

BIOREMEDIATION OF WOOD TREATED WITH CCA-C, ACQ, AND MCQ WOOD PRESERVATIVES BY *PHANEROCHAETE CHRYSOSPORIUM* FUNGAL STRAINS

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(RECEIVED AUGUST 2026)

ABSTRACT

This study investigated the bioremediation of wood treated with copper–chromium–arsenic (CCA-C), alkaline copper quat (ACQ), and micronized copper quat (MCQ) using two *Phanerochaete chrysosporium* fungal strains. Wood flour samples were incubated in fungal liquid cultures over 1 to 10 days. Fungal treatments removed substantially more copper than distilled water controls across all preservative types. Lower preservative loadings yielded copper extraction rates exceeding 90–95% for ACQ and MCQ, whereas higher loadings reduced efficiency. The *P. chrysosporium* Me strain demonstrated superior performance during later remediation stages. These findings confirm that *P. chrysosporium* effectively mobilizes copper from soluble and micronized formulations, offering a viable strategy for detoxifying waste wood and enabling resource recovery.

KEYWORDS: Micronized Cu, bioremediation, wood preservatives, *Phanerochaete chrysosporium*.

INTRODUCTION

Rising volumes of copper-treated waste wood pose major environmental and management challenges due to high metal content and limited recyclability. Bioremediation of wood treated with ACQ, micronized ACQ, nano-CuO, and CCA wood preservatives represent promising approaches for metal recovery and the production of decontaminated wood. Although chemical and biological remediation methods have been extensively studied and shown to be effective, there remains a need for cost-effective, easily managed strategies to reduce the volume of preservative-treated waste wood. Existing remediation approaches using chemical and biological techniques have been comprehensively reviewed by Kartal and Imamura (2003) and Clausen and Lebow (2011). Over the past decade, new copper-based preservative systems free of chromium and arsenic, such as ACQ, copper azole, copper citrate, and copper ethanolamine, have been introduced, offering reduced environmental impacts compared with traditional

heavy-metal preservatives. More recently, micronized copper systems, as well as nano-sized CuO and ZnO, have been evaluated as alternative preservative components (Kartal et al. 2009; Clausen et al. 2010,2011). These micronized and nanoparticulate copper formulations exhibit distinct chemical, physical, fixation, and distribution behaviours relative to water-soluble copper systems, suggesting that their remediation mechanisms may also differ (Kartal et al. 2013,2014).

Kartal et al. (2015) states that microorganisms, particularly fungi and bacteria, play a critical role in the natural remediation of treated waste wood, as metals can be mobilized through extracellular enzymatic activity and non-enzymatic oxidative processes (Kartal and Imamura 2003; Clausen and Lebow 2011). Previous studies have shown that oxalic acid produced by various fungi contributes significantly to the solubilization of metals in CCA-treated wood, with secretion levels dependent on environmental conditions (Kartal et al. 2004a). Furthermore, fungal copper tolerance has been identified as a key factor influencing the efficiency of bioprocessing copper-treated waste wood (Woodward and De Groot 1999). White-rot fungi are members of the Basidiomycetes group that possess the unique ability to extensively degrade lignin, a heterogeneous polyphenolic polymer within lignocellulosic structures. The selective or simultaneous removal of lignin from wood results in a characteristic whitish appearance of the substrate, from which the term “white rot” is derived. These fungi accomplish lignin depolymerization and subsequent wood biodegradation through the secretion of three major types of extracellular enzymes, collectively referred to as lignin-modifying enzymes. These enzymes include lignin peroxidase (LiP), manganese peroxidase (MnP), and laccase (Lac). Beyond their role in wood decay, lignin-modifying enzymes produced by white-rot fungi are of considerable importance in bioremediation applications due to their broad substrate specificity and strong oxidative capacity. These enzymatic systems are capable of degrading or mineralizing a wide range of recalcitrant organic pollutants, including pesticides, polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), dioxins and other halogenated aromatic compounds, various synthetic dyes, 2,4,6-trinitrotoluene (TNT) and other nitroaromatic explosives, as well as toxic inorganic and organochlorine compounds such as cyanide, azide, carbon tetrachloride, and pentachlorophenol (PCP). Consequently, white-rot fungi have attracted significant attention as effective biological agents for the degradation and bioremediation of environmentally persistent contaminants (Gao et al. 2010).

In recent years, *Phanerochaete chrysosporium* has been extensively investigated for its ability to degrade a wide range of environmental contaminants, including benzo(a)pyrene, pentachlorophenol (PCP), trichloroethylene, and various synthetic dyes. While the extracellular enzymatic systems described above play a central role in bioremediation processes, the fungal mycelium itself also contributes significantly through the adsorption of contaminant molecules. Given that heavy metals such as copper (Cu) and chromium (Cr) are commonly present in industrial wastewaters, the capacity of living fungal mycelia to adsorb these metals, as well as the potential for their transformation or mobilization by enzymes and other extracellular metabolites (e.g., organic acids) secreted by white-rot fungi, has become an important area of research (Sing and Yu 1998). *P. chrysosporium* is an aerobic, heterotrophic fungus that, in addition to producing lignin-degrading enzymes, is capable of secreting various low-molecular-weight organic acids, including oxalic, gluconic, and citric acids (Liu et al.

2020). The combined action of its oxidative enzymatic machinery, organic acid production, and strong adsorption capacity has long been recognized as conferring significant potential for the remediation of metal-contaminated agricultural residues, as well as wastewaters containing heavy metals and toxic organic pollutants (Zhao et al. 2016). Despite the extensive use of white-rot fungi in bioremediation applications, studies specifically addressing the recovery of heavy metals from lignocellulosic substrates such as wood using *P. chrysosporium* remain limited. Ilmann et al. (2000) evaluated the tolerance levels of 11 different *P. chrysosporium* strains toward wood samples impregnated with CCA, ACQ, PCP, and creosote, and assessed weight losses in these treated wood specimens through laboratory decay tests. However, no established bioremediation protocol was applied, and metal recovery was not investigated. More recently, Pandharikar et al. (2022) examined copper resistance in *P. chrysosporium* using wood specimens treated with copper azole preservatives, focusing primarily on fungal tolerance rather than metal remediation or recovery.

In this study, bioremediation processes, recognized as biotechnological approaches, was employed using the white-rot fungus *P. chrysosporium*. Impregnated wood specimens were exposed to fungal activity in order to investigate the recovery of elements such as Cu, Cr, and As, which have constituted copper-based wood preservatives. Both soluble copper-treated and micronized copper-treated wood samples were examined. The residual elemental concentrations in wood specimens subjected to biological remediation were quantitatively determined. The objective of the study included enhanced penetration and deeper migration of process fluids (liquid fungal cultures) into impregnated wood during biological remediation, thereby increasing the recovery efficiency of preservative elements.

MATERIAL AND METHODS

Sapwood specimens of Scots pine (*Pinus sylvestris* L.) with dimensions of 20 × 20 × 10 mm were prepared to ensure the absence of knots, checks, resin pockets, discoloration, and visible fungal or insect damage. In addition, growth ring characteristics were strictly controlled: transverse sections were selected to contain 2.5–8 annual rings per 10 mm, and the proportion of latewood within each annual ring did not exceed 30% (EN 113) (BS EN 1997).

The blocks were conditioned to a moisture content of 10–12% in a conditioning room at 22°C and 65% relative humidity (RH) for 2 weeks before preservative treatment. Prepared wood specimens were impregnated with copper–chromium–arsenic type C (CCA-C), alkaline copper quat (ACQ), and micronized copper quat (MCQ) wood preservatives (Tab. 1) at target retention levels of 0.7 and 7 kg m⁻³ using a vacuum impregnation method. For each preservative system, 360 wood specimens were treated at each retention level (i.e., 360 specimens at 0.7 kg m⁻³ and 360 specimens at 7 kg m⁻³). Groups of wood blocks were vacuum-treated for 40 min at 100 mm Hg with each treatment according to the AWP standard E10 method (AWPA 2012a). After treatment, the blocks were blotted-dry and re-weighed to determine preservative uptake. The treated blocks were conditioned for 2 weeks at room temperature to ensure that fixation was complete and then equilibrated to stable weight at 22°C and 65% RH for 2 weeks before weighing again (AWPA E10, AWPA 2012).

Tab. 1: Properties of wood preservatives used in the study.

Preservative	Commercial name	Producer	Composition (%) (m/m)
CCA-C	Osmose K-33	Osmose UK Protim Solignum Ltd	Copper oxide: 10.5%; Chromic acid: 29.9%; Arsenic penta oxide: 20%; water: 39.6%
ACQ	Osmose Celcure AC-500	Osmose UK Protim Solignum Ltd	Quat (benzalkonium chloride): 4.8%; Copper carbonate hydroxide: 16.53%; Boric acid: 5%
MCQ	Osmose Micro Pro (Celcure MC)	Osmose UK Protim Solignum Ltd	Quat (benzalkonium chloride): 10%; Micronized copper carbonate hydroxide: 17.39%; Boric acid: 5.23%

The impregnated and conditioned specimens were first reduced to matchstick-sized pieces and subsequently ground in a ball mill to produce wood flour with a particle size of 40 mesh. Two strains of *Phanerochaete chrysosporium* (ME 446 and SF-4), used during the bioremediation stage of the project, were obtained from the Fungal Culture Collection of the Department of Forest Biology and Wood Protection Technology, Department of Forest Industrial Engineering, Istanbul University, Cerrahpaşa Faculty of Forestry. The fungal strains were initially propagated on sterile Petri dishes containing 2% malt extract agar (MEA) and incubated in a climate-controlled chamber at 27°C and 65% relative humidity. Liquid culture media were prepared using peptone, glucose, and malt extract (40 g glucose, 3 g peptone, and 15 g malt extract per 1000 mL distilled water). Each *P. chrysosporium* strain was inoculated separately into the liquid medium and allowed to grow for 10 days to promote mycelial development. Subsequently, the mycelial biomass was homogenized under sterile conditions using a mechanical homogenizer to obtain a liquid bioremediation medium with a uniform mycelial distribution. Prior to treatment, samples were collected from the liquid culture medium for pH analysis. The prepared wood flour samples were then placed into polyester tea bags. The bags containing wood flour were immersed in the liquid fungal cultures and incubated for 1, 2, 5, and 10 days in a temperature-controlled shaking incubator (120 rpm, 27°C) to carry out liquid-phase bioremediation. For comparison, identical remediation procedures were conducted using distilled water. During process, pH values were measured in liquid fungal cultures before and after the remediation process. In liquid bioremediation systems, pH measurements were performed using a conventional pH meter probe.

Elemental analysis for treated sawdust was performed following extraction according to AWWA A21-08 (AWWA 2012), using microwave digestion procedures described in AWWA A7-12 (AWWA 2012). The resulting extraction solutions after microwave digestion of sawdust samples before and after remediation processes were analysed to determine Cu, Cr, and As concentrations. An inductively coupled plasma optical emission spectrometer (ICP-OES; PerkinElmer Optima 7000 DV) was used for elemental analysis. Analyses were conducted at a plasma gas flow rate of 15 L min⁻¹, a carrier gas flow rate of 0.2 L min⁻¹, and a plasma power of 1300 W. Each sample was prepared in triplicate, and three replicate measurements were performed; mean values were used to calculate elemental concentrations expressed as percentage composition. Following laboratory analyses, time-dependent Cu, Cr, and As removal efficiencies were calculated in percentage.

RESULTS AND DISCUSSION

The percentage removal of copper (Cu) from treated wood as a function of preservative formulation, concentration, and exposure time (1, 2, 5, and 10 days) is summarized in Fig. 1. Fig. 2 illustrates total Cu removal rates from the samples after 10-day-remediation process. Overall, Cu removal was strongly influenced by (i) the type of preservative (CCA, ACQ, MCQ), (ii) preservative concentration (0.1% vs. 1%), (iii) remediation agent (distilled water, *Phanerochaete chrysosporium* Sf and Me strains), and (iv) remediation duration.

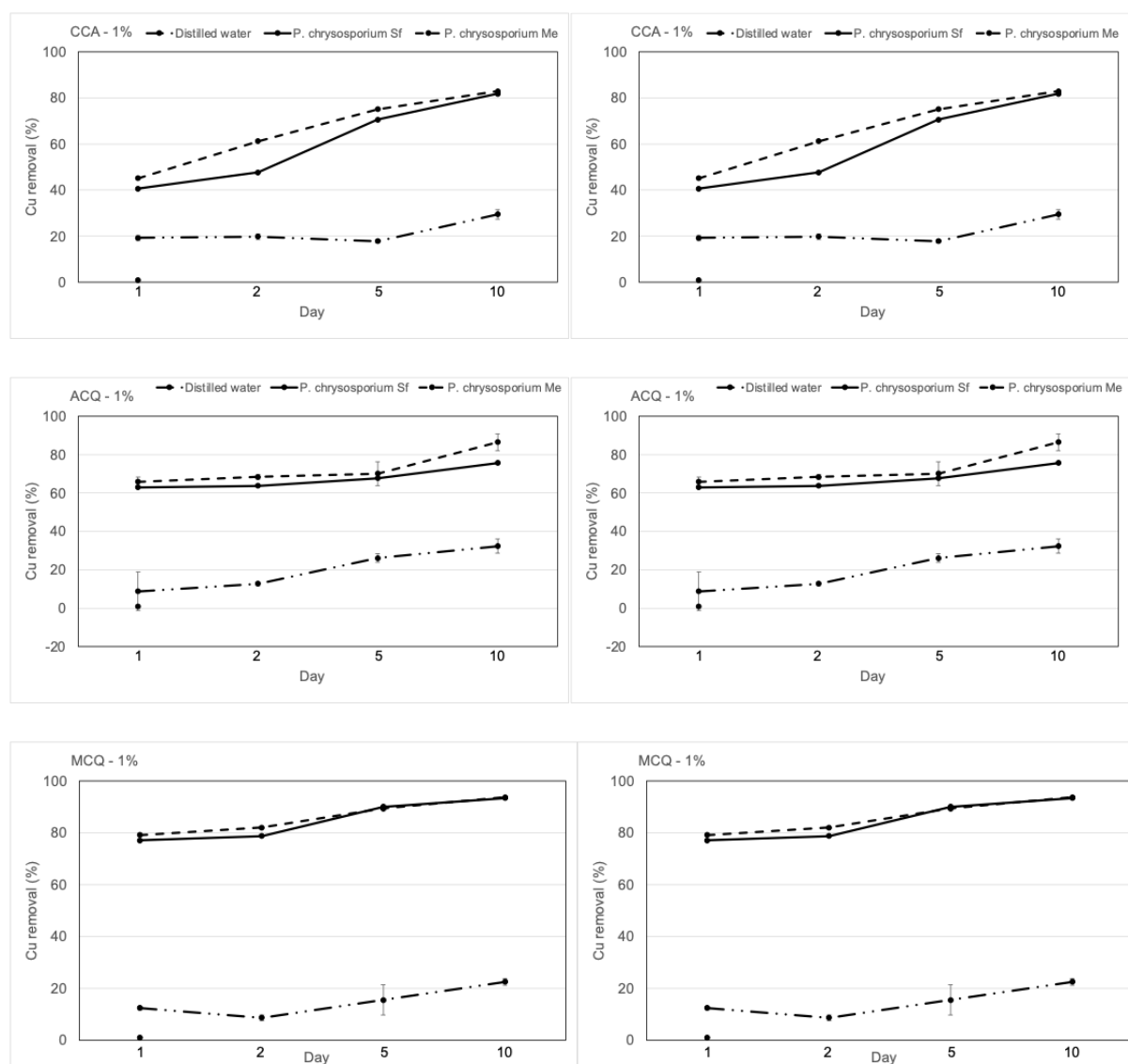


Fig. 1: Cu removal rates in bioremediation processes in CCA, ACQ and MCQ-treated wood ($n: 3$).

For CCA-treated wood at 0.1% concentration, distilled water resulted in limited Cu removal throughout the remediation period, with average values decreasing from approximately 27% at day 1–2 to about 18% by day 10. In contrast, fungal remediation markedly enhanced Cu removal. The *P. chrysosporium* Sf strain showed a clear time-dependent increase, achieving over 78% removal at day 1 and reaching approximately 93% by day 10.

The Me strain followed a similar trend, starting at about 68% on day 1 and increasing steadily to nearly 95% at day 10. These results indicate that both fungal strains were highly effective in mobilizing Cu from low-concentration CCA-treated wood, with slightly higher final removal efficiencies observed for the Sf strain. At the higher CCA concentration (1%), overall Cu removal efficiencies were reduced compared with 0.1% CCA. Distilled water again showed poor performance, with Cu removal remaining below 30% even after 10 days. Fungal treatments significantly improved removal, although absolute values were lower than those observed for 0.1% CCA. The Sf strain increased Cu removal from approximately 41% at day 1 to about 82% at day 10, while the Me strain achieved a comparable increase from roughly 45% to 83% over the same period. These findings suggest that increasing preservative loading limits Cu extractability, yet fungal remediation remains substantially more effective than abiotic leaching.

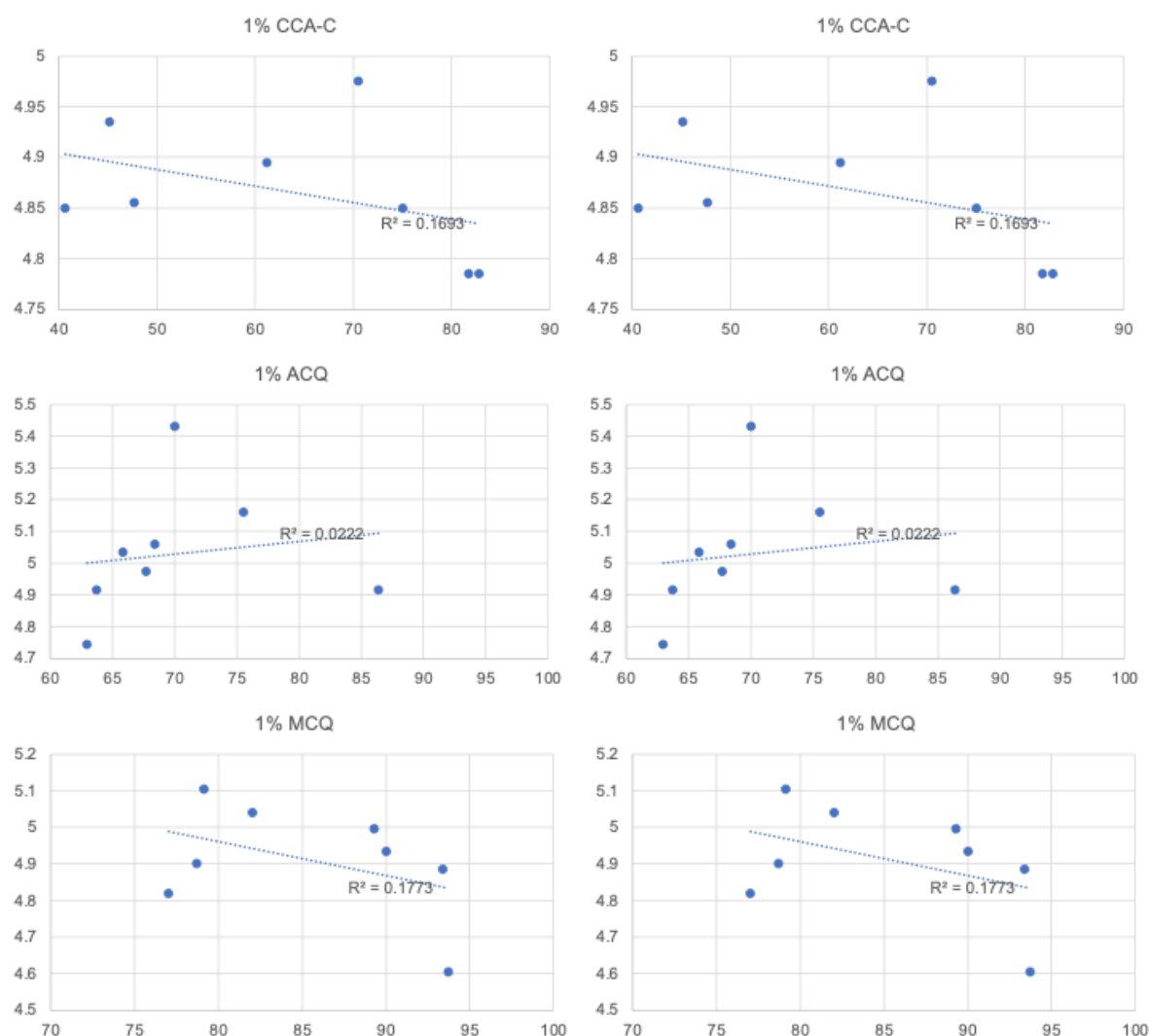


Fig. 2: Correlation between pH (y-axis) of remediated wood and Cu removal (%) (x-axis) from treated wood.

For ACQ-treated wood at 0.1% concentration, distilled water removed between 27–28% Cu during the first two days, with a slight decline to around 21% by day 10. In contrast, both fungal strains exhibited high and progressively increasing removal efficiencies. The Sf strain achieved approximately 84% removal at day 1, increasing to about 94% by day 10. Similarly, the Me strain showed strong performance, with Cu removal rising from nearly 79% at day 1 to about 98% by day 10, representing the highest Cu removal observed across all treatments and conditions. At 1% ACQ concentration, distilled water again resulted in minimal Cu removal, although a gradual increase with time was observed, reaching just over 30% by day 10. Fungal remediation significantly enhanced Cu removal, but efficiencies were lower than those at 0.1% ACQ. The Sf strain increased Cu removal from approximately 63% at day 1 to about 76% at day 10, while the Me strain improved removal from around 66% to nearly 87% over the same period. The consistently higher performance of the Me strain at longer exposure times indicates a strong capacity for Cu mobilization even at elevated ACQ concentrations.

In MCQ-treated wood at 0.1% concentration, distilled water achieved Cu removal values between 24–26% during the first two days, decreasing to approximately 20% by day 10. Fungal treatments again resulted in substantially higher Cu removal. The Sf strain exhibited a steady increase from about 82% at day 1 to nearly 96% at day 10, while the Me strain increased from approximately 80% to about 96% over the same period. Both strains performed comparably, indicating high effectiveness of fungal remediation for MCQ at low preservative concentration. At 1% MCQ concentration, distilled water showed the lowest Cu removal among all systems, remaining below 23% even after 10 days. In contrast, fungal remediation was highly effective. The Sf strain increased Cu removal from about 77% at day 1 to over 93% at day 10, whereas the Me strain demonstrated the highest efficiency, increasing from approximately 79% to nearly 94% by day 10. These results indicate that, among the preservative systems tested, MCQ-treated wood remained highly amenable to fungal Cu removal even at elevated preservative concentration.

Across all preservative types and concentrations, fungal remediation consistently outperformed distilled water, confirming the limited role of passive leaching in Cu removal. In general, Cu removal increased with remediation time, particularly for fungal treatments, indicating progressive Cu mobilization and/or bioaccumulation. Lower preservative concentration (0.1%) resulted in higher and faster Cu removal than 1% treatments, regardless of preservative formulation. Among preservatives, ACQ and MCQ at 0.1% showed the highest overall Cu removal efficiencies, particularly when treated with *P. chrysosporium* Me, which frequently achieved removal rates exceeding 95% by day 10. Standard deviation values were generally low for fungal treatments, especially at later time points, indicating good reproducibility and stability of the remediation process. In contrast, higher variability was occasionally observed for distilled water treatments, reflecting less controlled and less effective Cu release mechanisms. Overall, the results demonstrate that *P. chrysosporium*-based fungal remediation is a highly effective strategy for Cu removal from preservative-treated wood, with efficiency strongly dependent on preservative type, concentration, and exposure duration, and markedly superior to abiotic leaching with distilled water.

In a study conducted by Ilman et al. (2000), tolerance tests using the copper-containing preservatives CCA and ACQ revealed that the fungi *P. chrysosporium* (BKM-F1767) and

P. sordida (Kropp-36T2) did not exhibit tolerance to either preservative. In contrast, *P. chrysosporium* (F43G), *P. chrysosporium* (F65F), *P. chrysosporium* (O55B), *P. chrysosporium* (F66C), *P. omnivorum* (KKN-112-sp), and *P. omnivorum* (HHB-5969) were found to be tolerant to both preservatives. It was further determined that *P. chrysosporium* (P6G) exhibited tolerance only to CCA, whereas *Phanerochaete sordida* (FP-101975) showed tolerance only to ACQ. In the same study, fungal decay tests demonstrated that *Pycnoporus sanguineus* (RLG-10851) and *P. omnivorum* (HHB-5969-sp) caused a weight loss of 1.0% in ACQ-treated specimens and 0.2% in CCA-treated specimens. *P. omnivorum* (KKN-112) resulted in weight losses of 1.3% and 1.1% in ACQ- and CCA-treated wood, respectively. For *P. chrysosporium* (F65F) and *P. chrysosporium* (P6G), weight losses were reported as 1.6% and 0%, and 1.4% and 0% in ACQ- and CCA-treated samples, respectively. Pandharikar et al. (2022) also investigated the biological performance of copper azole-treated wood against *P. chrysosporium*. In their study, it was noted that copper-based formulations of wood preservatives are widely used in industry to protect wood materials from fungal deterioration; however, wood treated with these preservatives currently generates toxic waste that cannot be readily recycled. Although copper is highly effective as a fungicide against most fungi, certain species are capable of tolerating high metal concentrations. This was also observed for the white-rot fungus *P. chrysosporium*, which was able to grow efficiently on pine wood treated with Tanalith E-3474.

The results of the present study suggest that copper removal is primarily driven by fungal-mediated solubilization of Cu, followed by its translocation from the ground wood through hyphal transport. As previously discussed, oxalate produced by decay fungi functions both as an organic acid and as a chelating agent for Fe^{3+} and Cu^{2+} ions (Kartal et al. 2015). Although the precise mechanism governing Cu translocation in these organisms remains unclear, the transport of metal salts associated with oxalate-mediated solubilization in wood-decay fungi has been documented (Jellison et al. 1997). Jellison et al. (1997) further reported that fungal mycelia possess a strong capacity to absorb heavy metals from metal-containing growth media. The mobilization and release of metal ions are facilitated by the production of low-molecular-weight organic acids, such as oxalic and citric acids, which supply protons and metal-complexing ligands. In addition, extracellular immobilization of metal ions may occur through binding to fungal pigments, such as melanin (Kartal et al. 2015). Consequently, bioremediation of heavy metals may proceed through multiple pathways, including accumulation or biosorption by fungal mycelia, as well as chelation and precipitation mediated by secreted metabolites, including enzymes and organic acids. Clausen and Green (2003) emphasized that oxalic acid plays a central role in the colonization of copper-treated wood by copper-tolerant fungi and noted that the presence of copper may stimulate oxalic acid production. The findings of this study further indicate that copper solubilization and subsequent translocation, or sequestration by fungal-derived agents during wood degradation, may be critical processes contributing to the detoxification of certain copper-based preservative systems. Given that evidence for non-enzymatic degradation mechanisms involving chelators has been identified in at least three orders of Basidiomycota brown-rot fungi, namely the *Boletales*, *Gloeophyllales*, and *Polyporales*, it is plausible that mechanisms enabling

the detoxification of copper-based wood preservatives are widespread among fungal taxa (Kartal et al. 2015).

The copper removal efficiencies observed in this study can be attributed to multiple, synergistic mechanisms operating during fungal remediation. These include (i) secretion of low-molecular-weight organic acids (e.g., oxalic acid) that solubilize copper through chelation, (ii) oxidative modification of lignin and other wood polymers by lignin-modifying enzymes (LiP, MnP, and laccase), which disrupt preservative–wood complexes, and (iii) adsorption and accumulation of mobilized copper onto fungal hyphae and mycelial biomass. These mechanisms are well documented for white-rot fungi and have been shown to facilitate the mobilization of both organic pollutants and inorganic metals from complex matrices (Kartal and Imamura, 2003). The present findings confirm that these processes are equally effective for modern copper-based wood preservatives, including micronized formulations.

The initial pH of preservative-treated wood sawdust was acidic across all treatments (pH 4.31–5.38) (Tab. 2). CCA-treated sawdust exhibited the lowest pH values, reflecting the acidic nature of chromium-based fixation systems, whereas ACQ-treated samples showed higher initial pH, particularly at higher concentrations, consistent with amine-based copper formulations. MCQ-treated sawdust displayed intermediate pH values, indicating mildly acidic conditions despite the presence of micronized copper. These differences define distinct chemical environments likely to influence fungal activity and metal mobilization during bioremediation.

Tab. 2: pH of treated sawdust before bioremediation.

Sawdust	pH
CCA (0.1%)	4.31
CCA (1%)	4.37
ACQ (0.1%)	4.55
ACQ (1%)	5.38
MCQ (0.1%)	4.41
MCQ (1%)	4.83

Both *P. chrysosporium* strains maintained moderately acidic to near-neutral pH values, with strain-dependent trends observed (Tab. 3). The Sf strain generally exhibited more stable pH profiles, whereas the Me strain showed gradual acidification in the solid phase, particularly at lower preservative concentrations. At higher preservative loadings (1%), pH fluctuations were reduced for all treatments, indicating increased buffering capacity. Initial pH values of distilled water, growth media, and fungal liquid cultures were comparable (pH $\approx$ 5.1), ensuring consistent starting conditions. In liquid-phase systems, distilled water generally showed gradual pH increases over time. Fungal treatments exhibited distinct strain-specific responses, with the Sf strain maintaining near-neutral pH, while the Me strain caused pronounced alkalization in some systems, particularly at later stages of remediation. This effect was most evident at lower preservative concentrations and diminished at higher loadings, where liquid-phase pH remained near neutral.

Tab. 3: *ph values of treated wood sawdust after remediation.*

Preservatives	Remediation method	1 Day	2 Days	5 Days	10 Days
CCA (0.1%)	Distilled water	4.59 (0.16)	4.65 (0.13)	3.96 (0.12)	5.40 (0.35)
	<i>P. chrysosporium</i> Sf	5.17 (0.04)	5.33 (0.03)	5.27 (0.04)	5.28 (0.06)
	<i>P. chrysosporium</i> Me	5.27 (0.06)	5.22 (0.10)	5.04 (0.08)	4.87 (0.05)
CCA (1%)	Distilled water	5.00 (0.06)	4.88 (0.06)	4.99 (0.06)	4.87 (0.09)
	<i>P. chrysosporium</i> Sf	4.85 (0.01)	4.86 (0.02)	4.98 (0.02)	4.79 (0.15)
	<i>P. chrysosporium</i> Me	4.94 (0.09)	4.90 (0.04)	4.85 (0.04)	4.79 (0.01)
ACQ (0.1%)	Distilled water	4.80 (0.03)	4.83 (0.04)	3.74 (0.28)	5.34 (0.10)
	<i>P. chrysosporium</i> Sf	5.25 (0.08)	5.41 (0.17)	5.36 (0.08)	5.25 (0.08)
	<i>P. chrysosporium</i> Me	5.22 (0.03)	5.14 (0.02)	4.74 (0.06)	4.72 (0.06)
ACQ (1%)	Distilled water	5.46 (0.06)	5.30 (0.04)	5.33 (0.08)	5.55 (0.14)
	<i>P. chrysosporium</i> Sf	4.75 (0.22)	4.92 (0.02)	4.98 (0.02)	5.16 (0.16)
	<i>P. chrysosporium</i> Me	5.04 (0.05)	5.06 (0.00)	5.43 (0.68)	4.92 (0.04)
MCQ (0.1%)	Distilled water	4.72 (0.08)	4.80 (0.02)	4.07 (0.14)	5.17 (0.01)
	<i>P. chrysosporium</i> Sf	5.42 (0.01)	5.50 (0.06)	5.31 (0.11)	4.78 (0.40)
	<i>P. chrysosporium</i> Me	5.27 (0.01)	5.06 (0.08)	4.96 (0.06)	4.95 (0.02)
MCQ (1%)	Distilled water	5.14 (0.11)	5.26 (0.03)	5.28 (0.05)	5.43 (0.00)
	<i>P. chrysosporium</i> Sf	4.82 (0.23)	4.90 (0.03)	4.94 (0.04)	4.89 (0.11)
	<i>P. chrysosporium</i> Me	5.11 (0.01)	5.04 (0.01)	5.00 (0.01)	4.61 (0.15)

Overall, fungal remediation resulted in more controlled pH environments in both solid and liquid phases compared with distilled water. The observed pH stabilization suggests active buffering through fungal metabolism, organic acid production, metal complexation, and interactions with fungal biomass. Episodes of alkalization in liquid cultures, especially with the Me strain, indicate advanced metabolic activity and possible depletion or transformation of acidic metabolites during prolonged remediation. pH evolution emerged as a key indicator of fungal activity and remediation mechanisms. Prior to treatment, all preservative-treated wood samples exhibited acidic pH values, reflecting the inherent chemistry of copper-based preservatives. During remediation, fungal treatments produced more stable and biologically regulated pH environments than distilled water controls (Tabs. 4 and 5), which often showed abrupt and uncontrolled pH fluctuations. In solid wood sawdust, fungal remediation maintained pH values predominantly within the mildly acidic to near-neutral range (approximately pH 4.8–5.5), conditions known to favor ligninolytic enzyme activity and metal solubilization (Gao et al. 2010). In contrast, distilled water treatments frequently resulted in transient acidification, particularly at intermediate time points, likely due to uncontrolled release of preservative-derived ions without buffering capacity. In liquid systems, pronounced alkalization was observed in some fungal treatments, particularly with the Me strain at later remediation stages. This phenomenon may be associated with depletion of organic acids, assimilation of acidic metabolites, or release of alkaline by-products during advanced stages of fungal metabolism. Importantly, effective Cu removal occurred across a relatively broad pH range, indicating that *P. chrysosporium* can sustain remediation performance under varying chemical conditions.

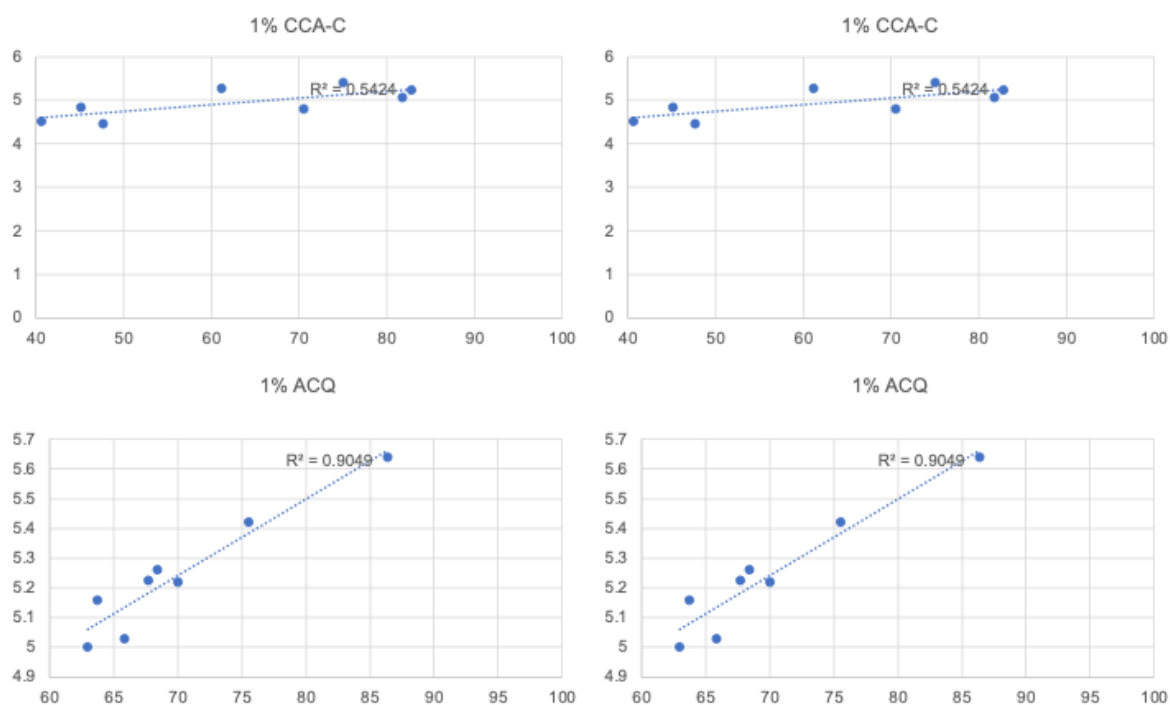
Tab. 4: *ph values of liquid fungal medium before remediation.*

Remediation medium	pH
Distilled water	5.08
<i>P. chrysosporium</i> Sf	5.1
<i>P. chrysosporium</i> Me	5.1

Tab. 5: *ph values of remediation medium after remediation.*

Preservatives	Remediation method	1 Day	2 Days	5 Days	10 Days
CCA (0.1%)	Distilled water	4.80 (0.13)	5.04 (0.01)	5.32 (0.11)	5.50 (0.28)
	<i>P. chrysosporium</i> Sf	5.11 (0.07)	4.93 (0.11)	5.23 (0.04)	5.31 (0.03)
	<i>P. chrysosporium</i> Me	4.86 (0.03)	4.92 (0.04)	5.95 (0.35)	7.72 (1.58)
CCA (1%)	Distilled water	5.06 (0.01)	5.28 (0.36)	5.04 (0.66)	5.01 (0.01)
	<i>P. chrysosporium</i> Sf	4.52 (0.04)	4.45 (0.08)	4.81 (0.11)	5.07 (0.06)
	<i>P. chrysosporium</i> Me	4.84 (0.01)	5.28 (0.03)	5.41 (0.23)	5.23 (0.49)
ACQ (0.1%)	Distilled water	5.06 (0.00)	5.17 (0.05)	5.28 (0.08)	5.58 (0.05)
	<i>P. chrysosporium</i> Sf	5.15 (0.06)	5.06 (0.08)	5.35 (0.02)	5.28 (0.19)
	<i>P. chrysosporium</i> Me	4.91 (0.06)	5.05 (0.02)	7.17 (2.35)	5.00 (1.13)
ACQ (1%)	Distilled water	6.30 (0.00)	6.28 (0.21)	5.41 (0.01)	5.19 (0.02)
	<i>P. chrysosporium</i> Sf	5.00 (0.01)	5.16 (0.04)	5.23 (0.01)	5.42 (0.06)
	<i>P. chrysosporium</i> Me	5.03 (0.01)	5.26 (0.04)	5.22 (0.07)	5.64 (0.47)
MCQ (0.1%)	Distilled water	4.95 (0.03)	5.21 (0.03)	5.25 (0.04)	5.42 (0.10)
	<i>P. chrysosporium</i> Sf	5.27 (0.02)	5.18 (0.04)	5.15 (0.23)	4.63 (0.61)
	<i>P. chrysosporium</i> Me	4.97 (0.01)	4.98 (0.02)	6.55 (0.21)	6.45 (0.01)
MCQ (1%)	Distilled water	5.40 (0.28)	5.50 (0.57)	5.39 (0.01)	5.51 (0.01)
	<i>P. chrysosporium</i> Sf	4.98 (0.02)	5.31 (0.01)	5.37 (0.02)	5.46 (0.01)
	<i>P. chrysosporium</i> Me	5.00 (0.06)	5.19 (0.21)	5.22 (0.16)	5.02 (0.59)

The correlation between remediated wood pH and copper removal provides insight into the chemical environment at the direct interface where fungal hyphae interact with the wood cell wall (Fig. 3).



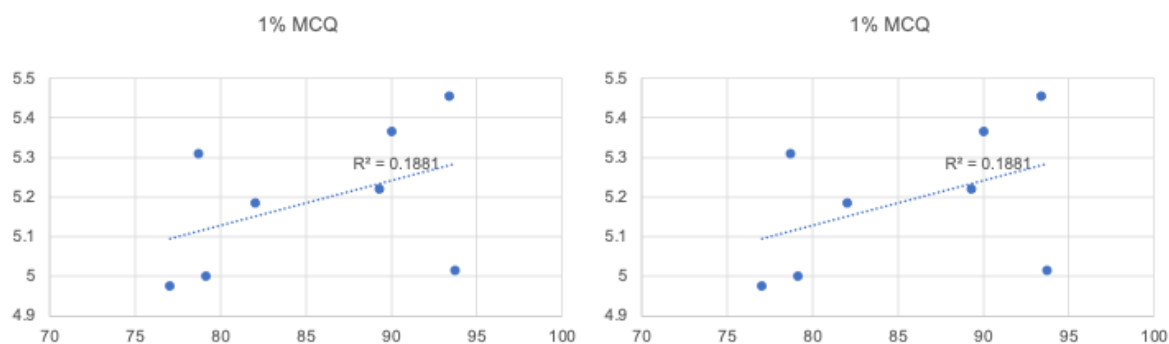


Fig. 3. Correlation between pH (y-axis) of remediation medium and Cu removal (%) (x-axis) from treated wood.

Across most treatments, particularly CCA and MCQ, a negative correlation was observed. This inverse relationship indicates that as copper removal efficiency increases, the wood matrix becomes more acidic. This is likely due to the proton-induced solubilization of fixed copper complexes. In CCA-treated wood, copper is fixed as insoluble copper arsenate; the secretion of organic acids by *P. chrysosporium* lowers the wood pH, providing the protons necessary to displace copper from the wood's functional groups (carboxylic and phenolic hydroxyl groups) and dissolve crystalline precipitates. However, the R^2 values for wood pH (ranging from 0.022 to 0.577) were generally lower than expected. This moderate correlation suggests that while wood acidification is a prerequisite for metal release, the wood pH measurement, taken after 10 min of immersion, is a “snapshot” of a highly buffered environment. The wood's natural buffering capacity, derived from lignin and hemicellulose, may mask the true extent of localized acidification occurring at the microscopic hyphal tips, thereby weakening the statistical correlation compared to the surrounding liquid.

The pH of the remediation medium serves as a cumulative indicator of the fungal metabolic state and the total acid production throughout the bioremediation period (Fig. 4). The correlations for the remediation liquid were significantly more robust in the amine-based systems, most notably for ACQ 1.0% ($R^2 = 0.905$). In ACQ and MCQ systems, copper is stabilized by ethanolamine or present as micronized basic copper carbonate. These forms are highly sensitive to the bulk pH of the surrounding solution. As the fungi secrete oxalic and citric acids into the medium, the bulk pH drops, causing a rapid and predictable release of copper ions into the liquid phase. The strong linear fit for ACQ 1.0% suggests that for this specific preservative, the remediation liquid pH can serve as a reliable predictive marker for the progress of the treatment. In contrast, the correlations for MCQ remained relatively low ($R^2 \approx 0.13 - 0.19$). This can be attributed to the micronized nature of the copper particles. Unlike the dissolved ions in ACQ, MCQ particles must undergo a physical dissolution process that may be rate-limited by particle surface area or the formation of secondary fungal-metal precipitates (like copper oxalate). In these cases, copper removal is not strictly a function of pH but is also influenced by the production of fungal extracellular polymeric substances and specific chelating agents that do not significantly alter the bulk pH. For industrial applications, these correlations suggest that monitoring the leachate (remediation liquid) is a more practical and statistically valid method for process control than measuring the internal wood pH.

CONCLUSIONS

This study demonstrates that pH evolution is a critical and dynamic parameter governing the bioremediation of copper-based wood preservatives using *P. chrysosporium*. Initial preservative-treated wood sawdust exhibited acidic pH values, with distinct differences among CCA, ACQ, and MCQ systems reflecting their underlying chemical formulations. During remediation, fungal treatments consistently produced more stable and regulated pH conditions in both solid and liquid phases compared with distilled water controls. Fungal remediation moderated extreme pH fluctuations, maintaining conditions favorable for enzymatic activity, metal solubilization, and fungal growth. The two *P. chrysosporium* strains exhibited clear strain-specific pH behaviour, particularly in liquid systems, indicating differences in metabolic pathways, organic acid production, and buffering capacity. Notably, the Me strain frequently induced pronounced alkalization at later remediation stages, suggesting advanced substrate utilization and potential exhaustion of acidic metabolites. The observed pH stabilization in fungal systems supports the proposed mechanisms of bioremediation, including organic acid-mediated metal mobilization, enzymatic degradation of lignocellulosic structures, and adsorption of released metals onto fungal biomass. Importantly, effective copper removal occurred within moderately acidic to near-neutral pH ranges, highlighting the suitability of fungal bioprocesses for treating preservative-treated waste wood without the need for aggressive chemical pH adjustment. Overall, the results confirm that *P. chrysosporium*-based bioremediation provides a chemically controlled, biologically resilient, and environmentally compatible approach for the recovery of copper from treated wood. The integration of pH monitoring with metal removal performance offers valuable insights for optimizing large-scale remediation systems and developing sustainable strategies for managing preservative-treated wood waste.

ACKNOWLEDGMENTS

This study was financially supported by the Coordination Unit for Scientific Research Projects (BAP), Istanbul University- Cerrahpaşa (Project No: FBA-2022-36138).

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