ANTIFUNGAL ACTIVITY OF GEOTHERMAL FLUIDS FROM DIFFERENT REGIONS OF TURKEY

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Antifungal effects of geothermal fluids obtained from the Ankara, Afyon, Denizli, and Eskişehir regions of Turkey on white-rot (*Trametes versicolor*, MAD-697) and brown-rot (*Coniophora puteana*, FPRL 11E) fungus (Basidiomycetes) were studied. Fungal experiments were performed on kraft paper and Scots pine wood (*Pinus sylvestris* L.). We used non-concentrated geothermal water and concentrated geothermal water (via evaporation) in ratios of 25%, 50%, and 75%. To evaluate the results, we measured the concentration of specific minerals in the geothermal fluids such as boron (B), arsenic (As), copper (Cu), sulfate (SO_4), sodium (Na), chloride (Cl), fluoride (F), potassium (K), and ammonia (NH_3). The highest antifungal effect was observed for a geothermal fluid from the Denizli region, followed by Ankara, Afyon, and Eskişehir, in decreasing order. Antifungal properties of GFs are thought to be associated with the type and amount of mineral substances. In addition, the antifungal effects increased with increasing concentrations of geothermal water.

Keywords: Wood protection; Impregnation; Geothermal fluid

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INTRODUCTION

Wood materials are subject to degradation, rot, and damage under external environmental conditions. One way to slow this process is to impregnate wood with chemical preservatives; however, many such perservatives have become targets of environmental organizations (Kartal and Kantay 2006), and those containing heavy metals are now restricted in Japan, United States, and various European countries because they are toxic to plants and animals (Kartal *et al.* 2004a). Hence, some studies have focused on identifying environmentally-friendly and efficient wood preservatives (Kartal *et al.* 2004b), such as geothermal fluids (Var 2009).

Geothermal fluids are applicable to a variety of disciplines. Turkey is rich in available geothermal sources and ranks among the top six countries globally (Ilgar 2005; Gürü 2005). According to the General Directorate of Mineral Research and Exploration (MTA) in Turkey, 277 areas are known to have geothermal formations (Erişen *et al.* 1996). Geothermal energy can melt magmatic rocks because of its high temperature,

which causes minerals and salts contained within the rocks to rise to the surface in hot water and in wet/dry steam (Mutlu 2004; Ilgar 2005; Gürü 2005). These minerals and salts include boron (B), chloride (Cl), sodium (Na), fluoride (F), potassium (K), magnesium (Mg), ammonia (NH_3), silicon dioxide (SiO_2), and sulfate (SO_4), and most of them can be used as wood-impregnating substances to effectively protect against biotic factors (Var 2009). Elements such as Cu, Cr, As, and B that are subcomponents of CCA, CCB are well known for their antifungal properties (Kartal 1998). Geothermal fluids have numerous advantages as protectants, such as low viscosity and no adverse effects on human health (Ilgar 2005). Water-soluble impregnating substances have concentrations ranging from 0.1% to 35–40%, similar to geothermal fluids (Berkel 1972; Bozkurt *et al.* 1993; Turner and Murphy 1998; Var 2009).

In the past quarter century, the direct use of geothermal resources has expanded significantly from industry to agriculture and medical treatment. However, no previous study has examined the effects of geothermal fluids to inhibit fungal wood decay. In the present study, we investigated the antifungal effects of geothermal fluids from four different regions of Turkey. This is the first study to evaluate the application of geothermal resources for wood protection.

EXPERIMENTAL

Experimental Materials and the Impregnation Process

The geothermal fluids (GFs) used were obtained from Afyon-Gazlıgöl, Denizli-Kızıldere, Ankara-Kızılcahamam, and Eskişehir-Kızılinler-Hasırca geothermal fields in Turkey. To increase the concentrations of the active ingredients, each GF was evaporated by 25% (Y), 50% (Z), and 75% (T). The evaporation took place in a 5-liter container of water at 100 ºC. In addition, samples impregnated with distilled water and non-concentrated (natural) GF (X) were used as controls. All samples were stored at 5ºC. Temperature and pH values of the GFs are shown in Table 1.

Table 1. Temperature and pH Values of GFs used in Experiments

Parameter	Geothermal Fields			
	Ankara-Kızılcahamam	Afyon-Gazlıgöl	Eskişehir-Kızılinler-Hasırca	Denizli-Kızıldere
Temperature (ºC)	62	72	43	121
pH	7.29	7.20	7.53	7.35

Fungal Strains

Brown-rot fungus (*Coniophora puteana*, FPRL 11E) and white-rot fungus (*Trametes versicolor*, MAD-697) were used as fungal strains. These fungi were provided by the US Forest Products Laboratory in Madison, WI, USA, and were used to initiate new cultures.

Antifungal Assay on Paper Disks

Tests were performed on disk samples prepared from thermomechanical pulp paper. This paper is similar to the chemical composition of wood and can provide rapid results (Wilkinson 1979; Bozkurt *et al.* 1993; Sen and Yalcin 2010). The experimental

papers were cut to approximately 9 cm in diameter disks. Each paper disk was treated with 4 mL of impregnation fluid in a vacuum desiccator. All test samples were placed in sterilized plastic Petri dishes.

Paper disks treated with different GF concentrations and distilled water were sterilized in an autoclave for 15 min at 121°C. Petri dishes were thoroughly cooled after sterilization. Fungal inoculum sections were cut approximately 10 mm in diameter from a Petri dish. After the samples were inoculated with the fungal species, they were incubated at 27°C and 72% relative humidity (RH) for 3 weeks. When the fungal mycelia completely covered the surfaces of control samples in the Petri dish, the antifungal effects of the GFs were evaluated. Mycelia growth on the surface of the samples was rated visually based on percentage of surface coverage. The antifungal effects were marked with plus (+) or minus (–) signs according to the degree of antifungal activity as follows (AWPA 2006; TS ENV 839, 2006; Yang and Clausen 2007; Sen and Yalcin 2010):

–: No visible growth of mycelia.
+: Mycelia covering about 25% of the Petri dish.
++: Mycelia covering about 50% of the dish.
+++: Mycelia covering about 75% of the dish.
++++: Mycelia covering about 100% of the dish.

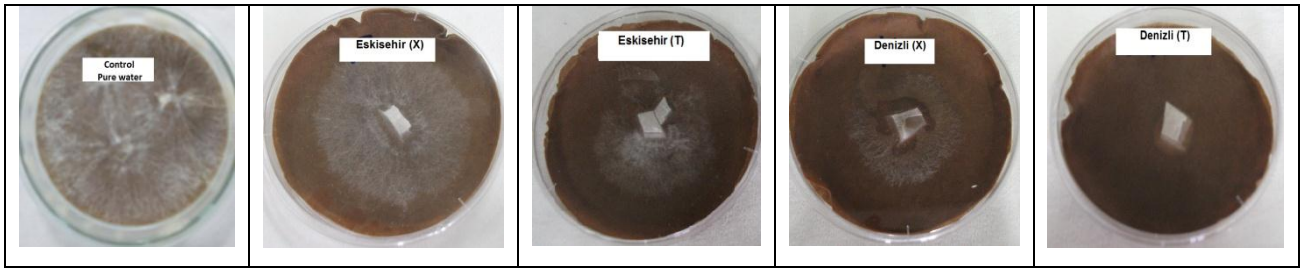

Fig. 1. Mycelial growth on the surfaces of disks of kraft paper treated with different GFs

Antifungal Assay on Wood Samples

Scots pine sapwood (*Pinus sylvestris* L.) was obtained from a natural plantation in the Duzce region. The wood samples were selected according to TS 2476. The decay tests were investigated according to European standard (1996) EN 113, based on the resistance against fungal decay by brown- and white-rot fungi. Scots pine sapwoods were cut into 5×10×30 mm (radial × tangential × longitudinal) blocks (Bravery 1978). Prior to the treatments, all wood specimens were conditioned at 20°C and 65% RH for 2 weeks. And the samples were dried at 60°C for 24 h and weighed to the nearest 0.01 g to determine the initial weight.

The wood samples were treated with GFs at different concentrations at room temperature. A vacuum treatment was used for impregnation. The specimens were submerged in treatment solution, applying a 6 kPa vacuum in a vacuum desiccator for 20 min. The treated wood blocks were immediately weighed to determine gross solution uptake. An air drying process was applied after the treatment. The retentions of treated specimens were calculated based on the following formula which takes changes in weight before and after the treatments and total mineral concentration of GF.

$$R = \frac{(M_1 - M_0) \times C}{V} \text{ g/cm}^3 \quad (1)$$

In this equation, M_0 is the specimen weight before treatment (g), M_1 is the specimen weight after treatment (g), C is the mineral concentration of GF, and V is the volume of wood blocks (cm^3).

The blocks were sterilized by autoclaving at 100±2°C for 20 min. Petri dishes with potato dextrose agar (4% for 500 mL jars) were inoculated with a mycelium agar disk taken from sub-margin old cultures of *C. puteana* and *T. versicolor* when the mycelia reached the edge of the Petri dish. The wood blocks were placed in the Petri dish medium under laminar airflow conditions. The Petri dishes were incubated for 16 weeks at 22±1°C and 75±5% RH to evaluate the efficacy of the treatments. After incubation, the wood blocks were removed, conditioned, and dried at 60°C for 24 h to determine the mass loss of wood blocks.

The percent mass loss (ML) was calculated as follows,

$$\text{Mass loss (\%)} = \frac{(M_1 - M_2)}{M_1} \times 100 \quad (2)$$

where M_1 represents the weight of specimens before the fungal test (g) and M_2 is the weight of specimens after the fungal test (g).

Chemical Analysis

Wood impregnating compounds in GFs were analyzed in the TUBITAK Marmara Research Center (MRC) in Turkey. The methods and equipment are shown in Table 2.

Table 2. GF Analysis Methods and Equipment

Elements	Analysis Method	Equipment
Arsenic, Copper, Sodium, Potassium	EPA 6020 A	ICP-MS
Chloride, Fluoride, Sulfate	SM 4500 B	Ion chromatography
Boron	SM 4500 C	Spectrophotometer
Ammonia	SM 4500 NH_3B	Distillation + titration

SM: Standard methods for the examination of water and wastewater, 21st edition (2005)
EPA: Environmental Protection Agency

Statistical Analysis

All results were evaluated using SPSS software (SPSS 19 2010). One-way analysis of variance (ANOVA) was used to evaluate changes in measured mass loss. Duncan's test was used to rank the average values of mass loss.

RESULTS AND DISCUSSION

Chemical Compositions of GFs

The concentrations of the chemical constituents of all GFs are shown in Table 3. There were nine minerals identified. The highest concentrations of boron (16.54 mg/L) and arsenic (0.699 mg/L) were found in the GF from the Denizli region, and this GF had the greatest antifungal activity. On the other hand, Ankara, Afyon, and Eskisehir GFs had much lower concentrations of most chemical constituents.

Table 3. Concentration of Chemical Elements for all Experimental GFs

Chemical Elements (mg/L)	Geothermal Fluids			
	Ankara	Afyon	Eskisehir	Denizli
Boron (B)	8.28	4.94	< 0.16	16.54
Arsenic (As)	0.531	0.141	0.021	0.699
Copper (Cu)	0.023	0.02	0.009	0.013
Sulfate (SO_4)	93.78	40.29	12.83	1033
Sodium (Na)	690.9	496.8	16.05	951.4
Chloride (Cl)	206.4	100.1	6.43	97.79
Fluoride (F)	2.36	1.51	0.2	12.25
Potassium (K)	57.72	34.03	1.661	101.7
Ammonia (NH_3)	< 0.2	< 0.2	< 0.2	< 0.2

Antifungal properties of GFs are thought to be associated with the type and amount of mineral substances. The lower mass loss found in Scots pine wood samples treated with Denizli GF may be correlated with the antifungal properties of its boron (16.54 mg/L), arsenic (0.699 mg/L), and sodium (951.4 mg/L) constituents.

Retentions

Mean total mineral retentions (g/cm^3) of treated wood blocks were calculated based on solution uptake and mineral concentration of GF (Table 4).

Table 4. Mean Total Mineral Retentions (g/cm^3) in Treated Wood Blocks (mean of six replicates; values in parentheses are standard deviations)

Samples	Evaporation Rate	Mean Total Mineral Retention (g/cm^3)
Afyon	0% (X)	0.02 (0.01) ***a***
	25% (Y)	0.07 (0.04) ***a***
	50% (Z)	0.15 (0.01) ***ab***
	75% (T)	0.27 (0.02) ***abc***
Denizli	0% (X)	0.37 (0.03) ***bc***
	25% (Y)	0.48 (0.03) ***c***
	50% (Z)	0.74 (0.05) ***d***
	75% (T)	1.6 (0.06) ***f***
Eskisehir	0% (X)	0.02 (0.00) ***a***
	25% (Y)	0.03 (0.00) ***a***
	50% (Z)	0.04 (0.00) ***a***
	75% (T)	0.08 (0.00) ***a***
Ankara	0% (X)	1.24 (0.08) ***e***
	25% (Y)	1.52 (0.1) ***f***
	50% (Z)	2.2 (0.16) ***g***
	75% (T)	4.42 (0.73) ***h***

The range of recorded retention values was between 0.02 and 4.42 g/cm^3. While total mineral retention was the highest in GF from the Ankara region, the most effective GF antifungal was from the Denizli region. This could be explained by calculation method of retention which takes into account the total mineral retention instead of effective mineral concentration.

Antifungal Effects of GFs on Kraft Paper

Table 5 and Figs. 1 and 2 show the results of the antifungal assay of the brown-rot *C. puteana* and white-rot *T. versicolor* in the presence of different GF concentrations from the four sites in Turkey. Control samples treated with distilled water showed the greatest mycelia growth for both fungi. It took both fungi 7 days to reach the edge of control dishes. As clearly seen in Table 4, the GF from the Denizli region had the highest antifungal activity, followed by that from Afyon, Ankara, and Eskisehir, in descending order. Non-concentrated GF (X)-treated *C. puteana* or *T. versicolor* did not show significant differences from those treated with pure water samples.

Table 5. Antifungal Effects of GFs towards the Wood-Decaying Fungi *T. versicolor* and *C. puteana* on Paper Disks

Samples		1 week		2 weeks		3 weeks	
		C. puteana	*T. versicolor*	*C. puteana*	*T. versicolor*	*C. puteana*	*T. versicolor*
Control	Distilled water	++++	++++	++++	++++	++++	++++
Afyon	Natural (X)	++	+++	+++	++++	+++	++++
	25% (Y)	+	++	++	++	++	+++
	50% (Z)	-	+	+	++	+	+++
	75% (T)	-	-	-	+	-	+
Denizli	Natural (X)	+	++	++	+++	++	+++
	25% (Y)	+	+	+	+	+	++
	50% (Z)	-	-	-	+	-	+
	75% (T)	-	-	-	-	-	-
Eskisehir	Natural (X)	+++	+++	++++	++++	++++	++++
	25% (Y)	++	+++	+++	++++	++++	++++
	50% (Z)	++	++	+++	+++	+++	+++
	75% (T)	+	+	+	++	++	+++
Ankara	Natural (X)	++	+++	+++	++++	+++	++++
	25% (Y)	++	++	++	+++	++	+++
	50% (Z)	+	+	+	++	+	+++
	75% (T)	-	-	-	-	-	+

– No visible growth of mycelia. + Mycelia covering about 25% of the Petri dish, ++ about 50%, +++ about 75%, ++++ about 100%; X: naturally, Y: 25%, Z: 50%, and T:75% show the GF concentration levels (AWPA, 2006; TS ENV 839, 2006; Yang and Clausen, 2007; Sen and Yalcin, 2010)

The paper disks treated with 75% GF (T) showed reduced mycelial growth compared to those treated with 50% (Z), 25% (Y), non-evaporated GF (X), and distilled water after incubation with both fungi.

In samples impregnated with low concentrations of GFs and with distilled water, mycelial growth started on the first day. Mycelial distribution occurred rapidly, especially for control samples, and reached the edge of the Petri plate by the end of the first week.

In contrast, mycelial growth for high-concentration GFs was not clearly observed until after the second week.

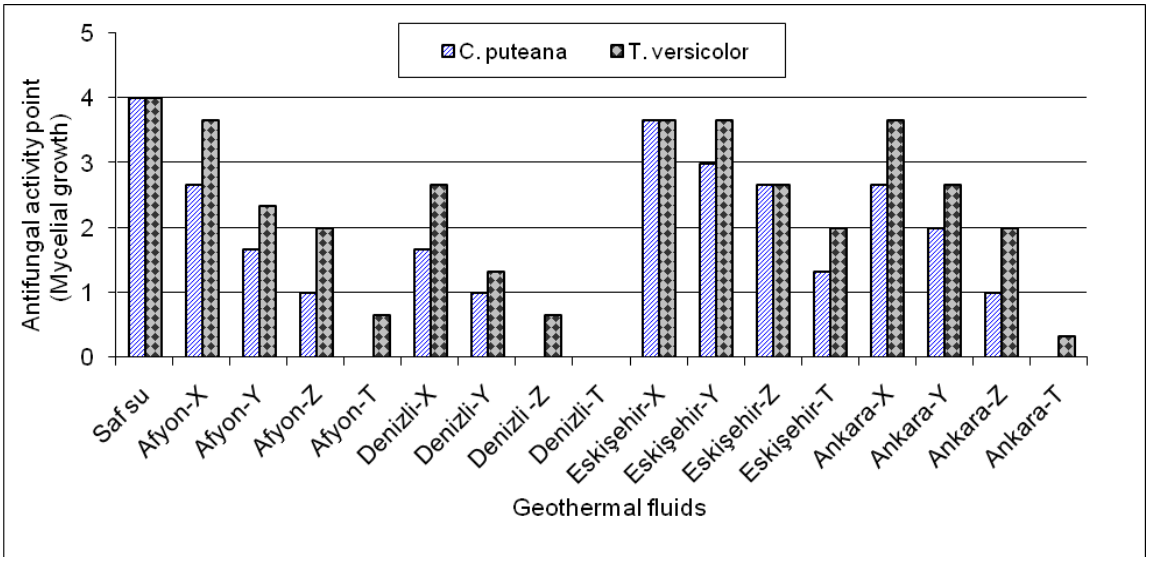

Fig. 2. Antifungal effects of GFs prepared at different concentrations against *C. puteana* and *T. versicolor* on paper disks (the mean values over 3 weeks)

Table 6. Mean Mass Loss of Scots Pine Wood Samples Caused by *C. puteana* and *T. versicolor* after a 16-Week Incubation (mean of five replicates, values in parentheses are standard deviations)

		C. puteana		*T. versicolor*	
Samples	**Evaporation Rate**	**Mass Loss (%)**		**Mass Loss (%)**	
Control	Pure water	43.2 (4.8)	***i*** [1]	37.7 (4.7)	***g***
Afyon	Natural (X)	29.7 (5.1)	***fg***	31.6 (4.5)	***efg***
	25% (Y)	25.2 (3.5)	***def***	30.3 (1.8)	***efg***
	50% (Z)	19.6 (3.5)	***cde***	30.1 (9.2)	***efg***
	75% (T)	7.4 (2.6)	***ab***	27.8 (5.7)	***efg***
Denizli	Natural (X)	16.2 (3.4)	***bcd***	18.2 (2.3)	***cd***
	25% (Y)	11.5 (3.9)	***bc***	8.0 (2.5)	***ab***
	50% (Z)	1.2 (0.8)	***a***	3.6 (1.4)	***ab***
	75% (T)	0.5 (0.4)	***a***	1.0 (0.1)	***a***
Eskisehir	Natural (X)	41.3 (1.5)	***hı***	23.4 (5.6)	***de***
	25% (Y)	41.7 (8.2)	***hı***	23.8 (3.9)	***de***
	50% (Z)	37.8 (3.6)	***ghı***	22.9 (1.0)	***de***
	75% (T)	36.8 (3.2)	***ghı***	12.3 (4.3)	***bc***
Ankara	Natural (X)	36.8 (7.4)	***ghı***	34.4 (2.5)	***fg***
	25% (Y)	32.2 (9.2)	***fgh***	34.9 (8.6)	***fg***
	50% (Z)	26.5 (5.1)	***ef***	26.2 (2.8)	***def***
	75% (T)	11.9 (0.8)	***bc***	11.0 (3.2)	***bc***

[1]Means within each column and factor followed by the same letter are not significantly different ($p<0.05$, Duncan's test).

Antifungal Effects of GFs on Wood Samples

Table 6 and Fig. 3 demonstrate the mass loss rating of Scots pine wood treated with GFs at different concentrations. The mean mass losses of control samples treated with distilled water were 43.22% and 37.70% from *C. puteana* and *T. versicolor*, respectively. *C. puteana* caused more mass loss than *T. versicolor*. These differences between fungi species may be due to differences in their decay mechanisms (Nemli 2006).

Statistical analyses showed that the effect of GF and its concentration levels on mass loss were significant at the 5% level. Increasing the GF concentration levels significantly decreased the mass loss of both fungi, as expected.

The Denizli GF showed the highest antifungal activity against *C. puteana*. The mean mass losses of Denizli GF were 16.17%, 11.48%, 1.17%, and 0.53% for treated Scots pine wood samples impregnated with natural GF or 25%, 50%, and 75% evaporated GFs, respectively. However, there were no significant differences between the mass losses of 50% and 75% evaporated GFs ($p>0.05$). Non-evaporated Denizli GF had the highest antifungal effects against *C. puteana*. The antifungal effects of Denizli GF at the 75% evaporation level increased to 98%, and that of Afyon, Ankara, and Eskişehir GF at the same evaporation level were 82%, 72%, and 15%, respectively (Table 6 and Fig. 3).

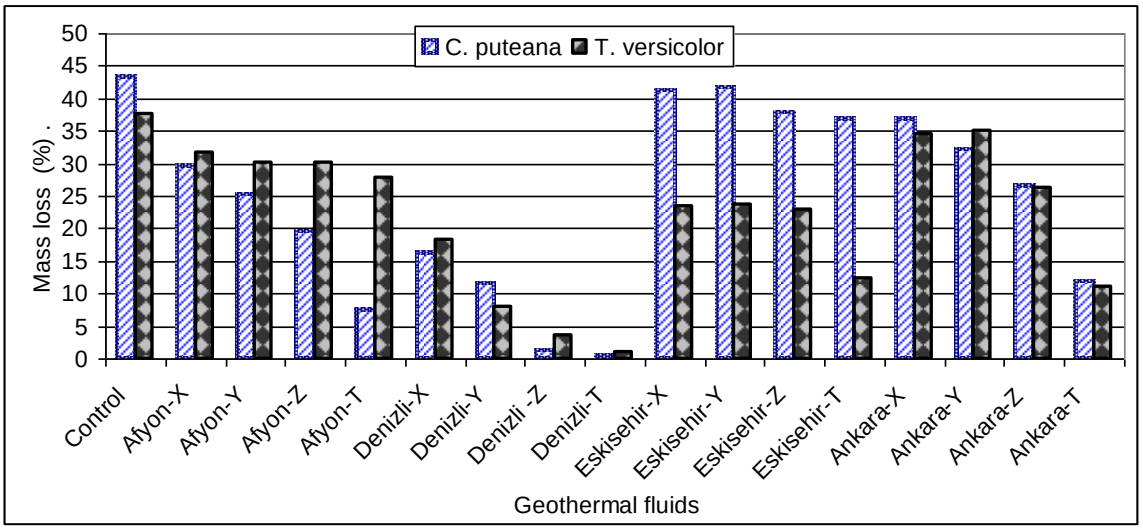

Fig. 3. Antifungal effects of GFs prepared at different concentrations against *C. puteana* and *T. versicolor* on wood blocks

The mass loss of Denizli GF against *T. versicolor* at non-concentrated, 25%, 50%, and 75% evaporation levels were 18.24%, 8.03%, 3.60%, and 1.01%, respectively, while that of Ankara and Eskişehir GF against *T. versicolor* at 75% evaporation levels were 11.03% and 12.32%, respectively. There were no significant differences between the mass losses of 25%, 50%, and 75% evaporated GFs ($p>0.05$). Ankara and Afyon GFs had the lowest antifungal effects against *T. versicolor* (Table 6 and Fig. 3).

Fig. 4. Effects of brown-rot (*C. puteana*) and white-rot (*T. versicolor*) on Scots pine treated with GFs at different concentrations (1: Afyon, 2: Denizli, 3: Eskisehir, 4: Ankara; X: non-concentrated and Y: 75% show the GF evaporation levels)

Figure 4 shows representative test specimens from each treatment for the observation of *C. puteana* and *T. versicolor* fungal decay. As shown in the figures, controls and some treated blocks (especially those treated with non-concentrated [X] GF, excluding Denizli GF) experienced high fungal damage. Although brown-rot fungi caused visual destruction of the wood, the destruction caused by white-rot was not visible externally. In addition, while the brown-rot fungus resulted in deep perpendicular cracks in the wood, the white-rot fungus induced a slight collapse along with spring wood zones, which easily crumbled.

CONCLUSIONS

1. The concentrations of chemical constituents of GFs increased after evaporation and had high antifungal activity on both kraft paper and wood samples.
2. The geothermal water obtained from different regions of Turkey had different antifungal activity depending on the wood-decaying fungi, probably because the mineral and salt concentrations differed between the GFs. That obtained from the Denizli region significantly reduced fungal growth compared to the other GFs.

3. Environmental and health concerns are increasing globally. The development of environmentally-friendly antifungal substances for wood preservation, especially for impregnation of indoor wood materials, is increasingly important. To this end, substances such as geothermal water, which have no adverse effects on the environment or on humans and do not decrease the strength and other physical properties of wood, could be used.

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