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EFFECT OF BLUE-STAINING ON THE RELEASE OF COPPER, CHROMIUM, AND ARSENIC FROM CCA-C TREATED WOOD (Pinus resinosa Ait.)¹

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Abstract

In this study, the effect of blue-staining on the leachability of CCA elements from CCA-C treated and stained red pine specimens was evaluated. The specimens were first blue-stained in laboratory conditions using *Ceratocystis pilifera*, *C. huntii*, *C. coerulescens*, and *Aurobasidium pullulans* fungi and then treated with 1% CCA-C solution. After fixation period, the specimens were subjected to 336-h of leaching according to modified AWPA standard method. The blue-staining fungi used in this study had no effect on major wood components, however subsequent degradation of ray parenchyma, tracheid walls and pits by blue-staining fungi used in this study caused increasing of permeability of wood. On the basis of all leached elements, *C. pilifera* and *C. huntii* caused somewhat more leaching of CCA from the specimens compared to percentage leaching rate of CCA in control specimens.

1.INTRODUCTION

The predominant inorganic compound used to preserve wood is chromated copper arsenate (CCA) in the United States. The copper, chromium, and arsenic elements in the CCA treated wood are resistant to leaching and removal when fixation reactions that occur to render the elements insoluble in water are completed (LEBOW/KARTAL 1999). Interest in leachability of preservative components from treated wood and its impact on the environment has increased in recent years in response to public concern about the environment since toxic ingredients from treated wood are recognized as a potential source of soil contamination and water pollution.

Leaching of elements from CCA-treated wood is affected by several factors such as fixation of elements in wood, wood species (hardwood/softwood, permeability, etc.), and conditions at surrounding environment. LEBOW (1996) stated that retention level, surface area, and grain orientation are all factors that could also affect the rate of preservative release from treated wood.

Bluestain or sapstain fungi are one of the most important colonizers of logs and sawn timber occurring soon after tree felling and sawing operations and mainly belong to the Ascomycetes

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and Fungi Imperfecti. The bluestain of softwood is a very common problem and is caused by various fungi including many species of Ophiostoma and Ceratocystis, black yeasts such as Aureobasidium and dark molds such as Alternaria (SEIFERT 1993). Bluestain fungi have capability of enhancing permeability of wood due to enzyme systems producing permeability enhancement. Considerable interest has been aroused for a long time by enhancement of permeability of refractory wood species (KING/EGGINS 1973). The use of such fungi to improve the permeability of wood is adventageous since these fungi are selective in their attack. Although more uniform treatment can be achieved with fungal pre-treatment of wood by blue-staining fungi, greater preservative loadings are also obtained. Greater preservative uptake and the larger surface area exposed by blue-stain fungi colonization may have the potential to increase the rate of preservative leaching. The objective of this study was to determine the release rates of copper, chromium, and arsenic from blue-stained and CCA-C treated wood.

2. MATERIALS AND METHODS

2.1 Wood specimens

The sapwood specimens, 7 by 20 by 70 mm long, were cut from a freshly felled red pine (*Pinus resinosa* Ait.) tree and immediately frozen in polyethylene bags prior to use.

2.2 Fungal inoculum

Four blue-staining fungi, *Ceratocystis pilifera* RWD-9472-B, *C. huntii* RD-776, *C. coerulescens* C-256, and *Aurobasidium pullulans* MDX-18 were inoculated on 2 percent malt extract agar (MEA) (20 g malt extract and 20 g Bacto agar in 1 liter distilled water) in petri dishes for 3 weeks at 23°C before inoculation of the specimens. All fungus cultures were obtained from the Forest Products Laboratory, Madison, WI, USA.

2.3 Inoculation of the specimens

Each petri dish was flooded with 20 ml sterile deionized water and the surface of fungal growth in the petri dish was rubbed with a transfer pipet in order to loosen fungal spores and hyphal fragments. Two specimens were placed on glass rods in a glass petri dish (100 mm diameter, 20 mm height) (Figure 1). To maintain high humidity in the petri dishes during the test period, 5 layers of filter paper were placed on the bottom of each dish. Then the papers were wetted with deionized water until free water appeared. The petri dishes including the specimens were then autoclaved at 121°C, 15 psi for 20 min and 5 ml of inoculum was taken into the transfer pipet and

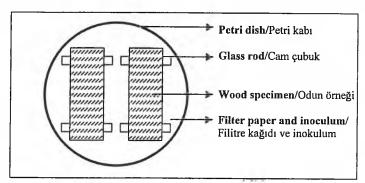


Figure 1: Placement of wood specimens into petri dishes Şekil I: Odun örneklerinin petri kaplarına yerleştiriliş şekli

Table 1. Chemical Analysis Results of Control and Blue-Stained Specimens
Tablo 1. Kontrol ve Mavi Renklenmiş Odun Örneklerinin Kimyasal Analiz Sonuçları

	Incubation time		W	ood carb	ohydrates					
Specimens Örnekler	Inkübasyon süresi (week/hafta)	K. Lignin	Arabinan	Galactan	Rhamnan	Glucan	Xylan	Mannan	Total Carbohydrate Toplam Karbonhidrat	
Control / Kontrol	-	28.0%	1.55%	1.35%	0.12%	44.78%	5.60%	12.26%	65.7%	93.7%
Ceratocystis pilifera	4	28.3%	1.63%	1.50%	0.21%	43.50%	6.38%	11.53%	64.7%	93.0%
	8	28.6%	1.58%	2.10%	0.17%	42.74%	6.67%	11.38%	64.6%	93.2%
C. huntii	4	29.3%	1.47%	1.86%	0.14%	43.41%	6.00%	11.82%	64.7%	93.8%
	8	28.6%	1.53%	1.42%	0.17%	43.75%	6.19%	11.94%	65.0%	93.5%
C. coerulescens	4	29.2%	1.53%	1.82%	0.13%	43.55%	6.03%	11.78%	64.8%	93.9%
	8	29.7%	1.49%	2.10%	0.14%	43.15%	6.09%	11.52%	64.5%	94.1%
Aurobasidium pullulans	4	28.3%	1.51%	1.46%	0.14%	44.05%	6.02%	11.88%	65.1%	93.2%
	8	29.0%	1.51%	1.50%	0.13%	43.49%	6.01%	11.95%	64.6%	93.5%

performed on the radial surface and cross sections of the specimens. The inoculated petri dishes were placed in polyethylene bags to prevent drying and incubated at 25°C and 70-80 percent relative humidity (RH). Incubation time was selected 4 and 8 weeks.

2.4 Preservative treatment of the specimens

After each incubation period, control and stained specimens were sterilized at 121°C, 15 psi for 20 min, conditioned to 12 percent moisture content at 20°C and 65 percent RH, and end-coated with two coats of a neoprene rubber sealant. For treatment, 1.0 percent CCA-C solution was used. The specimens were treated in a desiccator at 100 mm Hg of vacuum subjecting AWPA E10-91 standard method (AWPA 1997). Then the specimens were subjected to the fixation procedure at 20°C for 2 weeks.

2.5 X-ray spectroscopic analyses of treated specimens

In order to determine copper, chromium, and arsenic levels in the treated specimens, 2 cm long part was cut from one end of each specimen and these parts were ground to pass a 40-mesh screen. Resulting sawdust was assessed with an ASOMA X-ray fluorescence analyzer (ASOMA Instruments, Austin, Texas).

2.6 Leaching of the specimens

After fixation course, three replicate samples of four specimens were subjected to modified AWPA E11-97 standard leaching test (AWPA 1997). Copper, chromium, and arsenic concentrations in leaching solutions were determined with a Perkin Elmer 5100PC Atomic Absorption Spectrometer (AAS) equipped with a Zeeman Furnace Module. Leaching solutions collected from the 2-week leaching cycle were analyzed by either graphite furnace or flame atomization, as appropriate. Percentage of leached elements was estimated based on initial Cu, Cr, and As content in treated specimens determined by X-ray spectroscopic analyses.

2.7 Chemical analyses of the sound and decayed specimens

Lignin and carbohydrate content of both control and stained specimens was determined to investigate the effects of blue staining fungi. For lignin content in wood, wood specimens were milled to pass a U.S. Standard 30-mesh (589 mm) screen and vacuum dried at 45°C. Approximately 100 mg of sample was hydrolyzed with 1.00 ml 72% $\rm H_2SO_4$ for 1-h at 30°C. Samples were diluted to 4% $\rm H_2SO_4$ with deionized water, fucose was added as an internal standard, and a secondary hydrolysis was performed. A standard mixture of sugars was hydrolyzed in parallel with each batch of samples. Losses during primary hydrolysis were minimal and were ignored. Following secondary hydrolysis, samples were immediately filtered and three washes with 5 ml deinoized water were collected in 100 ml volumetric flasks and brought to volume with water. The acid-insoluble lignin residue (Klason lignin) was washed an additional six times with 10 ml hot deionized water and quantitated gravimetrically. Klason lignin values were corrected for ash content gravimetrically following incubation of the lignin at 575°C for less than 3 hours.

Wood carbohydrates were determined by subjecting the samples to acid hydrolysis, and then analyzing the hydrolysates using anion exchange HPLC (High Performance Liquid Chromatography) with pulsed amperometric detection. Sugar separation was achieved with Carbo-Pac PA1 guard and analytical columns connected in series. Sugars were quantitated using an internal standard method and results were reported in terms of percent of the original sample mass (DA-VIS 1998).

2.8 Scanning electron microscope (SEM) examinations of control and blue-stained specimens

The specimens were soaked in water and surfaced on a sliding microtome. The specimens were then dried and mounted on aluminum stubs with silver paste and coated with gold. The specimens were then imaged using a scanning electron microscope at 15 kV.

3. RESULTS AND DISCUSSION

3.1 Chemical analyses of control and blue-stained specimens

Klason lignin and arabinan, galactan, rhamnan, glucan, xylan, and mannan contents were about the same in both control and blue-stained specimens by *C. pilifera*, *C. huntii*, *C. coerulescens*, and *A. pullulans*. The results showed that blue-staining fungi did not affect the main composition of wood in terms of lignin and carbohydrates. It is well known that staining fungi are the initial colonizers of wood and they invade the parenchyma and epithelial cells, and assimilate the available nutrients, mainly non-structural wood components. It is also well recognized that soluble sugars and proteins are major nutrients for staining fungi in wood (BLANCHETTE et al 1992, GAO/BREUIL 1995).

3.2 CCA retention in control and blue-stained specimens

As expected, blue-staining improved the uptake of the treatment solution compared with the CCA uptake in control specimens (Table 3). Average CCA retention in control specimens was 6.084 kg/m³ while CCA retentions varied between 6.909 kg/m³ (*C. pilifera-*4 weeks) and 7.128 kg/m³ (*C. pilifera-*8 weeks).

3.3 SEM examinations of control and blue-stained specimens

In **Figure 2**, the penetration forms of fungal hyphae into tracheid walls and pits between tracheids are shown in blue-stained specimens. The SEM observations showed that there was a rapid growth of hyphae into longitudinal elements of the wood. The growth in longitudinal axis was via bordered pits and windows-like pits between rays and longitudinal tracheids. In 4 and 8-week blue-stained specimens by *C. pilifera*, several small holes in the tracheid walls around pits were observed.

3.4 Leaching of CCA elements

Blue staining appeared to have no big effect on the amount of CCA elements from the blue-stained specimens. On a percentage basis, the average amount of chromium leached from the all blue-stained specimens was actually less than that from the control specimens (Table 3). Leached As contents from 4 and 8 week stained specimens by *C. pilifera* were somewhat higher than those from control specimens. Although leached Cu contents from 4 and 8-week stained specimens by A. pullulans were somewhat lower than that from control specimens, all blue-stained specimens showed higher leaching rates in terms of Cu leachability. Percentage of Cu leached from 8 week stained specimens by *C. huntii* was the highest rate (3.05%) in all elements leached from the specimens however higher leaching rates were obtained in As element compared to the other elements leached.

Leaching rates of elements from the blue-stained specimens are higher during the early stages of leaching, especially 1, 2, and 4th days. The 6, 8, 10, 12, and 14-day leaching data from our study indicated that leaching from all the treated specimens stabilized at very low release rates (Figure 3).

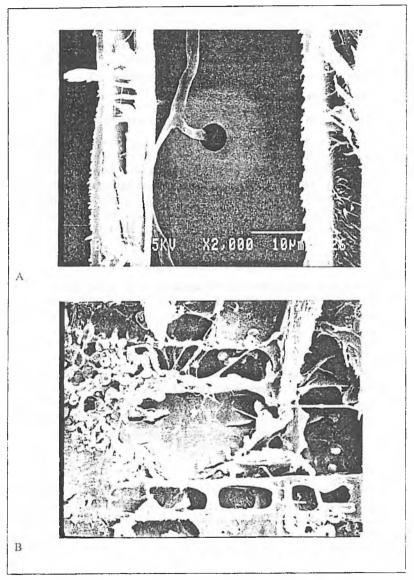


Figure 2: Scanning electron micrographs showing the structure of blue-stained specimens (A: C., pilifera on pits, B: A. pullulans on rays)

Şekil 2: Mavi renklenmiş örneklerin elektron miksroskop görünüşleri. (A: Geçitler üzerinde *C. pilifera*, B: Öz ışınlarında *A. pllulans*)

Leaching results showed although higher CCA retentions in blue-stained specimens by *C. coerulescens* and *A. pullulans* were obtained, percentages leached of all elements from these specimens were less than those of control specimens. Previous studies showed that leaching does not

Table 2. Percentage of leached elements from wood specimens treated with CCA-C.

Tablo 2. CCA-C ile emprenye edilmiş odun örneklerinden elementlerin yıkanma miktarları.

				Leached E	lements (ppm	ı) / Yıkanan l	Element Miktar	ı (ppm)		
Specimens / Örnekler	Leach				ing Duration (day) / Yıkama periyodları (gün)					
	Incubation time (week)/		11		2			4		
	İnkübasyon zamanı (hafta)	Cu	Cr	As	Cu	Cr	As	Cu	Cr	As
Control / Kontrol	•	0.91	1.50	0.65	0.14	0.12	0.52	0.15	0.09	0.65
		0.31	0.70	0.15	0.03	0.02	0.16	0.06	0.02	0.15
Ceratocystis pilifera	4	1.38	0.63	1.33	0.28	0.16	0.79	0.31	0.13	1.12
		0.34	0.10	0.08	0.05	0.02	0.05	0.13	0.01	0.11
	8	2.03	0.89	1.47	0.39	0.19	0.87	0.38	0.15	1.13
		0.17	0.17	0.10	0.04	0.03	0.04	0.13	0.01	0.05
C. huntii	4	1.69	1.28	0.88	0.42	0.21	0.54	0.46	0.17	0.67
		0.60	0.19	0.23	0.16	0.03	0.19	0.18	0.03	0.23
	8	2.29	1.18	1.03	0.95	0.19	0.60	1.09	0.18	0.79
		1.62	0.28	0.20	1.36	0.09	0.21	1.54	0.08	0.31
C. cereluscens	4	1.10	0.74	0.83	0.25	0.13	0.57	0.28	0.10	0.79
		0.42	0.20	0.14	0.10	0.03	0.14	0.05	0.02	0.08
	8	1.31	1.22	0.67	0.39	0.19	0.56	0.25	0.10	0.74
		0.21	0.85	0.10	0.26	0.04	0.17	0.09	0.01	0.10
Aurobasidium pullulans	s 4	0.85	0.60	0.90	0.23	0.15	0.57	0.17	0.10	0.76
		0.41	0.12	0.13	0.04	0.02	0.05	0.04	0.03	0.05
	8	0.90	1.75	0.77	0.16	0.14	0.57	0.16	0.07	0.77
		0.25	0.93	0.10	0.02	0.02	0.07	0.03	0.01	0.08

Each value represents the mean of three replicates of three specimens. Numbers in italics are standard deviations. Her deger uç örnekten oluşan üç yıkanma değerinin ortalamasını gösterir. İtalik numaralar standard sapmalardır.

Table 2: Extended Tablo 2: Devamı.

	- I Colombi													
						Elements (p)								
					Leachin	g Duration (day) / Yıkan	ıa periyodları	(gün)					
	6			8			10			12			14	
Cu	Cr	As	Cu	Cr	As	Сп	Cr	As	Cu	Cr	As	Cu	Cr	As
0.16	0.06	1.00	0.08	0.05	0.89	0.05	0.04	0.74	0.02	0.04	0.60	0.09	0.05	0.73
0.04	0.02	0.18	0.02	0.01	0.07	0.01	0.01	0.11	0.00	0.01	0.13	0.04	0.01	0.13
0.29	0.06	1.34	0.17	0.03	0.80	0.13	0.04	0.87	0.04	0.04	0.72	0.20	0.05	0.78
0.06	0.01	0.13	0.04	0.00	0.10	0.04	0.01	0.08	0.01	0.01	0.10	0.09	0.00	0.06
0.26	0.06	1.28	0.15	0.03	0.76	0.13	0.03	0.81	0.04	0.03	0.70	0.14	0.04	0.70
0.11	0.00	0.05	0.05	0.00	0.03	0.05	0.00	0.06	0.01	0.01	0.06	0.05	0.00	0.02
0.30	0.09	0.94	0.19	0.05	0.56	0.10	0.04	0.88	0.04	0.04	0.56	0.11	0.04	0.58
0.14	0.01	0.17	0.16	0.02	0.13	0.08	0.02	0.17	0.04	0.01	0.10	0.02	0.01	0.08
0.61	0.09	1.04	0.38	0.05	0.69	0.26	0.05	0.67	0.08	0.04	0.62	0.25	0.06	0.67
0.73	0.04	0.25	0.45	0.02	0.18	0.37	0.02	0.16	0.12	0.02	0.12	0.31	0.03	0.09
0.31	0.06	1.15	0.16	0.03	0.52	0.11	0.03	0.62	0.02	0.04	0.44	0.12	0.04	0.66
0.08	0.01	0.17	0.04	0.00	0.04	0.04	0.00	0.08	0.01	0.01	0.19	0.05	0.00	0.04
0.41	0.06	1.13	0.18	0.05	0.58	0.11	0.04	0.69	0.03	0.04	0.54	0.14	0.04	0.68
0.11	0.01	0.13	0.05	0.01	0.09	0.06	0.01	0.05	0.04	0.02	0.03	0.05	0.01	0.04
0.22	0.06	0.95	0.10	0.05	0.73	0.09	0.04	0.56	0.02	0.06	0.50	0.11	0.04	0.92
0.06	0.01	0.06	0.03	0.00	0.04	0.04	0.00	0.03	0.01	0.04	0.07	0.06	0.01	0.19
0.21	0.06	1.01	0.11	0.05	0.78	0.06	0.03	0.59	0.01	0.03	0.53	0.11	0.04	1.24
0.03	0.01	0.13	0.02	0.01	0.04	0.01	0.01	0.04	0.00	0.01	0.05	0.03	0.01	0.09

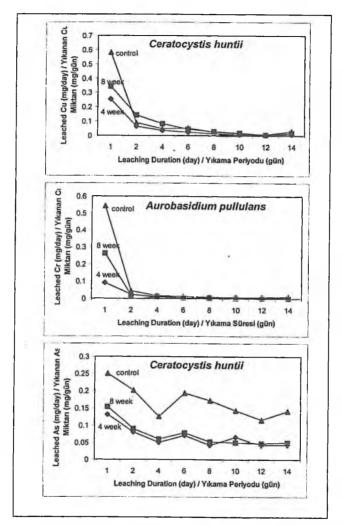


Figure 3: Leached Cu, Cr, and As content from control and some bluestained wood specimens Şekil 3: Kontrol ve bazı mavi renklenmiş örneklerden yıkanan Cu, Cr ve As miktarları

increase in direct proportion to retention (LEBOW 1996). On the basis of all leached elements, *C. pilifera* and *C. huntii* caused somewhat more leaching of CCA from the specimens compared to percentage rate of CCA in control specimens.

4. CONCLUSIONS

Blue-staining fungi had no effect on major wood components, however subsequent degradation of ray parenchyma, tracheid walls and pits by blue-staining fungi used in this study caused increased permeability of wood. Increasing permeability increased the retention of CCA in stained specimens. Although only one group (*C. huntii-8* week) showed significantly higher percentage of CCA compared to control specimens, effect of blue staining is not associated with increased leac-

Table 3: Total leached elements from CCA-treated specimens
Tablo 3: CCA ile emprenye edilen örneklerden yıkanan toplam element miktarları

	c	CA Retention (kg/m³)		Total Leached Elements (mg) Toplam Yıkana Elementler)	Total Leached Elements (%) Toplam Yıkanan Elementler (%)			
Specimens /	Incubation time (week)/ C	CA Retensiyon Miktarı		(mg)					
Örnekler	İnkübasyon zamanı (hafta)	(kg/m³)	Cu	Cr	As	Cu	Cr	As	
Control / Kontrol	-	6.084	0.24	0.29	0.87	1.02	0.71	2.23	
			0.153	0.106	0.335	0.006	0.021	0.000	
Ceratocystis pilifera	4	6.909	0.42	0.17	1.18	1.44	0.33	2.45	
			0.216	0.050	0.368	0.005	0.018	0.000	
	8	7.128	0.53	0.21	1.15	1.86	0.43	2.48	
			0.280	0.065	0.372	0.005	0.017	0.000	
C. huntii	4	7.005	0.50	0.29	0.84	1.74	0.57	1.78	
			0.260	0.086	0.267	0.002	0.001	0.000	
	8	7.077	0.88	0.28	0.92	3.05	0.54	1.92	
			0.458	0.081	0.289	0.002	0.001	0.000	
C. cereluscens	4	6.970	0.35	0.18	0.84	1.23	0.35	1.78	
			0.184	0.052	0.267	0.193	0.002	0.001	
	8	7.073	0.42	0.26	0.84	1.46	0.51	1.75	
			0.218	0.077	0.263	0.190	0.002	0.001	
Aurobasidium pullulans	s 4	6.975	0.27	0.17	0.88	0.94	0.33	1.88	
			0.140	0.050	0.281	0.004	0.026	0.001	
	8	7.016	0.26	0.33	0.94	0.89	0.66	1.99	
	191		0,133	0.100	0.298	0.004	0.026	0.001	

Each value represents the mean of three replicates of three specimens. Numbers in italics are standard deviations. Her deger üç örnekten oluşan üç yıkama değerinin ortalamasını gösterir. İtalik numaralar standard sapmalardır. hing of CCA from the specimens. Despite the fact that this study evaluated only one wood species-4 different blue staining fungi-2 different incubation period combination, it appears that the benefits of increased retention derived from blue-staining can be obtained without risk of increased leaching due to increased CCA retention in treated wood.

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ODUNDA MAVİ RENK OLUŞUMUNUN EMPRENYE EDİLMİŞ ODUNDAN BAKIR, KROM VE ARSENİK YIKANMASI ÜZERİNE ETKİSİ

Ar. Gör. Dr. S. Nami KARTAL.

Kısa Özet

Bu çalışmada mavi renk oluşumunun emprenye edilmiş ağaç malzemeden Cu, Cr ve As elementlerinin yıkanması üzerine etkisi incelenmiştir. Dört farklı mavi renk mantarı ile mavileşmiş örnekler %1 konsantrasyonda CCA-C çözeltisi ile emprenye edildikten sonra 14 gün süren yıkama işlemlerine tabi tutulmuştur. Araştırma sonucunda mavi renk oluşumun yıkanan element yüzdeleri üzerine önemli bir etkisi olmadığı belirlenmiştir.

ÖZET

CCA, odunu korumak amacıyla ABD'de en fazla kullanılan bir emprenye maddesidir. CCA ile emprenye edilmiş odundaki bakır, krom ve arsenik elementleri, bu elementlerin suda çözünmezliğini sağlayan fiksasyon reaksiyonları tamamlandığında yıkanmaya ve odundan ayrılmaya dirençlidirler. Emprenye edilmiş odundan yıkanan toksik karakterdeki elementler toprak ve su kirlenmesinin potansiyel kaynağı olarak kabul edildiklerinden dolayı, emprenye edilmiş odundan emprenye maddesi komponentlerinin yıkanması ve bunun çevreye etkileri son zamanlarda büyük önem kazanmıştır.

CCA ile emprenye edilen odundan elementlerin yıkanması, elementlerin oduna fikse olup olmaması, odun türü (yapraklı yada iğne yapraklı ağaç odunu, permeabilite) ve çevre şartları gibi çeşitli faktörler tarafından etkilenmektedir. Bununla birlikte, retensiyon seviyesi, odunun yüzey alanı ve kesit yönleri de önemli faktörlerdir.

Mavi renklenme yada genel anlamda renklenme oluşturan mantarlar, ağaç kesildikten ve odun biçildikten hemen sonra görülebilen ve esas olarak *Ascomycetes* ve *Fungi Imperfecti* grubuna dahil olan mantarlardır ve yuvarlak odun ve biçilmiş ağaç malzemenin en önemli zararlılarındandırlar. İğne yapraklı ağaç odunlarında mavi renklenme genellikle sık rastlanan bir problem olup, esas olarak *Ophiostoma* ve *Ceratocystis* mantarları, *Aurabasidium* gibi "black yeast"ler ve *Alternaria* gibi küf mantarlarının çeşitli türlerini içeren organizmalar tarafından oluşturulmaktadır. Mavi renk mantarları hüflerinde permeabilite artırıcı çeşitli enzim sistemlerine sahip olmalarından dolayı odunun permeabilitesini degradasyon sonucu artırmaktadırlar. Permeabilitenin mavi renk oluşumu ile artışı ile birlikte bu odunların emprenye işlemleri sırasında daha fazla retensiyon ve nüfuz derinlikleri elde edilebilmekte ve emprenye işlemleri iyileştirilebilmektedir. Fakat artan permeabilite, yıkanma sırasında odun içerisinde suyun hareket edebileceği alan miktarını ve emprenye maddesi absorpsiyonunu artırmakta ve bunun da yıkanan element miktarını etkileyebileceği düşünülmektedir. Bu araştırmanın amacı mavi renklenmiş ve CCA-C çözeltisi ile emprenye edilmiş

ağaç malzemeden yıkanan element miktarlarını kontrol örnekleri ile karşılaştırmak ve mavi renklenmenin yıkanma üzerine etkisini belirlemektir.

Araştırmada mavi renklenme oluşturan mantarlar olarak *Ceratocystis pilifera* RWD-9472-B, *C. huntii* RD-776, *C. coerulescens* C-256 ve *Aurobasidium pullulans* MDX-18 kullanılmış ve *Pinus resinosa* Ait. odunun diri odunundan hazırlanan örnekler petri kapları içersinde 4 ve 8 hafta süre ile bu mantarların etkisine bırakılmıştır. Daha sonra örnekler %1 konsantrasyondaki CCA-C çözeltisi ile emprenye edilmiş ve oda sıcaklığında fiksasyon reaksiyonları tamamlanmıştır. AW-PA E11-97 standarına göre 14 gün süreyle emprenye edilen örnekler yıkanarak yıkama suları her değişimde toplanmış ve AAS (Atomic Absorption Spectrometer) ile analiz edilmişlerdir. Örneklerin emprenye işlemleri sırasında absorbe ettikleri bakır, krom ve arsenik miktarı X-ışını spektroskopi analizleri ile belirlenerek örneklerden yıkanan element miktarları yüzde olarak hesaplanmış ve kontrol örnekleri ile karşılaştırılmıştır.

Ayrıca mavi renk mantarlarının odunun ana komponentlerinde yapmış oldukları değişiklikler kimyasal analizler ile ve hücre çeperleri ve geçitlerde yapmış oldukları değişiklikler ise SEM incelemeleri ile ortaya konmuştur.

Yüzde yıkanan krom miktarı esas alındığında, tüm mavileşmiş örneklerden yıkanan krom miktarının kontrol örneklerinden daha az olduğu bulunmuştur. *C. pilifera* ile 4 ve 8 hafta degrade olan örneklerden yıkanan arsenik miktarı kontrol örneklerinden biraz daha yüksek elde edilmiştir. *A. pullulans* ile 4 ve 8 hafta degrade olan örneklerde yıkanan bakır miktarı kontrol örneklerinden biraz daha düşük olmasına rağmen bütün mavileşmiş örnkler daha yüksek bakır yıkanma oranı göstermişlerdir. *C. huntii* ile 8 hafta degrade olan örneklerden yıkanan bakır yüzdesi tüm yıkanan elementler içinde en yüksek değere sahip iken, arsenik genel anlamda en fazla yıkanan element olmuştur.

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