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Technical Article

Electro-removal of copper, chromium, and arsenic from chromated copper arsenate treated waste wood

Eletro-remoção de cobre, cromo e arsênico de resíduos de madeira tratada com arseniato de cobre cromado

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ABSTRACT

Wood is a renewable material considered eco-friendly and used for various purposes. While wood treated with chromated copper arsenate (CCA) does not deteriorate, its final disposal may entail risks due to the concentration and toxicity of the components. The removal of CCA from wood can be achieved in different ways. This study focuses on the reduction of the concentrations of Cu, Cr, and As chemical species by the electro-removal technique, aiming to obtain biomass with low deleterious potential that would allow multiple uses or safe disposal in landfills. The analytical results showed reductions of 79.5, 87.4, and 81.3% in the mean concentrations of Cu, Cr, and As, respectively. It is worth mentioning the occurrence of the fungus *Xylaria* sp. after treatment 6 (60 min, 5 g, and 25 V), further suggesting that the method was effective. Samples of these fungi were identified from isolates by culture in medium, DNA extraction, and sequencing of the Internal Transcribed Spacer (ITS) region.

Keywords: environmental risk; solid waste; chemical species; dispersive energy x-ray fluorescence spectrometry.

RESUMO

A madeira é um material renovável, considerado ecologicamente correto e utilizado para diversos fins. Embora a madeira tratada com arseniato de cobre cromado (CCA) não se deteriore facilmente, seu descarte final pode acarretar riscos devido à concentração e toxicidade dos componentes. A remoção do CCA da madeira pode ser realizada de diferentes maneiras. Este estudo teve como foco a redução das concentrações de espécies químicas Cu, Cr e As pela técnica de eletro-remoção, visando obter uma biomassa com baixo potencial deletério que permitiria múltiplos usos ou disposição final segura em aterros sanitários. Os resultados analíticos mostraram reduções de 79,5; 87,4 e 81,3% nas concentrações médias de Cu, Cr e As, respectivamente. Vale ressaltar a ocorrência do fungo Xylaria sp. após o tratamento 6 (60 min, 5 g e 25 V), sugerindo ainda que o método foi eficaz. Amostras desses fungos foram identificadas dos isolados por meio de cultura em meio, extração de DNA e sequenciamento da região do espaçador transcrito interno (ITS).

Palavras-chave: risco ambiental; resíduo sólido; espécies químicas; espectrometria de fluorescência de raios x por energia dispersiva.

INTRODUCTION

Considered to be an environment friendly material for several purposes, wood has an organic, natural, and renewable origin. In addition, wood has a hydrophobic biological constitution and hydrophilic fibers, giving it physical-mechanical properties that differentiate it from other materials (MOHAJERANI; VAJNA; ELLCOCK, 2018; FERNÁNDEZ-COSTAS *et al.*, 2017; VIDAL *et al.*, 2015). However, wood has the disadvantage of being prone to deterioration and its durability is closely related to its service life when exposed to abiotic factors, such as variations in temperature, humidity, luminosity, and acidity of the soil or xylophagous organisms, such as bacteria, fungi, mollusks, crustaceans, and insects, which also act on their environmental degradation (MOHAJERANI; VAJNA; ELLCOCK, 2018; BOTOMÉ *et al.*, 2017; CHAGAS *et al.*, 2015).

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In order to avoid deterioration and provide an extension to its useful life, wood is subjected to treatments with chemical preservatives, especially water-soluble preservatives composed of a combination of water-soluble salts (BABAEE; MULLIGAN; RAHAMAN, 2018; OHGAMI *et al.*, 2015; MERCER; FROSTICK, 2014). Chromated copper arsenate (CCA) provides an insecticidal and fungicidal action when applied to the wood. It is currently considered to be the most efficient wood preservative, acting mainly on the permeable outer layers of wood and blocking deterioration by chemical, physical, and biological agents (FERNÁNDEZ-COSTAS *et al.*, 2017; BOTOMÉ *et al.*, 2017; VIDAL *et al.*, 2015).

However, concerns due to the high toxicity of the mixture of inorganic compounds with copper, chromium, and arsenic base, together with the possibility of leaching causing deleterious effects on the environment, led to the prohibition of CCA by countries such as the USA, England, Denmark, and Canada (OHGAMI *et al.*, 2015; MERCER; FROSTICK, 2014; JANIN *et al.*, 2012).

In Brazil, the Brazilian Institute of the Environment and Renewable Natural Resources (*Instituto Brasileiro do Meio Ambiente e dos Recursos Naturais Renováveis* –Ibama) allows the industrial use of the CCA with the formulation based on salt contents of chromium (45.7%), copper (18.5%), and arsenic (34. 0%), which is classified as CCA type C (CHAGAS *et al.*, 2015; OHGAMI *et al.*, 2015; VIDAL *et al.*, 2015). Brazil consumes approximately 700,000 m^3/year of treated wood, a large part of which comes from plantations of eucalyptus (93.5%) and pine (6.5%). Most of this wood is treated with CCA and applied in the production of stakes (62%), poles (30%), railroad ties (5%), and in civil construction (3%), demonstrating that CCA represents an excellent commercial alternative to increase the durability of wood (FERRARINI *et al.*, 2016; VIDAL *et al.*, 2015; FERRARINI *et al.*, 2015).

When removed from service life, a large proportion of copper (Cu), chromium (Cr), and arsenic (As) remains in treated wood and can enter the waste stream unless actions are taken to avoid it (FDEP, 2017; TANG *et al.*, 2015; OHGAMI *et al.*, 2015).

After its use, CCA-treated wood generates waste categorized in class I — hazardous, according to the Brazilian Association of Technical Standards (*Associação Brasileira de Normas Técnicas* – ABNT, 2004), NBR 10004, whose objective is to classify solid waste according to their potential risks to the environment and to public health so that they can be properly managed. The solid and semi-solid residues resulting from anthropogenic activities, including the liquid effluents and debris originated from the wood preservation process, must be subjected to treatments with economically viable techniques and good removal efficiency so they can be discarded in class II — non-hazardous landfill sites.

The final disposal of CCA-treated wood becomes a challenge to the environmental sciences as it is a solid residue with high concentrations of Cu, Cr, and As, which are toxic components with possible harmful interactions with local communities (MOHAJERANI; VAJNA; ELLCOCK, 2018; FDEP, 2017; OHGAMI *et al.*, 2015). Incineration of those wastes is prohibited in many countries, such as Denmark and Brazil, and may pose a risk to human health (FERNÁNDEZ-COSTAS *et al.*, 2017; BOTOMÉ *et al.*, 2017; OHGAMI *et al.*, 2015).

Although the specialized literature points to studies on wood preservatives that are less aggressive to the environment, there are currently no efficient and economically viable substitutes in relation to CCA, whose consumption should continue to increase in the coming decades (MOHAJERANI; VAJNA; ELLCOCK, 2018; VIDAL *et al.*, 2015; FERRARINI *et al.*, 2015). In Brazil, there is no regulation targeted at the disposal of these solid residues, and one of the current challenges is the removal of CCA from treated wood for reuse or recycling (FERRARINI *et al.*, 2016; VIDAL *et al.*, 2015; FERRARINI *et al.*, 2015).

According to the National Solid Waste Policy, Law Number 12.305/2010, waste management has the objective of protecting public health and environmental quality, whose responsibility falls on generators and public authorities (BRASIL, 2012). As stated by D'Almeida and Vilhena (2000), the management of solid waste, including its treatment and final disposal, is the responsibility of generators.

Therefore, it is important to develop methodologies for the removal of toxic elements in those types of waste, predicting the difficulties related to their use and proposing viable alternatives for reuse or disposal (SANTOS *et al.*, 2018; TANG *et al.*, 2015; FERRANI *et al.*, 2015). Numerous researchers have turned their attention to the efficacy and use of electro-removal processes in CCA-treated wood compounds as well as avoiding the possible contamination of soils and water from the use of discarded CCA-treated wood at the end of its service life (FDEP, 2017; KUMPIENE *et al.*, 2016; OHGAMI *et al.*, 2015; FERRARINI *et al.*, 2015).

Studies in the last decades have shown concern over the remediation of CCA-treated wood by the use of different extractive solvents, such as oxalic acid, phosphoric acid, sulfuric acid, EDTA, hydrogen peroxide, and their mixture in different proportions (MOHAJERANI; VAJNA; ELLCOCK, 2018; KUMPIENE *et al.*, 2016; FERRARINI *et al.*, 2015; JANIN *et al.*, 2012). Janin *et al.* (2012) show that the best cost-benefit ratio lies in the use of sulfuric acid-based electrolytic extractive solutions, but state that oxalic acid has a higher extraction yield when compared to EDTA, phosphoric acid, or sulfuric acid alone. There is also a great interest in the development of effluent recovery technologies resulting from these extractive treatments through techniques, such as coagulation/precipitation with ferric chlorides, route-evaporation of compounds, and exchange of ions through ion exchange membranes (BABAEE; MULLIGAN; RAHAMAN, 2018; FERRARINI *et al.*, 2016; COUDERT *et al.*, 2014; JANIN *et al.*, 2012).

The electro-removal technique is based on the emergence of an electric field in a conductive solution created by a voltage source between the electrode terminals. The electromotive force acting between the terminals of the graphite electrodes through the action of the voltage source constitutes an electric field capable of polarizing the CCA salts. This is achieved by the production of polarization voltages between the molecules and also with the acid electrolytic conductive medium, guiding the movement of ions in the solution and "pulling" the CCA from the wood (TICIANELLI; GONZALEZ, 2015; BRETT; BRETT, 2000). The reactions involved in the electron translation process are:

$$CuO + C_2H_2O_4 \leftrightarrow 2\ CO_2 + H_2O + Cu$$
$$K_2Cr_2O_7 + C_2H_2O_4 \leftrightarrow H_2\ Cr_2O_7 + K_2\ C_2O_4$$
$$As_2O_3 + 2\ H_3PO_4 \leftrightarrow P_2O_3 + 2\ H_3AsO_4$$

This electric field is capable of mediating an electric force on the ions of the system, which provides electrokinetic movement (MERCER; FROSTICK, 2014; COUDERT *et al.*, 2014; CHRISTENSEN *et al.*, 2006). Thus, the cleaning agent used in the electro-removal process is provided by an electric current that transports the soluble ions (Cu, Cr, and As) that are present in the wood (SANTOS *et al.*, 2018; MOHAJERANI; VAJNA; ELLCOCK, 2018; FERRARINI *et al.*, 2016; TANG *et al.*, 2015; CHRISTENSEN *et al.*, 2006).

Energy dispersive X-ray fluorescence (EDXRF) is one of the techniques recommended by the American Association of Environmental Impact Researchers for Treated Wood, at the Bill Hinkley Center for Solid and Hazardous Waste Management, for quality-quantification of CCA in wood samples (FDEP, 2017). Furthermore, several studies use EDXRF for the quantification of compounds in the wood (BARATA *et al.*, 2013; COL; BUENO, 2009; SOLO-GABRIELE *et al.*, 2004).

In this sense, the electro-turning technique should not be confused with the process of adsorption of metals in liquid medium by sawdust of wood since it deals precisely with the process of removal of Cu, Cr, and As metals from wood treated with CCA. The metals removed from the wood (solid material) pass into an electrolytic fluid and can be used again in the production of CCA for the preservation of new wood or to be recovered from the medium by different methods (BABAEE; MULLIGAN; RAHAMAN, 2018; FERRARINI *et al.*, 2016; TANG *et al.*, 2015; JANIN *et al.*, 2012).

In this context, the objective of this study is to investigate the electro-removal of Cu, Cr, and As from CCA-treated wood waste by an electro-removal technique, aiming at the formation of biomass with low deleterious potential that allows multiple uses of the material or even its safe disposal in landfills.

METHODOLOGY

Protocol of clean techniques and preparation of solutions

Clean technique protocols were adopted for the cleaning of glassware and the preparation of storage containers. These were treated in Extran (10%, 24 h), triple washed with distilled water (Quimis®, Q341-25), bathed with nitric acid (10%, 24 h), and triple washed with ultrapure water. In accordance with the cleaning techniques protocol, the samples and other materials were manipulated in a granite worktop after being cleaned in order to minimize the effects of possible contaminations by chemical species according to the clean techniques protocol.

All reagents used were of analytical grade, and ultrapure water was used for the preparation of solutions (Millipore equipment, Simplicity — UV).

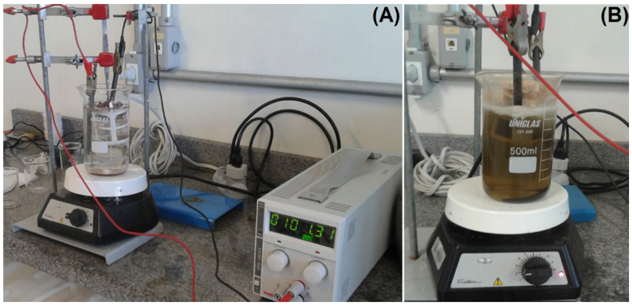
Collection and reparation of samples

The samples destined for the electro-removal treatment were obtained from a public lighting pole donated by the Procel Company of the Frederico Westphalen City. The CCA-treated wood from *Eucalyptus grandis* has been in use for over 20 years for electric power transmission lines. With the aid of an electric chain saw (Electric chain 2200 W Saber 18”45 cm — Tekna) the material was fractionated into smaller parts and sent to the joinery, where the chip reduction (< 2 cm in length) was performed with the aid of an equipment for shredding organic waste (Trapp brand, TR 200 model), as recommended by Christensen *et al.* (2006).

Parts of these samples were sent directly to perform the electro-removal process in different experimental conditions according to the factorial design. The remainder were stored in a double Ziplock (2 L) polypropylene bag, identified with labels (external/internal) made in pencil, and sent for further quantification of the average concentrations of the chemical species of interest.

Electro-removal treatments

The electro-removal treatment system used in this study was mounted on the bench scale (Figure 1) and operated through the use of a voltage source (Ametek, Sorensen, XPD 120–4.5). During the experiment, different electrical potentials were applied between the electrode terminals of graphite immersed in an electrolytic conducting solution, between which some mass of CCA-treated wood was deposited. Graphite was chosen as the base material of the conduction electrodes because it is an excellent conductive material to use in electrodes, it is cheap when compared to other types of materials and it also enables the reuse of the electrode without the loss of its capacity (MANOSSO, 2006).



Source: prepared by the authors (2019).

Figure 1 – Detail of the electro-removal experiment: (A) initial state of the treatment system, (B) final state after the treatment system.

The base of the system was formed by a magnetic stirrer that provided smooth movement of the electrolytic conductive solution in order to avoid excessive heating during the treatment. This study opted for the formulation proposed by Christensen *et al.* (2006), a mixture of oxalic acid and phosphoric acid, which is considered the one with the highest extraction yield.

For each test, samples were prepared in volumes of 450 mL using ultrapure water, 150 mL of oxalic acid at 5% concentration, and 300 mL of phosphoric acid at a concentration of 0.5 mol L^{-1}. Separate batches of wood samples were packed in a porous polypropylene filter system aligned between the terminals in order to prevent the free dispersion of the sample in the conductive solution.

Quantification of Cu, Cr, and As concentrations

Two control samples were obtained by the mechanical pruning of branches using a metal blade (machete), with a diameter greater than 50 and 250 mm in length, of a tree from the Eucalyptus grandis forest in the *Universidade Federal de Santa Maria* (UFSM) area. These were taken to the laboratory, where they were devoid of the internal and external peels and dried in a circulation oven at 50°C for 120 h. They were then fragmented into powder fractions (sawdust) and sent to mechanical screening in a sieve agitator system, with only fine fractions (< 0.063 mm) being used as white control (without CCA) and in production of reference material in this study.

For the analysis, data were taken in triplicate, with samples of 1 g of mass. Samples were fragmented in a knife mill equipment (Solab brand, SL 31 model), sieved in a mechanical sieve agitator system (Edutec brand, EEQ9029A model) for the separation of fine fractions (< 0.063 mm), and supported on a sample port (31 mm Closed X-Cell — SPEX) with thin film (*Mylar*, 6 µ-SPEX). Materials from the white samples (1 g mass; in fine fractions) were used as a reference, doped with 1 mL of Fluka brand ICP spectroscopic multi-element standard solution at concentrations of 50 ± 0.2 mg L^{-1} for chromium and 10 ± 0.04 mg L^{-1} for copper. Then, 1 ml of an Arsenic solution prepared at the concentration of

63.5 ± 2.7 mg L^{-1} was added following the dissolution of 2.095 mg of analytical standard reagent As_2O_3 (Vetec brand) in 2 ml of ultra-pure nitric acid (Merck label) and a 25 mL flask with aliquots of ultrapure water.

The fine fractions doping was deposited in the sample holder and dried in a circulation oven (40°C, 72 h) to ensure the solution penetration into the wood fibers. The quantification of the mean concentrations of Cu, Cr, and As chemical species was performed before and after the electro-removal treatment in an X-ray spectrometer by dispersive energy (Shimadzu brand, EDX-720 model), allowing the comparison of the results.

The operating conditions were: X-ray tube: Rh 3 kW, excitation: 15 kV for Si Ka and 50 kV for ULα, collimator: 10 mm, detector: Si (Li) cooled with liquid nitrogen, and integration time: 100 s. Simultaneous data collection in the wood samples was performed using the Fundamental Parameters Method (YOUNIS *et al.*, 2017; ROUSSEAU, 2013), with 15 retrievals of the reference standard to establish the mean analytical figures of merit, standard deviation, precision by determining the coefficient of variance (%), accuracy by determination of relative error (%), limit of determination of the method, and Z-Score test (INMETRO, 2018; SILVA *et al.*, 2016).

Experimental planning, statistical analysis, and graphic constructions

A factorial scheme depends, in part, on the range of factors investigated. Thus, differences at levels large enough to cause alterations in the responses were defined, allowing the evaluation of the experimental error (BARROS NETO; SCARMINIO; BRUNNS, 2001). The values of electrical voltage, treatment time, and mass of CCA treated wood were varied in order to allow an experimental arrangement by a factorial design 2^3, which allowed identifying the ideal operating conditions for the system. In addition, values inconsistent with the reality of the experimental apparatus were avoided, since they would make data acquisition impossible. Table 1 presents the experimental factors and the levels used in the study.

For data analysis and the creation of graphics, the open-source multi-platform SciDAVis software was used (BENKERT; FRANKE; STANDISH, 2007). The ANOVA and Tukey's pairwise test were performed in the PAST software, version 3.08 (HAMMER; HARPER; RYAN, 2001). The results of the study of the relationship between factors, levels, and interactions associated with the different treatments are presented in Table 2.

Table 1 – Factors and levels used in experimental planning.

Factors	Levels	
	(-)	(+)
1. Time (minutes)	20	60
2. Mass (grams)	5	15
3. Voltage (Volts)	10	25

Source: prepared by the authors (2019).

Table 2 – Factorial planning of electro-removal of Cr, Cu and As from CCA treated wood.

Treatment	Factor 1	Factor2	Factor 3	R1	R2	R3	Rm
(Chrome)	(Time)	(Mass)	(Voltage)	(mg kg^{-1})	(mg kg^{-1})	(mg kg^{-1})	(mg kg^{-1})
1	-	-	-	61.6	64.1	71	65.6
2	+	-	-	81.9	60.9	72.8	71.9
3	-	+	-	66.3	89.3	82.1	79.2
4	+	+	-	38.6	39.2	49.1	42.3
5	-	-	+	40.5	42.5	39	40.7
6	+	-	+	13.7	17	17	15.9
7	-	+	+	17.4	16.3	18	17.2
8	+	+	+	16.4	16.6	16.7	16.6
(Copper)							
1	-	-	-	27	27.8	28.2	27.6
2	+	-	-	31.4	24.7	29	28.4
3	-	+	-	32.3	41.7	39	37.7
4	+	+	-	18.2	18	20.3	18.8
5	-	-	+	15.5	16.1	15.1	15.6
6	+	-	+	10.3	13	13	12.1
7	-	+	+	15	15.1	16.3	15.5
8	+	+	+	21.4	30.9	15.7	22.7
(Arsenic)							
1	-	-	-	89.8	101.1	119.1	103.3
2	+	-	-	131.9	95.8	102.3	110
3	-	+	-	105.1	130.3	134.3	123.2
4	+	+	-	82	66.3	81.7	76.7
5	-	-	+	34.9	51.9	42.4	43.1
6	+	-	+	15.8	20.4	20.8	19
7	-	+	+	32.5	42	32.9	35.8
8	+	+	+	33.6	39.3	35	36

R1, R2, R3: Concentrations of Cr / Cu / As (Triplicate); RM: Mean concentrations of Cr / Cu / As.
Source: prepared by the authors (2019).

Identification of the fungal isolate

It is worth mentioning that the onset of the fungus *Xylaria* sp. in the samples occurred after treatment 6 (60 min, 5 g, 25 V) and 7 (20 min, 15 g, 25 V), suggesting that the method may be effective. The methodology for extracting DNA from isolates was described by Vicente *et al.* (2008).

The material was triturated and chloroform was added. The aqueous phase was collected, transferred to a new tube, and had ethanol added to precipitate the DNA. The DNA obtained was quantified by spectrophotometry (260 nm) and its integrity verified in 0.8% agarose gel.

Amplicons were purified with Exonuclease I and Alkaline Phosphatase (SAP) and sequenced using a BigDye Terminator Cycle Sequencing Kit v. 3.1 (Applied Biosystems, Foster City, CA, USA), according to the manufacturer's instructions. Reactions were purified with Sephadex G-50 (GE Healthcare BioSciences, Uppsala, Sweden) and the sequences were analyzed in an ABI Prism 3,500 DNA Sequencer (Perkin-Elmer, Norwalk, Foster City, CA, USA). The sequences obtained were edited with the BioEdit software (HALL, 1999) and compared to the reference sequences in the GenBank (Supplementary Material) datasets. The alignment was performed with MAFFT (KATOH *et al.*, 2005) and visual inspection was performed with the MEGA v7 software (KUMAR; STECHER; TAMURA, 2016). The best evolutionary model for each dataset was estimated using MEGA v7. A phylogenetic tree was constructed with 1,000 bootstrap replicates using the maximum probability method implemented in Mega v7.

RESULTS AND DISCUSSION

Analytical figures of merit and mean concentrations of analytes obtained by dispersive energy x-ray fluorescence spectrometry

The EDXRF technique was demonstrated to meet the requirements of the analytical applications according to the merit figures, ensuring the reliability of the results for the Cu, Cr, and As chemical species. Table 3 shows the results of the mean, confidence interval, precision CV (%), error (%), the determination limit of the method (DML), and the Z-Score test obtained from data on the standard sample.

The results presented in Table 3 show a good agreement between the material's reference and tracing, and also point to the precision of the chemical species analysis by the EDXRF technique, which was inferior to 20%, an acceptable standard for environmental samples (INMETRO, 2018). The error was estimated to be less than 10%, while the limit of determination of the method was compatible with the mean concentrations of the chemical species in the reference material (INMETRO, 2018; ROUSSEAU, 2013).

Table 3 – Analytical figure of merit of the process of taking data by EDXRF.

Analyte	MR (mg kg^{-1})	*x* (mg kg^{-1})	CV (%)	E (%)	DML	Z-score
Cu	10 ± 0.04	10.1 ± 0.3	3.4	0.6	0.3	0.1
Cr	50 ± 0.2	52.8 ± 1.7	6.4	5.7	8.9	0.8
As	63.5 ± 2.7	63.1 ± 1.6	4.9	-0.7	4.5	-0.1

EDXRF: energy dispersive X-ray fluorescence; MR: reference material; : mean concentrations in the reference sample; CV: precision; E: error; DML: determination limit of the method; Z-SCORE: Z-Score test.
Source: prepared by the authors (2019).

Experimental planning of the electro-removal technique

The best treatment for the removal of the chemical element chromium (Table 2) was the sixth treatment, which was performed with the longer time (60 min), the lower mass (5 g), and the higher electrical voltage between the terminals (25 V). This treatment resulted in an mean concentration of 15.9 mg kg^{-1}, providing greater removal of chromium than the control (mean concentration of 124.1 mg kg^{-1}), *i.e.*, a reduction of 87.2% over the initial concentration. The eighth electro-removal treatment, performed with the use of the longest time factor (60 min), the greatest mass (15 g), and the highest voltage between the terminals (25 V), provided an average after-removal concentration of 16.6 mg kg^{-1}. Finally, the seventh electro-removal treatment, performed with the shortest time (20 min), the greatest mass (15 g), and the highest tension (25 V), reduced the mean concentration to 17.2 mg kg^{-1}.

For Cu, it is highlighted that the seventh electro-removal treatment presented a mean concentration of 15.6 mg kg^{-1} after the treatment (Table 2). The treatment had shorter applied time (20 min), lower mass (5 g), and higher tension between the terminals (25 V). In relation to the control, the best electro-removal treatment for copper was the sixth. This treatment used the greatest time factor (60 min), the smallest mass (5 g), and the highest voltage between the terminals (25 V) and resulted in a mean concentration of 12.1 mg kg^{-1} in the treated wood, leading to a greater copper removal than the control (mean concentration of 59.1 mg kg^{-1}), *i.e.*, a reduction of 79.5% in relation to the initial concentration.

The highest reduction of mean arsenic concentration in the samples (Table 2) was evidenced in the seventh treatment, which used the lowest time factor (20 min), the largest mass (15 g), and the highest voltage between the terminals (25 V). This treatment resulted in a mean concentration of 35.8 mg kg^{-1} in the treated wood, providing greater removal of arsenic than the control (mean concentrations of 191 mg kg^{-1}), *i.e.*, a reduction of 81.3% relative to the initial concentration.

Thus, the electro-removal process proved to be more advantageous, mainly due to the use of graphite electrodes, which are low-cost materials and have a much shorter process time. These results corroborate those of Christensen *et al.* (2006) who, in electro-dialysis experiments under different conditions, obtained the removal of 87% of Cu, 81% of Cr, and 95% of As by means of electrolytic membranes and platinum electrodes. Pedersen *et al.* (2005), working on a small-scale pilot plant, found reductions of 82% of Cr, 88% of Cu, and 96% of As after 21 days of an electro-dialysis treatment with ion-exchange membranes in a glass chamber and platinum electrodes. In a 30-day treatment experiment, Ribeiro *et al.* (2000) obtained yields of CCA removal from treated wood in the order of 93% for Cu, 95% for Cr, and 99% for As due to process optimization of experimental conditions with the use of membranes and ion-exchange electrodes.

The factorial design shows that the treatment conditions for Cu, using greater time, lower mass, and higher tension obtained a better agreement, with a mean of 15.9 mg kg^{-1}. The best treatment for Cr, also due to the factors mentioned above, obtained a mean of 12.1 mg kg^{-1}. For As, the best conditions were also represented by greater time, lower mass, and higher tension. Considering these results, it can be concluded that the treatment conditions of the sixth electro-removal treatment were ideal for the removal of Cu, Cr, and As.

Figure 2 shows the efficiency of the electro-removal treatment for the removal of Cu (A), Cr (B), and As (C) chemical species in relation to the control samples. The most effective removals were observed in the 20 min, 5 g, and 25 V; 20 min, 15 g, 2 V; and 60 min, 5 g, 25 V conditions for Cu (A), Cr (B), and As, respectively, all related to treatment 6.

In addition, the study emphasizes the efficiency of the electro-removal treatment of chemical species by reducing Cu (by more than four times in the best treatment in relation to the untreated control), Cr (by more than five times in the best treatment in relation to the untreated control), and As (by more than ten times in the best treatment in relation to the untreated control). The ANOVA test (Table 4) demonstrates the existence of significant differences ($p < 0.05$) between the different treatments when considering each chemical species in relation to the untreated sample.

Electro-removal post-treatment biological responses

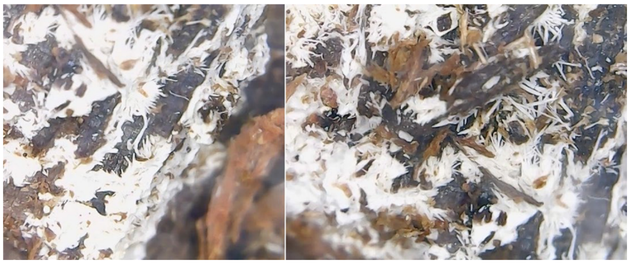
After the wood samples dried out in the circulation oven, it was observed that the formation of fungi occurred in treatment 6, a fact that may be associated with a biological response to the greater efficiency of this treatment in the removal of highly toxic elements present in the samples. Figure 3 shows the formation of fungi in the samples (image taken with a manual microscope; Wireless Microscope, magnification of 50–500x).

Based on the sequence analysis of the internal transcribed spacer (ITS) regions of the rDNA gene, it was possible to identify the isolated fungus *Xylaria* sp. This region is widely used for the identification of fungi because it is highly intra-specifically conserved but variable among different species, which makes it possible to distinguish it at the specific level (FUNGARO, 2000; MORESCHI, 2013). The phylogenetic tree was constructed by the Maximum Probability method implemented with the standard Kimura 2-parameter model. The proportion of completely indeterminate gaps and characters in this alignment was 0.22, with 33 distinct alignment patterns. The frequencies of the empirical basis were pi (A): 0.231, Pi (T): 0.231, Pi (C): 0.299, and Pi (G): 0.269 with 1,000 bootstrap replicates.

Table 4 – Anova test applied to the data of each of the treatments of electro-removal.

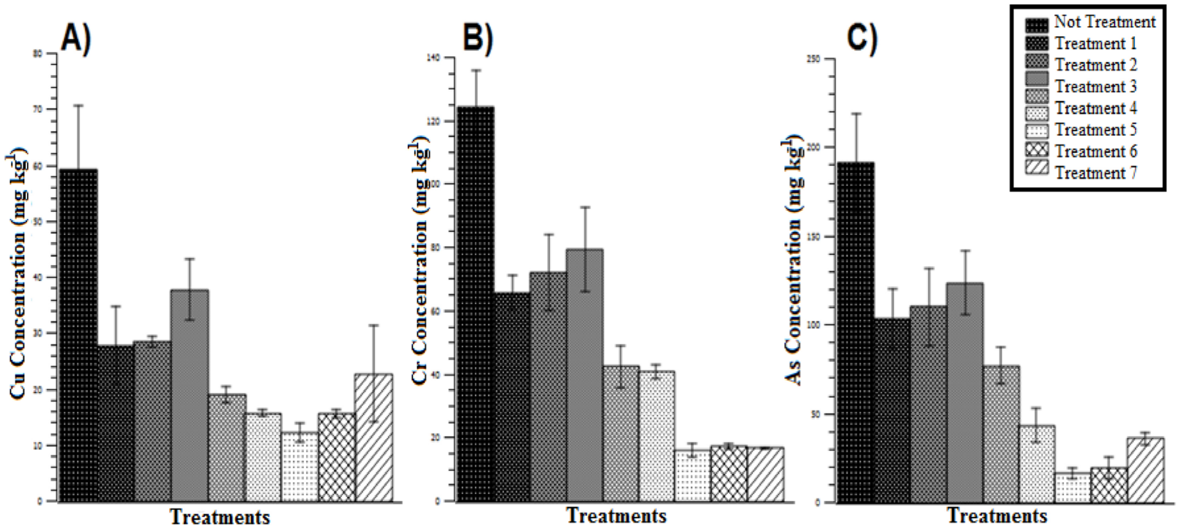
	CHROME			
	Sum of sqrs	*DF*	*F*	*p*
Between groups	31,530.8	8	84.3	1.1×10^{-12}
Within groups	841.847	18		
Total	32,372.7	26		
	CoPPER			
	Sum of sqrs	*DF*	*F*	*p*
Between groups	5,136.3	8	42.2	4.2×10^{-18}
Within groups	273.9	18		
Total	5,410.2	26		
	ArseniC			
	Sum of sqrs	*DF*	*F*	*p*
Between groups	73,761.2	8	50.8	8.7×10^{-11}
Within groups	3,267.41	18		
Total	7,7028.6	26		

df: degrees of freedom; *F*: statistics; *p*: probability.
Source: prepared by the authors (2019).



Source: prepared by the authors (2019).

Figure 3 – Formation of fungi.



Source: prepared by the authors (2019).

Figure 2 – Mean concentrations of (A) Cu, (B) Cr, and (C) As in the wood samples.

The Xylariaceae family is the largest of the Ascomycota phylum, comprising 86 genera, seven of which are uncertain, and more than 1,343 species (LUMBSCH; HUHNDORF, 2010; STADLER *et al.*, 2013). In Brazil, 24 generic names, 213 specific epithets, and 10 infra-specifics ones were listed (PEREIRA, 2015). Members of Xylariaceae play a functional role in terrestrial ecosystems, decomposing a wide range of substrates such as wood, leaves, seeds, and fruits. The great majority live as saprophytes soon after tree deaths, while other species act as phytopathogenic and endophytic (ROGERS, 1979; WHALLEY, 1985).

Included in the order Sphaeriales and the family Xylariaceae, *Xylaria* is classified as a white-rot fungus due to the degradation of the lignin present in the chemical constitution of the wood. It can sometimes be described as soft rot, requiring concise macroscopic and microscopic analyses and chemical characteristics to define the type of degradation.

In general, *Xylaria* sp. has been described as wood-degrading due to its habit of colonizing decomposing material and/or living as saprophytes of woody plants (ALEXOPOULOS; MIMS; BLACKWELL, 1996). As a general characteristic, the fungi of the species *Xylaria* sp. have black and rough clavulated stromas, with a diameter and length ranging from 2 to 5 mm and 5 to 7 cm, respectively, simple or branched, and sooty and white dotted surfaces.

CONCLUSIONS

The best Cr removal conditions were obtained with the highest stress, lowest mass, and longest time (60 min, 5 g, and 25 V) of treatment. After the treatment, a contaminated chemical residue composed of Cu, Cr, and As is generated. This residue can be processed for its reuse in the form of CCA preservative, which allows the restart of the treatment cycle of the wood.

Electro-removal, including the use of alternative materials that provide good results, produced reductions in Cu, Cr, and As mean concentrations of 79.5, 87.4, and 81.3%, respectively, than in other techniques commonly used for the removal of CCA. The electro-removal of the CCA preservative present in treated wood proved to be technically feasible. The results showed the proliferation of the fungus *Xylaria* sp. in the wood samples from treatment six, as well as the satisfactory removal of chemical species from the wood.

This study could represent the basis for other studies aiming to develop a pilot plant that could allow the study of the best treatments for larger amounts of CCA-treated wood.

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AUTHORS' CONTRIBUTIONS

Castro, R. J.: Formal Analysis, Investigation, Writing — Original Draft. Kehl, G. D.: Formal Analysis, Investigation, Writing — Original Draft. Candaten, L.: Investigation, Writing — Original Draft. Santos, G. D.: Data Curation, Formal Analysis, Investigation, Software, Validation, Writing — Original Draft. Silva, P. R. B.: Conceptualization, Data Curation, Formal Analysis, Investigation, Methodology, Project Administration, Software, Supervision, Validation, Visualization, Writing — Original Draft, Writing — Review & Editing. Trevisan, R.: Investigation, Writing — Original Draft. Wastowski, A. D.: Funding Acquisition, Resources.

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