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Biological and chemical remediation of CCA treated eucalypt poles after 30 years in service

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HIGHLIGHTS

- Fungi, organic acids and EDTA were tested to remediate CCA treated eucalypt waste.
- Among copper tolerant fungi, *W. cocos* showed high arsenic removal of treated wood.
- Combination of EDTA and oxalic acid removed 98 % of copper from treated wood waste.

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ABSTRACT

The aim of this study was to evaluate the efficacy of biological and chemical remediation of chromated copper arsenate (CCA) treated *Corymbia citriodora* poles, removed from service after 30 years. The presence of arsenic (As), chromium (Cr) and copper (Cu) was quantified by inductively coupled plasma optical emission spectrometry (ICP–OES). Twelve species of decay fungi were used for the biological remediation assay. For chemical remediation oxalic, citric, maleic and ethylenediamine tetraacetic (EDTA) acids were used for 24 and 48 h. In biological remediation, copper-tolerant brown-rot fungi, *Wolfiporia cocos*, *Antrodia xantha* and *Fibroporia radiculosa*, performed the best results, with the highest removals for As (59–85 %) and Cr (38–61 %). Cu was the most easily extracted, with removals above 60 % among the tested fungi, with the best results (90–98 %) for *F. radiculosa*, *Coniophora puteana*, *Antrodia vaillantii* and *Postia placenta*. In chemical remediation, the extraction time of 48 h was the most effective, and oxalic acid generally reached the highest removals. The EDTA + oxalic acid combination reached the highest value for Cu extraction (98 %).

1. Introduction

Preservative treatments are required for non-durable wood to ensure performance and improve its service life. In Brazil, the most common methods for wood treatment are those that use waterborne preservatives (mixture of salts and metal oxides) under vacuum and pressure. Among wood preservatives, chromated copper arsenate (CCA) was widely used not only in preservation plants in Brazil but worldwide (Sierra-Alvarez, 2009; Vidal et al., 2015).

Concerns related to public health and the environment have led several countries to create restrictions on products that use arsenic in their formulation (Townsend et al., 2005; Sierra-Alvarez, 2009), however, no restrictions were applied for CCA-treated wood in Brazil (Vidal et al., 2015). There are growing concerns about the risk of leaching and environmental contamination by CCA-treated timber in-service, especially when freshly treated, and when treated wood residues are reused (Wang et al., 2016; Frédette et al., 2019; Liu et al., 2019).

The amount of chemicals in wood removed from service will depend on its initial retention (proposed use/standards requirements) and also on the weathering processes (Townsend et al., 2005). According to Brazilian Association of Technical Standards (ABNT) the amount of preservative for CCA-treated wood varies from 4.0 to 6.5 kg m^{-3} for

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above ground contact to 6.5–12.8 kg m^{-3} for ground contact and 24–40 kg m^{-3} for exposure in marine environments (ABNT-NBR 16143, 2013; Vidal et al., 2015).

Most of treated wood waste is reused or disposed of in landfills, thus, care must be taken during and after the end of treated wood service life, as its reuse and inadequate disposal become a problem. According to Robinson et al. (2006) soil CCA concentrations increased with time in a three-year leaching experiment with treated wood posts. Liu et al. (2019) found CCA metals above the maximum levels recommended by some food safety standard regulations when CCA treated wood waste was simulated as garden materials. Yanitch et al. (2020) showed that plants grown near wood preservatives residues were able to translocate the metals from contaminated site to their aerial parts. Also, other studies mentioned contaminated sites and the risk of contamination with CCA metals, including soil and groundwater (Hirata et al., 1993; Wang et al., 2016; Frédette et al., 2019; van der Kallen et al., 2020; Gosselin and Zagury, 2020).

Thus, treated wood removed from service and containing remaining toxic metals requires proper treatment to decontaminate wood prior to disposal or reuse (Solo-Gabriele et al., 1998; Helsen and Van Den Bulck, 2005; Clausen and Lebow, 2011; Liu et al., 2019). Leachates generated during the treated wood decontamination process contain toxic metals and also require proper treatment. Inside the different methods of treated wood remediation, the recovery and reuse of metals after the remediation of treated wood has been studied by several authors and is an interesting approach that allows the metal recovery and reuse of wood residues in various production chains (Kazi and Cooper, 2006; Janin et al., 2009b, 2021, 2021; Chen, 2015; Bertuol et al., 2020; Gschwend et al., 2020).

Treated utility poles are included in the various types of wood waste. The failure in treated poles is related to decay or weathering, and determines its end of service life and replacement. Thus, due to the high preservative retention, more attention should be given to this material (Khan et al., 2006; Robinson et al., 2006; Potvin et al., 2015).

The decontamination of treated wood has been studied for waste management, either by chemical extraction methods (organic and inorganic acids; chelating agents) or bioremediation using fungi and bacteria (Sierra-Alvarez, 2009; Kartal et al, 2014, 2015, 2014; Wang et al., 2016). However, further studies are needed to assess the remediation of treated wood waste, either through the use of biological organisms or effective chemicals. The aim of this study was to evaluate the effectiveness of biological and chemical remediation of CCA treated eucalypt poles, removed after 30 years in service.

2. Materials and methods

2.1. Wood samples and preparation

Two *Corymbia citriodora* (Hook.) K.D. Hill & L.A.S. Johnson utility poles treated with CCA–A were removed after 30 years in service by a service company of the electric sector in the city of Alegre, Espírito Santo state, and sent to the Department of Forest and Wood Sciences (DCFM), Federal University of Espírito Santo (UFES), Jerônimo Monteiro, ES, Brazil. Each pole had an identification plate with the treatment information (under pressure with CCA–A Osmose K–33), length (10 m) and the preservation date, August 1988.

The deteriorated parts of the top, where it was not possible to remove discs, were not used, as well as the bottom of the poles below groundline region, which was retained in the soil after removal of the poles. Only integral parts below the regions of deterioration were used for test samples. The poles were sectioned into eight sections (1 m in length) from the groundline region (bottom) to the undamaged top parts. The sections were used to obtain discs of 5 cm in thickness. Then, the discs from each position and pole were used to obtain sapwood portions.

The sapwood portions were sent to the United States Department of Agriculture–Forest Service (USDA–FS), Forest Products Laboratory (FPL), Research Unit on Wood Durability and Protection (RWU 4723), Madison, Wisconsin, United States of America (USA), for the remediation tests. The sapwood portions obtained were converted into chips and transformed into sawdust in a Willey mill using a 10 mesh screen (2 mm opening). Approximately 250 g of chips were produced from each disc. The sawdust from both poles was completely homogenized and classified through a 40 mesh (0.42 mm) sieve prior the remediation tests.

2.2. Fungal growth and biological remediation of treated wood

2.2.1. Quantification of fungal biomass

Before starting the remediation test, a separate experiment was conducted to assess the fungal mass during 10 days of incubation. Selected cultures were obtained from the Culture Collection of the Center for Forest Mycology Research, Northern Research Station in Madison, WI, and quantified for fungal biomass production before the remediation tests. The fungi were selected based on data from literature on their tolerance to CCA metals and their uses in remediation tests, mostly found for softwoods (Arango et al., 2009; Clausen et al., 2000; Humar et al., 2004; Kartal et al., 2015; Ohno et al., 2015). Some white-rot fungi were selected due to preference for hardwoods (eucalypts).

The brown-rot fungi *Fibroporia radiculosa* (Peck) Parmasto. (L-9414-Sp), *Wolfiporia cocos* (F.A. Wolf) Ryvarden & Gilb. (MD-106-R), *Coniophora puteana* (Schumach.) P. Karst. (ME-715), *Antrodia xantha* (Fr.) Ryvarden (Mad-5096-35), *Fomitopsis palustris* (Berk. & M.A. Curtis) Gilb. & Ryvarden (TYP-6137), *Postia* (= *Rhodonia*) *placenta* (Fr.) M.J. Larsen & Lombard (Mad-698-R), *Antrodia vaillantii* (DC.) Ryvarden (RWD-63-263), and the white-rot fungi *Phanerochaete chrysosporium* Burds. (ME-461), *Phlebia brevispora* Nakasone (MD-192), *Pycnoporus sanguineus* (L.) Murrill (FP-105829-Sp), *Abortiporus biennis* (Bull.) Singer (508), *Schizophyllum commune* Fr. (ME-441), were used for the biomass quantification and remediation tests.

For the quantification of fungal mycelial biomass, a 5 mm plug obtained from the edge of a Petri dish, with active fungal growth on malt extract agar medium (MEA), was inoculated into 250 mL Erlenmeyer flasks as recommended by Clausen et al. (2000), with some modifications. The flasks contained 100 mL of sterile liquid culture medium (20 min, 103 kPa and 121 °C), which was prepared with 40 g glucose, 3 g peptone, 15 g malt, per liter of deionized (DI) water.

After inoculation in liquid medium, the flasks, with three replicates for each fungus, were kept in a shaker incubator at 120 rpm and 27 °C for 10 days in absence of treated sawdust. After this period, the inoculated culture was filtered on Whatman No. 1 filter paper and washed with 200 mL of deionized water on a Büchner funnel coupled to a side arm flask connected to a vacuum pump, where the mycelial biomass of each fungus was retained. The filter papers were removed from the Büchner funnel and oven-dried at 60 °C for 72 h, after which they were weighed to obtain the dry mass of the fungal biomass by discounting the initial dry mass of the filter paper.

2.2.2. Fungal remediation of CCA treated wood

Treated sawdust was used in the remediation processes, following the procedures used by Kartal et al. (2004a), with some modifications. The fungi used in the biological remediation process were inoculated again under the same conditions described in section 2.2.1 for 10 days. After this period, the remediation bag (sachet type), made of polyester fibers measuring 6 × 7 cm, containing 3 g of sawdust treated with CCA, was sterilized (20 min, 103 kPa, 121 °C) and added to Erlenmeyer flasks containing the 100 mL medium grown with the fungus. Two control treatment situations were used for remediation bags with treated sawdust, one including sterile non-inoculated culture medium and the other with deionized water. The flasks, with three replications for each fungus, were kept for 10 days in a shaker incubator under the same conditions previously mentioned.

After this period, the culture medium was removed from the flasks,

and the remediation bags were washed 3 times with 100 mL of deionized water to remove the fungal hyphae that were in contact with the bags. The remediation bags were oven-dried at 60 °C for 72 h and then the sawdust was removed and stored in glass jars for quantification of the remaining arsenic (As), chromium (Cr) and copper (Cu) by inductively coupled plasma–optical emission spectroscopy (ICP–OES), as described in section 2.4.

2.3. Chemical remediation of treated wood

In chemical remediation, oxalic (pH: 1.4), citric (pH: 2.2), maleic (pH: 2.3) and ethylenediamine tetraacetic acids – EDTA (pH: 4.6) (MilliporeSigma, St. Louis, MO, USA) were prepared with deionized water at 1 % concentration. For the experiment, 250 mL Erlenmeyer flasks containing 100 mL of each acid were sterilized prior the addition of remediation bags containing 3 g of CCA treated sawdust. Remediation occurred in a shaker incubator at 120 rpm and 27 °C for 24 and 48 h. Additionally, combinations of each acid with EDTA were tested for 48 h under the same conditions mentioned above. Sterile deionized water (100 mL) was used for the control treatment (no chelator) and three repetitions were used for each situation tested.

For 48 h extraction time, the experiment was conducted in two steps. After the first 24 h, the bags were washed as already described and exposed again to fresh reagents for another 24 h of extraction under the same conditions mentioned above. After completing the 48 h, the bags were removed from the Erlenmeyer flask and washed three times as described previously and oven-dried (60 °C for 72 h). Finally, the sawdust was removed from the remediation bag and stored in glass jars for quantification of the remaining elements As, Cr and Cu by ICP–OES (Section 2.4).

2.4. ICP–OES analysis

The quantification of As, Cr and Cu was performed by ICP–OES at the Analytical Chemistry and Microscopy Laboratory at FPL. The ICP–OES analysis was performed in duplicate for each sample extracted. Before analysis, the remediated and control sawdust samples were oven-dried (103 ± 2 °C) for 24 h. The digestion of sawdust samples (approximately 1 g) was carried out in an Anton Paar (Ashland, VA) microwave digester. For this purpose, 3 mL of 70 % HNO_3 (Sigma-Aldrich (Milwaukee, WI) ACS-grade) was added to each digestion flask and left to rest for approximately 15 min in an exhaust hood for pre-digestion. Then, 4 mL of deionized water was added, and the flasks were closed properly.

The microwave digestion temperature program was composed of four stages, with an initial heating ramp from 15 min to 145 °C, maintaining the temperature for 10 min, final heating ramp from 15 min to 190 °C and maintenance temperature for 10 min. After cooling, the digested solutions were filtered and transferred to polypropylene flasks, where the volume was adjusted to 50 mL with deionized water.

The determinations of As, Cr and Cu were made using a Horiba (Edison, NJ) ULTIMA II high resolution spectrometer. The equipment contained a water cooled 40 MHz radio frequency source, Czerny Turner monochromator with 1 m focal length, nitrogen purged optical system (6 L min^{-1}), holographic grid (2400 grooves mm^{-1}) with resolution < 5 p.m. (high resolution) in the 120–320 nm wavelength range (1st order) and <10 p.m. (good resolution) in the 320–800 nm range (2nd order) and a vertical torch with radial viewing. Power level was 1000 W. Gas flows were: plasma gas 12 L min^{-1}, sheath gas 0.2 L min^{-1}, nebulizer gas 0.28 L min^{-1}.

The device was turned on approximately 30 min before starting readings to minimize instrumental deviation. Calibration was performed using external standards with a correlation coefficient $r > 0.999$ for As, Cr and Cu. The standards were prepared from 1000 ppm stock solutions without serial dilution. Standard curves were obtained using external standards for As 188.983, 193.695; Cr 276.654, 284.325; Cu 224.700, 324.754, 327.396 nm. Acquisition was done in Max mode peak shape, with 1 s integration time and three replicate readings averaged for the reported measurement. With the values obtained by reading by ICP–OES, the final retention of the CCA in the wood was calculated according to Equation (1). Removal percentages were calculated based on the amount of each element present in the unextracted sawdust.

$$R = \frac{L \times F_d \times F \times \rho_a}{m_a} \quad (1)$$

where R = Retention of the element in the sample (kg a.i. m^{-3}); L = Reading obtained by ICP–OES (mg L^{-1}); F_d = dilution factor 0.05 L; F = Stoichiometric factor used to transform As, Cu and Cr to oxides, being 1.5340; 1.2518 and 1.9230, respectively; ρ_a = anhydrous density (g cm^{-3}); and m_a = mass of the sample (g).

2.5. Statistical analysis

To assess the amount of As, Cr and Cu removed by biological and chemical remediation methods, a completely randomized design was used. Metal removal data for biological and chemical remediation were analyzed by one-way and two-way analysis of variance (ANOVA), respectively. The parameters affected significantly (F test with $P \leq 0.05$) were evaluated with the Scott-Knott test ($P \leq 0.05$).

For all cases, the normality of the data was verified by the Kolmogorov-Smirnov test, and the homogeneity of the variances was verified by the Cochran's C test. When necessary, the values of metal removal were transformed in $arcsin\sqrt{Removal_{(\%)}/100}$. Pearson correlations were also performed between the fungal biomass and metal removal and between elements (As x Cr x Cu) for the tested remediation methods.

3. Results and discussion

3.1. Fungal growth and biological remediation of treated wood

A variation in the amount of mycelial growth of each fungus was observed after 10 days of growth without the presence of treated wood. The results of fungal biomass production are shown in Fig. 1. Notably, some fungi, such as *S. comune*, *W. cocos*, *C. puteana* and *P. chrysosporium*, produced considerably more hyphae than others.

Several approaches are described in the literature on the mechanisms of the fungal remediation process, the main ones being the performance of hemicellulolytic and ligninolytic enzymes, the production of organic acids (e.g., oxalic acid) and biosorption through fungal biomass (Kartal and Imamura, 2003). Additionally, according to Rudakiya et al. (2019), fungal biomass has the highest capacity for absorbing contaminants compared to other microorganisms. However, in the present study, there was no relationship between mycelial growth among different fungal species evaluated and the removal of the metal elements present in CCA treated wood (Figure S1 – Supplementary material).

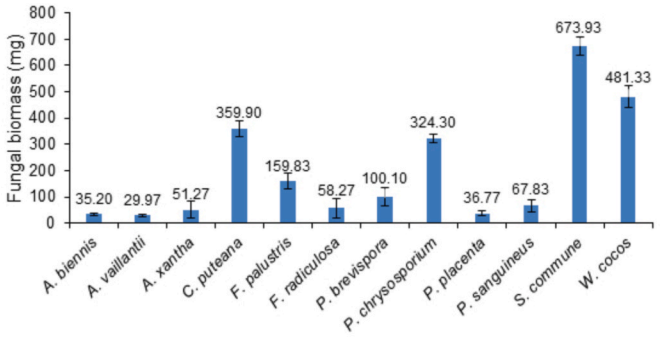

Fig. 1. Quantification of mycelial growth of fungi in liquid culture medium after 10 days of incubation without treated sawdust.

When analyzing the percentage of removal of the elements for each fungus, a significant difference was observed among the tested fungi (Fig. 2). *W. cocos* removed the largest amount of As (85%), differing from *A. xantha* (71%) and *F. radiculosa* (59%), which obtained the second best removal. The other fungi tested showed a similar performance when compared to control treatments (culture medium: 29%; H_2O: 30%), removing approximately 25–46% of As. The removal of the Cr element reached lower values when compared to the As and Cu elements, ranging from 15% (*P. brevispora*) to 61% (*W. cocos*). As previously observed for As, *W. cocos* obtained the highest percentage of removal, followed again by fungi *A. xantha* (46%) and *F. radiculosa* (38%).

Among the elements analyzed, Cu was the most easily removed by the tested fungi, which achieved a removal above 60%. Unlike the previous elements, *F. radiculosa* showed the best performance for the removal of Cu (96%), followed by *A. vaillantii*, *C. puteana* and *P. placenta*, which removed approximately 90%. *A. xantha*, *F. palustris*, *S. commune* and *W. cocos* showed an intermediate performance in removing Cu (75–78%), while the other fungi (*A. biennis*, *P. brevispora* and *P. sanguineus*) did not show differences in relation to the control culture medium.

Although the fungus *P. Chrysosporium* has not obtained good performance for Cu removal, Besserer et al. (2021) stated that this fungus, when incubated with a bacterial consortium, promoted promising results in Cu removal from treated wood. Another alternative pointed out to improve the bioremediation performance is the use of brown-rot fungi associated with a pre-extraction with weak acids (Sierra-Alvarez, 2009; Besserer et al., 2021).

When considering the final retention after remediation (Table 1), we observed that *W. cocos*, *A. xantha* and *F. radiculosa* obtained the highest total removals (50–70%). In general, tested controls removed considerable amount of CCA from sawdust (21–27%) and culture medium was able to remove more preservative, mainly Cu, than DI water. Kartal et al. (2015) found similar values for culture medium and DI water used as control for total remediation of CCA treated wood. According to the authors culture medium containing glucose, peptone and malt extract has some ability to remove CCA metal from treated wood, mainly Cu.

When studying 19 isolates of copper-tolerant *W. cocos*, Clausen et al. (2000) found that some isolates had mycelial growth and improved oxalic acid production in a culture medium enriched with copper. The authors suggested that this acid may contribute to the fungus ability to colonize treated wood and remove Cu; however, no mention of other elements such as As and Cr were made.

Oxalic acid has been described in the literature as an important metabolite produced mainly by brown-rot fungi (Cameselle et al., 1998; Kartal and Imamura, 2003). Following this hypothesis, Kartal et al. (2004a), tested brown-rot fungi in the bioremediation of CCA treated Scots pine poles and found that *F. palustris*, *Laetiporus sulphureus* and

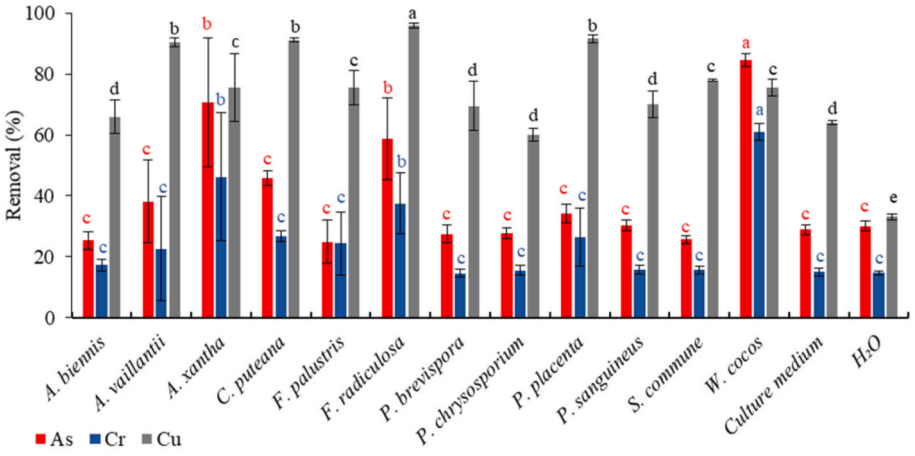

Fig. 2. Removal of the elements arsenic (As), chromium (Cr) and copper (Cu) after the biological remediation process.

Table 1
Final retention after biological remediation with the tested fungi.

Fungus	Final retention (kg m^{-3})				Total removal (%)
	As_2O_5	CrO_3	CuO	Total	
W. cocos	0.25 (0.04)	2.38 (0.17)	0.49 (0.05)	3.12 (0.16)	68
A. xantha	0.48 (0.35)	3.29 (1.29)	0.49 (0.22)	4.26 (1.40)	56
F. radiculosa	0.68 (0.22)	3.82 (0.61)	0.08 (0.02)	4.58 (0.84)	53
C. puteana	0.89 (0.04)	4.48 (0.11)	0.18 (0.01)	5.55 (0.16)	43
P. placenta	1.08 (0.05)	4.50 (0.58)	0.17 (0.03)	5.74 (0.57)	41
A. vallianti	1.01 (0.22)	4.74 (1.05)	0.19 (0.03)	5.94 (1.30)	39
F. palustris	1.23 (0.12)	4.63 (0.64)	0.49 (0.11)	6.34 (0.80)	35
S. commune	1.22 (0.02)	5.16 (0.08)	0.68 (0.01)	6.82 (0.08)	30
P. sanguineus	1.14 (0.03)	5.16 (0.09)	0.68 (0.08)	6.90 (0.18)	29
A. biennis	1.22 (0.05)	5.06 (0.12)	0.68 (0.11)	6.96 (0.16)	29
P. brevispora	1.19 (0.05)	5.23 (0.08)	0.61 (0.16)	7.02 (0.29)	28
P. chrysosporium	1.18 (0.03)	5.16 (0.10)	0.80 (0.04)	7.14 (0.13)	27
Culture medium (Control)	1.16 (0.03)	5.20 (0.08)	0.72 (0.01)	7.08 (0.12)	27
H_2O (Control)	1.14 (0.03)	5.22 (0.04)	1.34 (0.02)	7.70 (0.07)	21
Sawdust–CCA–A (Initial retention)	1.64 (0.01)	6.12 (0.05)	2.00 (0.00)	9.75 (0.05)	–

Numbers in parentheses represent the standard deviation.

C. puteana produced approximately 0.42, 0.32 and 0.03%, respectively, of oxalic acid in liquid medium after 10 days, removing 100, 85, and 18% of As and 72, 50, and 67% of Cu. However, in the present study *C. puteana* (46%) removed a greater amount of As than *F. palustris* (25%) (Fig. 2). Fungi of the genus *Antrodia* sp. are also described as efficient in oxalic acid production (Humar et al., 2004). As observed in the present study, *Antrodia xantha* showed the second best performance in removing As (Fig. 2).

The ascomycete fungus *Aspergillus niger*, also produces high amounts of oxalic acid, and was studied by Clausen (2004) and Kartal et al. (2004b) with similar concentrations of this acid (~1.4%) after 10-day incubation period. However, the results from both studies for metal removal were contrasting, with the removal of As being 52 and 97% and Cu being 84 and 49%, respectively. Several factors could be pointed out in mentioned studies that affected the results such as different nutrient medium, sawdust/solution ratio, treated wood retention and different strains.

Kartal et al. (2008) also observed that Cr was the most resistant to removal in treated wood. According to these authors, the limited removal of Cr can be explained by the high power of fixation in the wood and the low capacity of the agents produced by the fungi on its removal. It is known that chromium acts as a fixing agent for As and Cu in wood by reducing Cr^{+6} to Cr^{+3}, forming strong insoluble complexes with lignin (Kartal et al., 2008; Lepage et al., 1986). In addition, the formulation used for the utility poles treatment (CCA–A) is the one with the highest proportion of Cr among the existing formulations.

Regarding the Cu removal, most basidiomycetes are described as not copper–tolerant (Kim et al., 2007). However, some brown-rot fungi have been reported in the literature to be more tolerant to CCA than white-rot fungi (Clausen et al., 2000; Kartal et al., 2015). Since 1952, the brown-rot fungus *F. radiculosa* has been reported as tolerant to some copper-based wood preservatives, reaching up to 40% mass loss in CCA treated pinewood (Clausen and Jenkins, 2011; Ohno et al., 2015). Genetic regulation of copper tolerance and copper removal in *F. radiculosa* appears to involve increased expression of genes related to oxalate production (Tang et al., 2013; Akgul and Akgul, 2018; Akgul et al., 2018), which was corroborated by the accumulation of copper oxalate crystals in the wood (Tang et al., 2013). However, no studies were found with the present remediation methods to associate and quantify the metal removal by *F. radiculosa* oxalate production. In fact, other studies (Jenkins et al., 2014; Ohno et al., 2015, 2016, 2017, 2020, 2016, 2020) suggest high oxalic acid production may not be the sole requirement for the mechanism of copper-tolerance and metal removal in *F. radiculosa*.

Among brown-rot fungi, species of the genus *Antrodia*, *Coniophora*, *Postia*, *Serpula, Fibroporia*, and *Wolfiporia* are among the most tolerant and aggressive to copper treated wood, while *Gloeophyllum* species are more sensitive (Akgul et al., 2018; Clausen et al., 2000; Clausen and Jenkins, 2011; Ohno et al., 2017, 2020, 2020). These statements corroborate the results of the present study, in which *F. radiculosa*, *C. puteana*, *A. vailiantti* and *P. placenta* removed more than 90% of Cu, while white-rot fungi obtained the worst performances (Fig. 2). The results showed by Kartal et al. (2015) also corroborate the above-mentioned facts, where brown-rot fungi *T. palustris*, *G. trabeum*, *P. placenta* and *C. puteana* removed approximately 80% of Cu from the CCA–treated wood, while the white-rot fungus *Trametes versicolor* and *Irpex lacteus* removed around 60%. The results found in the literature for fungal remediation show a wide variation within and among the species of fungi studied, which can be explained by the fact that the removal of heavy metals depends on several factors, such as pH, temperature, nutrient medium, time of exposure and concentration.

3.2. Chemical remediation of treated wood

In general, there was an increase in metal removal from 24 to 48 h (Fig. 3); however, ANOVA showed significant interaction between the acid and time factors. When comparing the mean values (Table 2), the exception occurred for As and Cr removal, which were not significant with time when maleic acid, EDTA and H_2O (control) were used.

In the comparison between the acids used (Table 2), the removal of As was achieved best using oxalic acid at both extraction times (24 and

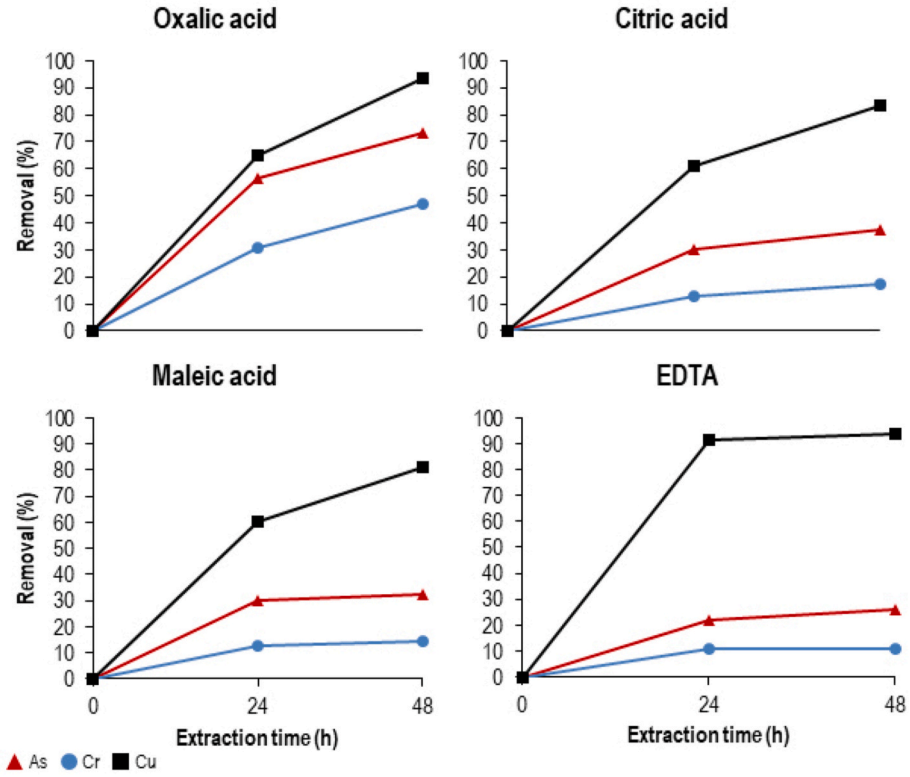

Fig. 3. Removal of arsenic (As), chromium (Cr) and copper (Cu) after the chemical remediation process for extraction times of 24 and 48 h.

Table 2
Removal of the elements arsenic (As), chromium (Cr) and copper (Cu) after the chemical remediation process for extraction times of 24 and 48 h.

Treatment	Removal (%)					
	As		Cr		Cu	
	24 h	48 h	24 h	48 h	24 h	48 h
Oxalic acid	56.64 Ba (2.14)	73.39 Aa (3.39)	31.01 Ba (1.31)	47.27 Aa (3.33)	65.10 Bb (1.26)	93.33 Aa (1.05)
Cítric acid	30.03 Bb (2.91)	37.67 Ab (2.21)	12.55 Bb (2.31)	17.46 Ab (1.22)	60.96 Bc (0.80)	83.41 Ab (0.37)
Maleic acid	29.86 Ab (2.37)	32.24 Ab (2.72)	12.68 Ab (1.34)	14.56 Ab (2.22)	60.49 Bc (0.99)	80.94 Ac (1.16)
EDTA	21.73 Ac (1.72)	25.94 Ac (3.14)	10.56 Ab (0.38)	10.80 Ac (0.96)	91.26 Ba (0.04)	94.07 Aa (0.14)
H_2O (Control)	17.30 Ac (6.23)	22.24 Ac (3.83)	9.69 Ab (4.11)	7.75 Ac (2.60)	26.63 Bd (3.15)	38.64 Ad (0.91)

Means followed by the same letter, for each element (As, Cr and Cu), uppercase (row) or lowercase (column), do not differ (Scott-Knott, $p > 0.05$). Numbers in parentheses represent the standard deviation.

48 h), differing from the other extracting solutions. The second highest As removals were attributed to citric and maleic acids, however, with values below 50%. The lowest performance for the removal of As occurred with EDTA, which did not differ from the control (H_2O).

Oxalic acid again obtained the highest removal for Cr in both extraction times, differing from the other extraction solutions. The other extraction solutions achieved removals below 20% and did not differ from the control samples within 24 h, while after 48 h, only EDTA did not differ from the control. The highest Cu removals were obtained with EDTA (>90%), which differed significantly from the other extraction solutions at an extraction time of 24 h. However, after 48 h, oxalic acid reached Cu extraction values statistically similar to EDTA. In 48 h, citric acid (83%) and maleic acid (80%) achieved intermediate performance in the removal of Cu. The control treatment (H_2O) reached the lowest averages of Cu removal in both extraction times with removal below 40%.

When comparing the removal of As, Cr and Cu by the acids alone or in combination with EDTA for 48 h (Fig. 4), it was observed that the EDTA combinations did not increase the As and Cr removal. Removals by oxalic acid alone (As: 74%; Cr: 47%) were superior to the other treatments. The combination of EDTA + oxalic acid obtained the second best removal of As and Cr (58 and 33%, respectively), while the combinations of EDTA with citric and maleic acids did not differ from the chemicals when used alone.

For the Cu removal, it was noted that there was an increase when oxalic, citric and maleic acids were combined with EDTA, exceeding the values achieved when used alone, suggesting a slight synergistic effect. The best performance was obtained for the combination EDTA + oxalic acid (98%). The combinations EDTA + citric acid and EDTA + maleic acid reached 96% removal (Fig. 4).

In addition, it was noted that the removal of As and Cr was dependent on each other, with an average removal of As two to three times greater than the removal of Cr. This fact can be explained by Cr forming compounds with wood in trivalent and hexavalent forms. In addition, it is estimated that in treated wood, approximately 85% of arsenic reacts with chromium in trivalent form, forming $CrAsO_4$ (Christensen et al., 2006; Lepage et al., 1986). A strong significant correlation was observed for the dependence on the removal of As and Cr, while the removal of Cu was more independent of these two elements (Figure S2 – Supplementary material).

In general, the extraction in oxalic acid for 48 h obtained the lowest CCA final retention in the wood, which represented a total removal of 61% (Table 3). The combination EDTA + oxalic acid showed the lowest retention for CuO and the second largest total removal (51%). When observing the general performance of biological remediation (Table 1), with the exception of CuO retention, chemical extraction was not superior to the results obtained with the fungus *W. cocos* (68% of total removal).

Oxalic acid showed satisfactory results, mainly for the removal of CuO. In the approach carried out in this study, those, copper–tolerant fungi, that are mentioned in the literature for the production of oxalic acid, were also the ones that removed the most of this element. However, other mechanisms of fungal action probably led to greater removal of As and Cr compared to chemical remediation.

In contrast with our results, Sierra-Alvarez (2009) found poor performance of oxalic acid and copper-tolerant brown-rot fungi

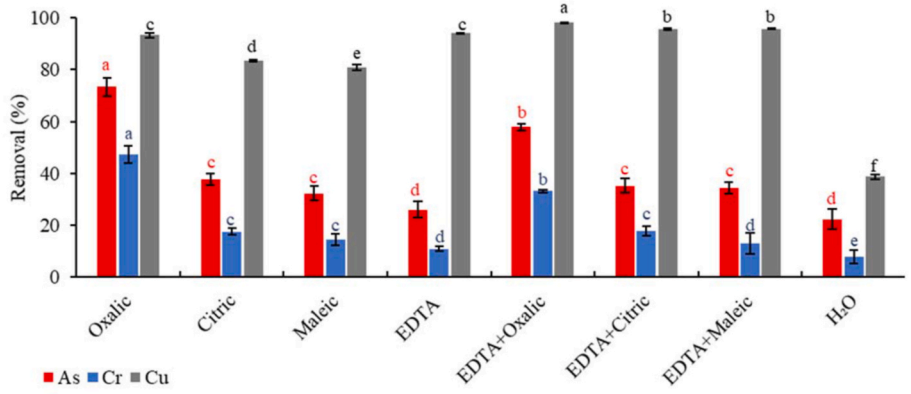

Fig. 4. Removal of the elements arsenic (As), chromium (Cr) and copper (Cu) after the chemical remediation process for 48 h of extraction.

Table 3
Final retention after chemical remediation.

Treatment	Final retention (kg m^{-3})				Total removal (%)
	As_2O_5	CrO_3	CuO	Total	
Oxalic acid (48 h)	0.44 (0.06)	3.22 (0.07)	0.13 (0.02)	3.79 (0.26)	61
EDTA + Oxalic acid	0.69 (0.02)	4.09 (0.16)	0.04 (0.00)	4.82 (0.04)	51
Oxalic acid (24 h)	0.71 (0.04)	4.22 (0.72)	0.70 (0.03)	5.63 (0.14)	42
EDTA + citric acid	1.06 (0.04)	5.03 (0.24)	0.09 (0.01)	6.18 (0.15)	37
Citric acid (48 h)	1.02 (0.04)	5.05 (1.08)	0.33 (0.01)	6.40 (0.11)	34
EDTA + maleic acid	1.07 (0.04)	5.33 (0.03)	0.08 (0.01)	6.48 (0.29)	33
Maleic acid (48 h)	1.11 (0.04)	5.23 (0.17)	0.38 (0.02)	6.71 (0.20)	31
EDTA (48 h)	1.21 (0.05)	5.46 (0.24)	0.12 (0.00)	6.79 (0.11)	30
EDTA (24 h)	1.28 (0.03)	5.47 (1.23)	0.17 (0.00)	6.92 (0.05)	29
Citric acid (24 h)	1.14 (0.05)	5.35 (0.23)	0.78 (0.02)	7.27 (0.20)	25
Maleic acid (24 h)	1.15 (0.04)	5.34 (1.23)	0.79 (0.02)	7.28 (0.13)	25
H_2O (48 h)	1.27 (0.06)	5.64 (0.07)	1.23 (0.02)	8.14 (0.22)	17
H_2O (24 h)	1.35 (0.10)	5.52 (0.17)	1.47 (0.06)	8.34 (0.41)	14
Sawdust–CCA	1.64 (0.01)	6.12 (0.05)	2.00 (0.00)	9.75 (0.05)	–

Numbers in parentheses represent the standard deviation.

(*A. vailiantti*) for Cu removal in CCA and ACC treated wood. The author found that citric acid alone performed better than oxalic acid in Cu removal and found the best result when citric acid was combined with biological remediation (*A. vaillantii*), which removed 100, 80 and 87% of As, Cr and Cu in CCA treated wood, respectively. As pointed out by Xing et al. (2020), the application of combined techniques in chemical or biological remediation process can be an effective way to improve the metal removal of treated wood waste.

The results obtained by Clausen and Smith (1998) for the chemical remediation of *Pinus* sp. treated with CCA (6.4 kg m^{-3}) showed an average removal of 89, 62 and 81% for As, Cr and Cu, respectively, after 24 h of extraction in oxalic acid (1%). In the present study, the results found for the same acid after 24 and 48 h of extraction were lower than those obtained by Clausen and Smith (1998), with the exception of Cu removal after 48 h. Chelating agents have been cited as potential alternatives for removing heavy metals in contaminated areas due to their ability to separate metals from contaminated waste, forming stable soluble metal-chelate complexes (Chang et al., 2013; Papassiopi et al., 1999). Among them, EDTA has been cited as effective in removing Cu but less effective for As and Cr (Janin et al., 2009b; Kartal, 2003).

In the present study, although oxalic acid showed better results in general, EDTA was more effective in removing Cu. Kartal (2003) found approximately 38, 36 and 93% removal of As, Cr and Cu, respectively, after extraction in EDTA (1%, 24 h) for *Pinus sylvestris* wood treated with CCA–C (21 kg m^{-3}), confirming the effectiveness of EDTA for removing Cu even in high retentions. Similar to the authors mentioned above, Janin et al. (2009a) obtained 100% removal of Cu in CCA–treated wood after EDTA extraction (2%, 22 h) and only 20% As and 4% Cr, while oxalic acid reached removals of 80, 61 and 49% of As, Cr and Cu, respectively.

In another study, Kartal et al. (2014) tested the removal of Cu in the wood of *Pinus* sp. treated with CCA–C (~7.5 kg m^{-3}) and obtained a removal of 47% and 97% after 24 h of extraction in oxalic acid (1%) and EDTA (1%), respectively. Although removal for EDTA was similar to that found in the present study, removal for oxalic acid was less efficient. In general, the studies found in the literature show a greater efficiency and stability of EDTA for the removal of Cu, reaching constant values above 90%, while the removal with oxalic acid presents removal values varying from 47% (Clausen and Smith, 1998) to 81% (Kartal et al., 2014) after 24 h of extraction. Although oxalic acid has a higher performance for removing the other tested elements (As and Cr), such facts suggest that its performance is more influenced by factors such as species, wood particles size, diffusion of the chemical in the wood and initial CCA concentration.

4. Conclusions

In biological remediation, copper-tolerant brown-rot fungi performed the best. Those fungi with higher mycelial biomass production did not show a direct relationship with the removal of the metal elements. *W. cocos* showed the best performance in As removal while *F. radiculosa*, *C. puteana*, *A. vaillantii* and *P. placenta* were better at removing Cu. The extraction time of 48 h was the most effective for chemical remediation and oxalic acid, in general, achieved the highest metal removal.

Overall, biological remediation achieved superior results compared to chemical remediation, except when EDTA + oxalic acid was used, where Cu was almost entirely removed. The bioremediation of treated wood, despite having achieved greater removals in relation to chemical remediation, requires a longer process. In addition, more information is needed on the remediation mechanisms by the tested fungi. The analysis of the effectiveness of the combination of biological and chemical remediation methods is another approach that should be investigated.

Authorship contributions

Conception and design of study: Costa, L.G.; Paes, J.B.; Brocco, V·F.; Kirker, G.T. **Acquisition of data:** Costa, L.G. Brocco, V·F.; Bishell, A.B.; Kirker, G.T. **Analysis and/or interpretation of data:** Costa, L.G.; Brocco, V.F. **Drafting the manuscript:** Costa, L.G.; Brocco, V.F. **Critical revision:** Paes, J.B.; Kirker, G.T.; Bishell, A.B.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

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