscopic study of bioincised wood (Bakır *et al*. 2021). In addition, Schwarze *et al.* (2006) have found a significant increase in heartwood permeability as a result of *P. vitreus* activity for 6 weeks in *Picea abies* and *Abies alba* woods. In contrast, Lehringer *et al.* (2010) have reported that the fungus did not show remarkable activity in Norway spruce (*Picea abies*) heartwood but was higher in the sapwood. It is believed that the increase in solvent (water) uptake in this study may also be related to water condensation in the capillary spaces within the cell walls as a result of *P. vitreus* activity, which can simultaneously rot in the wood; however, it should be noted that different rates and the number of cell wall components remaining from the fungal activity may be related to their components' water retention or binding capacity (pectin > hemicellulose > cellulose > lignin) (Ek *et al.* 2009). Volkmer *et al.* (2010) have emphasized that pretreatment with *P. vitreus* enhances the uptake of water in wood samples, thus causing an increase in wood moisture and promoting colonization by blue-stain fungi.

Overall, the correlations between weight losses (%) and CuO retention (kg/m^3) occurred in the samples by vacuum treatments in spruce sapwood and heartwood samples are seen in Figure 2. In general, as weight losses increased, CuO retention levels increased; however, in spruce sapwood samples, the lower weight loss group had the higher CuO retention level.

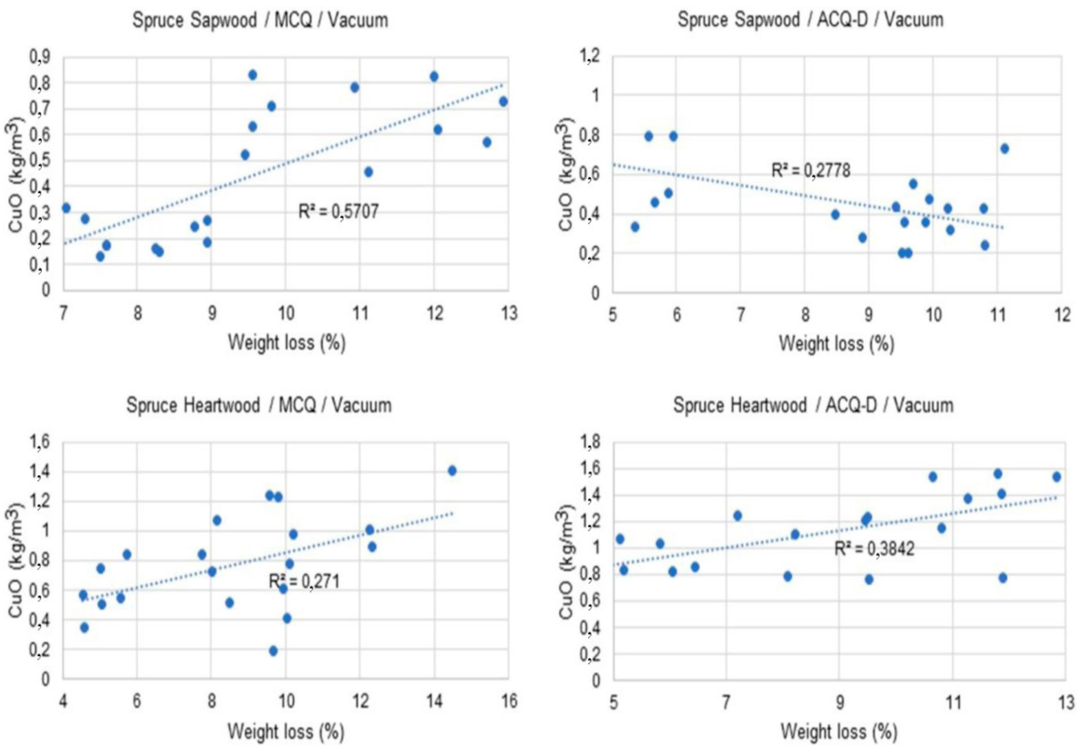

Figure 2: Correlation between weight losses and CuO retention in wood samples by vacuum treatment.

The leaching tests showed that as weight loss increased in the sapwood and heartwood samples, the leaching MCQ and ACQ-D (%) increased both in the vacuum and dipping treatments. But for the dipping method in the sapwood and vacuum method in the heartwood, there was no significant difference among the wood samples in both weight loss groups (Table 5 and Table 6). In addition, the amount of Cu release was higher in the bioincised spruce sapwood and heartwood samples than in control and Scots pine samples. The results showed that the increase in the amount of Cu leached from spruce wood was statistically significant in both the sapwood and heartwood samples compared to that in the control and Scots pine samples after the bioincising process. Petrič *et al.* (2000) and Stirling *et al.* (2008) have described that the lignin functional groups within the wood are targeted adsorption sites for the transition metal ions regardless of the carrier solvent; therefore, as a result of degradation of these lignin groups by *P. vitreus*, it is expected that leaching would increase, especially during the weight loss of 5-10 %.

Table 5: Cu element leaching (%) from treated Spruce and Scots pine sapwood samples before and after bioincising process based on the weight loss groups occurred by *P. vitreus*.

Wood species		MCQ		ACQ-D	
		Dipping	Vacuum	Dipping	Vacuum
Scots Pine Sapwood	Control (Unincised)	0,75	0,59	1,12	1,20
		(0,49)b	(0,53)c	(0,92)b	(0,78)c
Spruce Sapwood	Control (Unincised)	0,40	1,70	0,80	0,70
		(0,30)b	(0,30)b	(0,50)b	(0,50)c
	5-10 % WL	2,02	1,90	2,10	5,10
		(0,40)a	(0,40)b	(0,50)a	(0,50)a
	10-15 % WL	1,90	2,50	2,20	3,30
		(0,40)a	(0,40)a	(0,50)a	(0,50)b

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 ≤0,05). WL: Weight loss.

Table 6: Cu element leaching (%) from treated Spruce heartwood and Scots pine sapwood samples before and after bioincising process based on the weight loss groups occurred by *P. vitreus*.

Wood species		MCQ		ACQ-D	
		Dipping	Vacuum	Dipping	Vacuum
Scots Pine Sapwood	Control (Unincised)	0,75	0,59	1,12	1,20
		(0,49)bc	(0,53)b	(0,92)bc	(0,78)b
Spruce Heartwood	Control (Unincised)	0,30	0,30	0,80	0,90
		(0,20)c	(0,20)b	(0,40)c	(0,40)b
	5-10 % WL	1,10	1,80	1,60	3,01
		(0,30)b	(0,30)a	(0,40)b	(0,40)a
	10-15 % WL	2,01	1,90	2,50	2,90
		(0,30)a	(0,30)a	(0,40)a	(0,40)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 ≤0,05). WL: Weight loss.

The amount of Cu leached from spruce sapwood, and heartwood samples increased after the bioincising process. This increase was well correlated with the increase in CuO retention after the bioincising process, particularly in the heartwood. We also found that Cu leaching in the bioincised spruce sapwood samples was higher than those in the bioincised spruce heartwood samples even though the bioincised heartwood samples had greater CuO retention when compared to the bioincised sapwood samples (Table 3, Table 4, Table 5, and Table 6). Wang and Kamdem (2012) stated that the amount of Cu leached from treated wood was proportional to the amount of Cu absorbed during the treatment and is in agreement with the published data for ACQ-D and MCQ. In addition, Schubert *et al.* (2011) have emphasized that when bioincising is applied, leaching the active substances in the preservative also increases because of the increase in the permeability of the wood; however, different amounts of Cu leached from the sapwood and heartwood may be the result of the chemical components (pectin, cellulose, hemicellulose, and lignin) and substances (extractives, lignans, and phenolic compounds) consumed by *P. vitreus*, which might be different in sapwood and heartwood. In other words, it is believed that the rate of the unaspirated pits in heartwood, which allows for the increase in CuO retention up to a weight loss of 10-15 % from *P. vitreus* activity, is more than the amount of degraded lignin in heartwood, which plays a key role in the retention of preservatives. If the amount of degraded lignin is more than the number of pits that were unaspirated, all of the preservative taken into the wood would be adsorbed and leached away regardless of the amount; therefore, in such a situation, as in sapwood, the leaching will be increased.

Schubert *et al.* (2011) and Volkmer *et al.* (2010) conducted detailed studies on the movement of wood preservatives into the wood to different depth levels. In both studies, the penetration of 3-iodo-2-propynyl butylcarbamate (IPBC) into bioincised Norway spruce wood with preservatives was analyzed by high-pressure liquid chromatography (HPLC). Although average IPBC content at different depths in Norway spruce wood with and without pre-treatment with *Physisporinus vitreus* after 6 months' exposure to weathering was measured by Volkmer *et al.* (2010), no weathering of the analyzed specimens was performed by Schubert *et al.* (2011). Here, bioincised Norway spruce wood samples without weathering revealed higher concentrations of IPBC but recorded the highest IBPC concentrations in not bioincised wood samples with weathering. One explanation for this discrepancy is that, because of the increased permeability of the bioincised Norway spruce wood, the leaching of the active substance (IPBC) in the preservative increases during weathering. Besides, a study by Kartal and Lebow (2002) employing mechanical incising on Eastern hemlock showed that incising greatly increased both the penetration and retention of preservative in the incised wood compared to unincised wood. Incising did not increase the percentage of Cu, chromium (Cr), or arsenic (As) that leached from the wood. Thus, incising was not associated with the leaching of elements from the wood. It is suggested that the appearance of higher leaching from the unincised samples might be an effect of their lower original retention, as previous studies have shown that leaching does not increase in direct proportion to retention (Lebow 1996). They discuss that the lack of difference in leaching rates at the higher retention levels may be more reasonable if one considers the realistically little difference in retention in the outer 5 mm. Preservative elements in the outer shell are most accessible during the early leaching periods when most losses occur**.** Higher retentions in incised samples might result in increased leaching over the very long term, but such changes may have little practical importance. Another study by Kartal (2001) using a number of blue-staining fungi found that the fungi had no effect on major wood components; however, degradation of ray parenchyma, tracheid walls, and pits by the fungi resulted in increasing permeability of wood and *Ceratocystis pilifera* and *C. huntii* caused more leaching of preservative elements compared to control specimens.

CONCLUSIONS

CuO retention levels in bioincised spruce samples increased when compared to Scots pine sapwood samples. Moreover, there was much more CuO retention in the heartwood samples than in the sapwood samples; however, leaching from the heartwood samples after the bioincising process was lower than that from the incised sapwood samples. Considering the CuO retention levels and Cu leaching in the samples, it is more appropriate to use ACQ-D wood preservative to treat spruce wood followed by bioincising processes. Further studies are needed to understand the reasons that *P. vitreus* increases the permeability of heartwood more than sapwood. In order to interpret the results, additional studies should emphasize the changes in bordered pits and other pectin-containing wood cell elements caused by *P. vitreus*.

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