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Application of textile (PAN-based) activated carbon fibers decorated with silver nanoparticles in water treatment

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Introduction: Water treatment deficit and poor health, hygiene and sanitation infrastructure can contribute to disease transmission by dissemination of contaminants and microorganisms. As an alternative, carbon-based materials coated with antimicrobial molecules have been proposed for water treatment, but few supporting data are available so far. Hence, this study investigates the potential use of PAN-based activated carbon fibers (ACF) decorated with silver nanoparticles in water treatment.

Methods: Silver nanoparticles were incorporated into the material using a cheap and electroless method. Field emission scanning electron microscopy (FEGSEM), Raman spectroscopy and X-ray photoelectron spectroscopy (XPS) characterized the whole material. The textile was mounted on a water filter prototype and had its capacity to remove bacterial (*Escherichia coli*) and fungal (*Candida albicans*, *Aspergillus niger* and *Penicillium funiculosum*) cells evaluated. Composition and toxicity of the filtered water were determined.

Results: Water filtered by Ag@ACF for 2 and 24 h contained 0.254 mg/L and 0.964 mg/L Ag, respectively. Ag@ACF filtering successfully removed *E. coli*, *C. albicans*, and *A. niger* from the suspensions, but not *P. funiculosum*. Treated water was non-toxic for Vero cells and *Drosophila melanogaster*, but toxic for *Raphidocelis subcapitata*. Ag@ACF showed efficient microbial elimination when applied in water treatment. Silver nanoparticles released in aqueous medium may be responsible for *R. subcapitata* toxicity. Future studies should be conducted to reduce silver nanoparticles release from the carbon fiber.

KEYWORDS

water treatment, decontamination, silver nanoparticles, antimicrobial effect, toxicity

1 Introduction

As a result of a combination among population growth, socio-economic development, and changing consumption patterns, the use of water has increased worldwide at a rate of approximately 1% per year since the 1980s (UNESCO, 2019). Access to water in quantity and quality is considered a basic human right and is essential to effective health protection

policies (UNESCO, 2017). Despite this, water has a unique ability to transmit diseases and can contain a wide variety of constituents that can be harmful to health. Unfortunately, 30% of the world's population does not have access to safe drinking water (UNESCO, 2019), in other word water with adequate quality for human use and consumption is becoming increasingly scarce.

Pathogenic microorganisms from infected humans and animals can enter the aquatic environment through waste or feces as a result of inadequate sanitation and can compromise water quality and health (Tran et al., 2015). UNESCO estimates that, on a global scale, more than 80% of wastewater is discharged without proper treatment into the environment, containing several microorganisms and contaminants that can cause diseases and imbalance in the ecosystem (UNESCO, 2017). In addition, 60% of the world's population does not have access to safely managed sanitation services (UNESCO, 2019). Health, hygiene, and sanitation infrastructure conditions are factors that can influence environmental contamination by different pathogens (Silva et al., 2016) and public health, affecting the quality of life and human rights (WHO, 2019).

The assessment of bacteria indicative of fecal contamination, such as *Escherichia coli* and *Enterococcus spp.*, in water bodies, is the standard methodology adopted worldwide for monitoring the microbial quality of water (WHO, 2017). The water delivery with these bioindicators can cause major threats to human health (WHO, 2017; Bianco et al., 2020), as problems of food poisoning, gastroenteritis, and urinary tract infections (Ahmed et al., 2019; Pichel et al., 2019).

Fungi can be abundant in water sources, can grow on the surface of materials in water supply systems and produce substances that result in unpleasant tastes and odors in water (WHO, 2017). For this reason, between the 2000s, the occurrence of filamentous fungi in water has been had special attention and are now are accepted as contaminants in drinking water (Hageskal et al., 2009; Oliveira et al., 2013). A study carried out by Oliveira et al. (2013) reported that *Penicillium* and *Aspergillus* were the most frequently isolated from surface water. However, to date, they are not considered in international water quality guidelines and are not monitored in water treatment facilities. It is not known how fungi develop in water, as this is not their natural habitat, but preventive measures are important and the presence of fungi in water may require adequate regulations (Hageskal et al., 2009).

Disinfection generally constitutes the final step of drinking water treatment and aims to eliminate pathogenic microorganisms causative of waterborne diseases, using methods such as ultraviolet radiation (UV), filtration, pasteurization, and chemical methods such as chlorination, chloramination, treatment with chlorine dioxide and ozonation. Chlorination is the most frequently used method (Pichel et al., 2019). However, these methods have limitations, such as efficiency, cost, product demand, and toxicity (Pichel et al., 2019). Disinfection effectiveness can also be affected by the presence of pathogens encased in flakes or particles, protecting them from the action of disinfectants (WHO, 2017). Considering that water is an indispensable resource for life, the interest of private and public sectors on these issues is very high, motivating the development of alternative and innovative methods for water treatment (WHO, 2017).

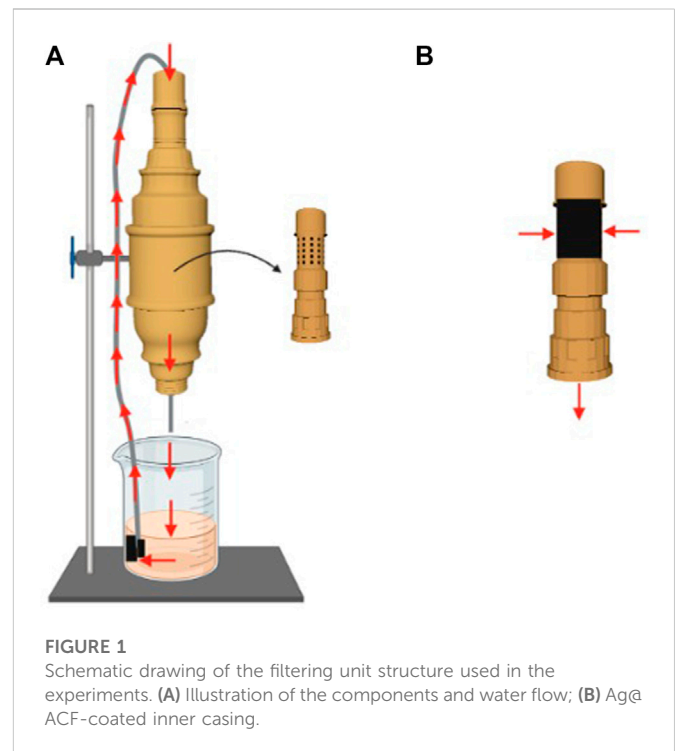


FIGURE 1
Schematic drawing of the filtering unit structure used in the experiments. (A) Illustration of the components and water flow; (B) Ag@ACF-coated inner casing.

Within this context, the activated carbon fiber (ACF) based on textile polyacrylonitrile polymer (PAN) is a fiber intended for the production of clothing and fabrics in general and represents a low-cost raw material with the potential for transformation into an activated fiber with a high surface area, a high fraction of micropores (Marcuzzo 2016) and high strength (Lee et al., 2014). ACF-PAN textile is a product of varied applicability, with fibers that have a microporous structure, giving it faster and more selective adsorption kinetics to molecular contaminants, standing out in the removal of water pollutants (Marcuzzo, 2016; Lee et al., 2014).

The high surface area and micropore fraction make the material interesting for the adsorptive function, which favors the adhesion of fungi and bacteria. However, microbial proliferation due to adhesion can occur in polymer fiber mesh (Abrigo et al., 2015) and ACF felts (Yoon et al., 2008), which may represent a long-term disadvantage. Thus, antibacterial and antifungal coating agents, such as silver (Park and Jang, 2003), may be advantageous. Silver (Ag) is an effective broad-spectrum antimicrobial agent, capable of causing cell damage and oxidative stress. In addition, it has long-term stability and activity that advantageously assists in the application of coatings for medical devices, bone prostheses, dental compounds and water and air filters (Chaloupka et al., 2010). Studies have shown that detachment of Ag from commercial products to wastewater and the aquatic environment may occur, raising concerns about possible effects of silver on biota (Benn et al., 2010; Lorenz et al., 2012; Kalantzi et al., 2019).

Thus, this study aimed to evaluate the potential application of activated carbon felt produced from PAN and decorated with silver nanoparticles for water treatment, by assessing the microbial retention efficiency and toxicity of the filtered water.

2 Materials and methods

2.1 Synthesis of activated carbon fiber (ACF)

Activated carbon fibers were produced from oxidized PAN textile fiber felts carbonized in an argon atmosphere at 900°C for 20 min and activated in a CO₂ plus water vapor atmosphere at 1000°C for 2 h. Electroless plating was performed by submerging part of the ACF in a solution containing silver nitrate (AgNO₃) at a ratio of 2 g of metal/L in deionized water (DI) for 24 h, then washed with deionized water and dried in a vacuum oven at 100°C for 4 h for AgNPs incorporation on the felt surface (Ag@ACF) (Rodrigues et al., 2018).

2.2 Characterization of activated carbon fiber (ACF)

Samples underwent chemical and morphological characterization. Field emission scanning electron microscopy (FEG-SEM) was performed using a TESCAN MIRA3; Raman spectroscopy using a Horiba Labram Hr Evolution, with a 514.6 nm laser; and X-ray photoelectron spectroscopy (XPS) using a Kratos Axis Ultra XPS analyzer, with monochromatic Al-K α X-ray radiation (1486.5 eV). Vacuum was stabilized at 10⁻⁷ Pa. Mean diameter of Ag particles present on the Ag@ACF surface was determined by analyzing 30 particles based on Ag@ACF FEG-SEM sample images using Image J software (United States, version 1.53a).

2.3 Filtration unit

Antimicrobial action was analyzed using a PVC filter unit, made according to the schematic design shown in Figure 1. Inner area consisted of a perforated tube coated with an Ag@ACF sample (area 3 cm × 15 cm). The outer side comprised a protective casing connected to a hydraulic pump that allows water recirculation at a flow rate of approximately 3.33 L/min.

2.4 Detection of chemical elements in water sample filtered by Ag@ACF

Water filtered by Ag@ACF for 2 and 24 h was analyzed via ICP Optima 7300 DV Spectrometer to verify and quantify the presence of chemical elements using 188–670 nm wavelengths. 0.5 M nitric acid was added to the treated water for sample conservation. Presence of chemical elements in the solution was compared with the concentration established by the WHO's Guidelines for Drinking Water Quality (2017) and CONAMA—National Council for the Environment and Resolution, 2005 Distilled water was used as a negative control.

2.5 Evaluation of Ag@ACF microbial retention efficiency

Reference strains of *Escherichia coli* (ATCC 10799) and *Candida albicans* fungi (ATCC 18804), *Aspergillus niger* (CCIBT 2261), and

Penicillium funiculosum (ATCC 8725) were used. For *E. coli* and *C. albicans*, the strains were plated on Brain Heart Infusion (BHI) agar and Sabouraud dextrose (SD) agar, respectively, and incubated at 37°C for 24 h. Then, spectrophotometrically standardized suspensions in saline solution (0.9% NaCl) containing 10⁹ cells/ml ($\lambda = 530$ nm and OD = 1.306) for *E. coli* and 10⁶ cells/ml ($\lambda = 530$ nm and OD = 0.380) for *C. albicans* were diluted in BHI broth to a concentration of 10⁷ cells/ml for bacteria and 10⁵ cells/ml in SD broth for fungal species.

Suspensions were then filtered separately through a sterile Ag@ACF mounted on the filtration unit. Samples were analyzed at 15, 30, 45, 60, 120 min, and 24 h after filtration. After serial dilution, filtrates were plated onto specific culture media (BHI agar for *E. coli* and SD agar for *C. albicans*), and the number of colony forming units per milliliter (CFU/mL) was determined after 24-h incubation at 37°C. All tests were performed in triplicate.

Filamentous fungi were analyzed according to Cronin et al. (2014). Standardized suspensions of 10⁵ spores/ml in RPMI 1640 broth and 10⁴ spores/mL in SD broth for *A. niger* and *P. funiculosum*, respectively, were obtained in a Neubauer chamber. Ag@ACF filtration occurred for 2 h. Samples were collected every 30 min and quantified in a Neubauer chamber to verify the number of spores/mL. We plated 20 μ l of *A. niger* and *P. funiculosum* solutions onto potato and SD agar, respectively. Plates were incubated at 30°C for 7 days, for visual analysis of fungal growth regarding hyphal expansion and pigmentation variation. All tests were performed in triplicate.

2.6 Field emission scanning electron microscopy (FEG-SEM) of Ag@ACF after filtration

After final filtration of the contaminated solution, Ag@ACF samples were fixed with glutaraldehyde for 24 h, followed by dehydration at increasing ethanol concentrations for 1 min, and then analyzed by field emission scanning electron microscopy to visualize the adhesion and morphology of the studied microorganisms.

2.7 Toxicity analysis of Ag@ACF-filtered water samples

Ag@ACF-filtered water toxicity was evaluated using three experimental models: cytotoxicity for Vero cells, and ecotoxicity for *Drosophila melanogaster* and *Raphidocelis subcapitata*.

2.7.1 Cytotoxicity evaluation for Vero cells

Following ISO 10993-5 methodology, 200 μ l of a suspension containing 10⁴ Vero cells (ECACC 84113001) were seeded into a 96-well plate and exposed to Dulbecco's modified Eagle's medium (DMEM) enriched with 10% inactivated fetal bovine serum (FBS) and supplemented with penicillin/streptomycin 1% Ag@ACF-treated medium. For analysis, the DMEM medium was filtered through Ag@ACF for 2 and 24 h. Cell viability was determined 24 and 48 h after exposure to Ag@ACF-treated medium (2 and 24 h) using MTT assay (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium; Sigma, St Louis, United States) at 570 nm (EL808IU, Biotek, Vermont,

United States). Two separate experiments were performed in triplicate. Results were expressed as percent viable cells (%), using the number of cells grown in wells without exposure to Ag@ACF-treated DMEM medium as a negative control.

Cell viability percentages were classified as: 0 (non-cytotoxic), no reduction in cell growth; 1 (mild), only slight observable growth inhibition; 2 (mild), no more than 50% growth inhibition; 3 (moderate), more than 50% observable growth inhibition; 4 (severe), almost complete or complete destruction of cell layers. Negative control was set as 100% cell viability (ISO 10993).

2.7.2 Toxicity evaluation for *Drosophila melanogaster*

Evaluation of treated water toxicity for the *D. melanogaster* model was performed according to Sampaio et al. (2018) using 4–10-day old wild type (WT) Canton-s females. Distilled water treated by Ag@ACF for 2 and 24 h was used to prepare the base medium for fly food, replacing the distilled water making up the original base medium. For the analysis, 80 flies were randomly divided into four independent experimental groups and previously stored in tubes for 6 h without food. They were then transferred to tubes with medium containing Ag@ACF-treated water (treatment group) and untreated water (control group). Ecotoxicity was analyzed for eight consecutive days, checking the number of survivors to obtain a survival curve.

2.7.3 Toxicity evaluation for *Raphidocelis subcapitata*

Chlorophyceae *R. subcapitata* was cultivated, in an exponential growth phase, on a sterile L.C Oligo culture medium (AFNOR—Association Française de Normalisation, 1980) without ethylenediaminetetraacetic acid (EDTA) inside Erlenmeyer flasks. The culture was kept under illumination (with a 12/12-h photoperiod) of $80 \mu\text{mol m}^{-2} \text{s}^{-1}$ and controlled temperatures at $25^\circ\text{C} \pm 2^\circ\text{C}$ (ABNT Brazilian Association of Technical Standards, 2011). Algae cells were collected by centrifugation and counted in a Neubauer chamber. Treatment was performed at the following concentrations: a) 100% culture medium (control), b) 50% treated water and 50% culture medium, and c) 100% treated water. All treatments used a cell density of 5×10^4 cells/ml, in a final volume of 100 ml, stored in a polycarbonate Erlenmeyer flask. Performed in triplicate and under aseptic conditions, the experiments were conducted in 96 h under the same photoperiod, light intensity and temperature conditions used to maintain the microalgae. We extracted 2 ml aliquots of the samples at the beginning and end of the experiments. An optical microscope evaluated algal density by cell counting in a Neubauer chamber at 400× magnification. Microalgae growth rate in the different treatments was calculated according to Fogg (1975). The toxic effect referred to the reduction in algal growth rate values after treatment with Ag@ACF-treated water in the filtration unit, compared to that obtained in the control (L.C. Oligo medium).

2.8 Statistical analysis

Statistical analysis was performed via GraphPad Prism 7.0 and Toxstat 3.3 software. Normality tests were performed. Ag@ACF filtering potential data relative to baseline ($t = 0$) and scores obtained at each evaluation period were compared using the Kruskal–Wallis test, followed by Dunn's post-hoc multiple comparison test for *E. coli*, *C.*

albicans, *A. niger*, and *P. funiculosum*. Toxicity results from the *D. melanogaster* model were evaluated by a log-rank test (Mantel–Cox) to compare the survival curve plotted via Kaplan–Meier test. Toxicity for *R. subcapitata* was evaluated by ANOVA, followed by Tukey's post-hoc test. Significance level was set at 5%.

3 Results

3.1 Material characterization

3.1.1 Field emission scanning electron microscopy (FEG-SEM)

Both ACF and Ag@ACF samples analyzed by FEG-SEM showed similar morphologies: massive needled felt with grooves all over their surface, probably from their manufacturing process and porosity (Figure 2) (Rodrigues et al., 2018). We can also observe that ACF has a slightly rough and intact surface with no apparent defects. In the Ag@ACF sample, we can see white dots scattered throughout the filaments, possibly related to Ag deposition. Ag deposition did not alter the morphology of the felt filaments, and presented a non-uniform particulate distribution and mean particle diameter of 121 ± 0.062 nm.

3.1.2 Raman spectroscopy

Regarding the Raman shift spectra of both felt samples, we observed two bands characteristic of amorphous carbon materials: the D band at approximately 1350 cm^{-1} and the G band at approximately 1600 cm^{-1} (Figure 3A). These bands provide information about the degree of disorder of the material and possible defects in its carbon structure (D band), as well as about its degree of graphitization (G band) (Cançado et al., 2004; Claramunt et al., 2005).

3.1.3 X-ray photoelectron spectroscopy (XPS)

We performed XPS analysis and high-resolution spectra. Figure 3B shows the elements present on the surface of the samples, from which we quantified the concentrations for each element identified (Table 1). After spontaneous deposition of Ag, XPS identified this element on the surface of the Ag@ACF sample, in addition to oxygenated functional groups. Figures 3C, D show the high-resolution spectra of Ag present in ACF and Ag@ACF, respectively. In case of deposition, Ag^0 or metallic silver was identified only in the Ag@ACF sample (Tjeng et al., 1990). According to the XPS spectra, we can observe C–OH and/or C–O–C functional groups on the carbon felt surface, which act as the main active sites for deposition of silver nanocrystals. Thus, Ag^+ ions (from the aqueous AgNO_3 solution) are perhaps electrostatically adsorbed onto the carbon fiber surface and then reduced by these oxygenated functional groups (Vanitha et al., 2015).

3.2 Detection of chemical elements in the water sample filtered by Ag@ACF

According to spectrophotometric analysis, silver (Ag) showed the highest concentrations (0.25 mg/L – 0.96 mg/L at 2 h and 24 h, respectively). Important for *R. subcapitata* development, we also analyzed copper (Cu) (0.02 – 0.04 mg/L at 2 and 24 h, respectively), zinc (Zn) (0.11 – 0.09 mg/L at 2 and 24 h, respectively), phosphorus (P) (0 mg/L for both times). Supplementary Table S1 summarizes all the analyzed elements.

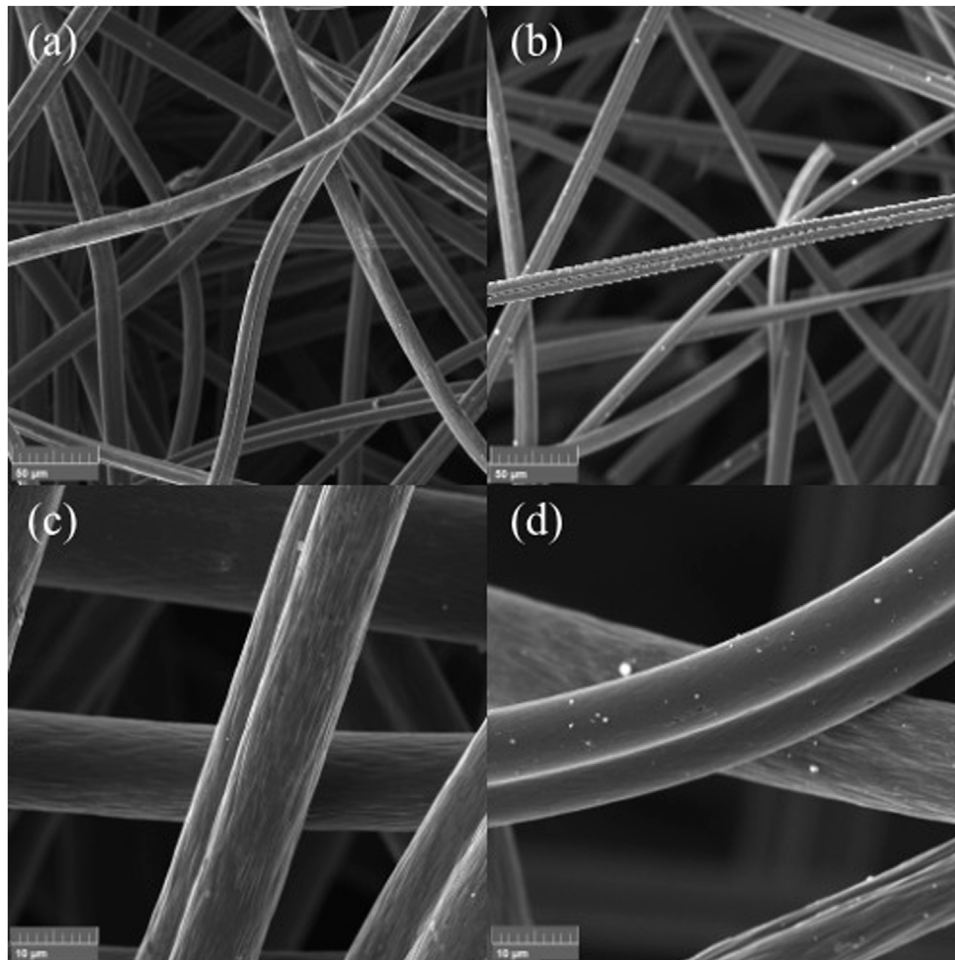


FIGURE 2
ACF and Ag@ACF FEG-SEM micrographs: (A) ACF at 1kx magnification; (B) Ag@ACF at 1kx magnification; (C) ACF at 5kx magnification; (D) Ag@ACF at 5kx magnification.

3.3 Evaluation of Ag@ACF microbial retention efficiency

We investigated the microbial reduction by Ag@ACF filtration of suspensions containing *E. coli*, *C. albicans*, *A. niger* and *P. funiculosus*. Figure 4 shows the results of microbial counts after Ag@ACF filtration from 15 min to 24 h of treatment. Its capacity to reduce *E. coli*, *C. albicans*, and *A. niger* counts was time dependent. Supplementary Table S2 presents the mean, standard deviation and median of the antimicrobial effect of Ag@ACF.

We identified a significant reduction of 97.8% in *E. coli* counts after a 24 h ($p = 0.0012$) filtration compared to control (0 h) (Figure 4A). However, reduction was observed between 30 min ($p = 0.0004$) and 1 h ($p = 0.0076$) when compared 24 h of filtration with 22.3 and 40.4% of bacterial reduction, respectively. *C. albicans* counts showed a significant reduction after 24 h (100% reduction) of treatment when compared to the control ($p = 0.0290$) group (Figure 4B). Similarly, *A. niger* presented significant reduction at 1.5 h ($p = 0.0022$; 99.4% of reduction) and 2 h ($p = 0.0006$; 99.8% of reduction) compared to control (Figure 4C). For *P. funiculosus* (Figure 4D), significant difference was observed only after 30 min ($p = 0.0123$), however there was no reduction observed in filtration process.

We evaluated the effect of Ag@ACF filtration on *A. niger* and *P. funiculosus* suspensions by visual analysis (Figure 5). Fungal growth showed reduction between 30 min and 2 h for *A. niger*, but a similar effect was not observed for *P. funiculosus*. These results are in agreement with the statistical analysis. Moreover, during *P. funiculosus* growth, we observed variations in the pink pigmentation of the colonies, with yellow halos and flattening of the central area (black and gray arrows). The white and, filamentous outer margin showed a reduction in that edge over time, demonstrating changes in colony expansion (yellow arrow).

3.4 Field emission scanning electron microscopy (FEG-SEM) of Ag@ACF after filtering

Micrographs of the Ag@ACF sample after final filtration of the *E. coli*, *A. niger*, and *P. funiculosus* suspensions were taken. Figure 6A shows the arrangement of the carbon fibers in the textile. Figure 6C shows *E. coli* cells retained on the felt without morphological changes, whereas Figure 6D shows unaltered bacterial cells. Figure 6B shows a matrix possibly formed by the reaction between the carbon fibers and

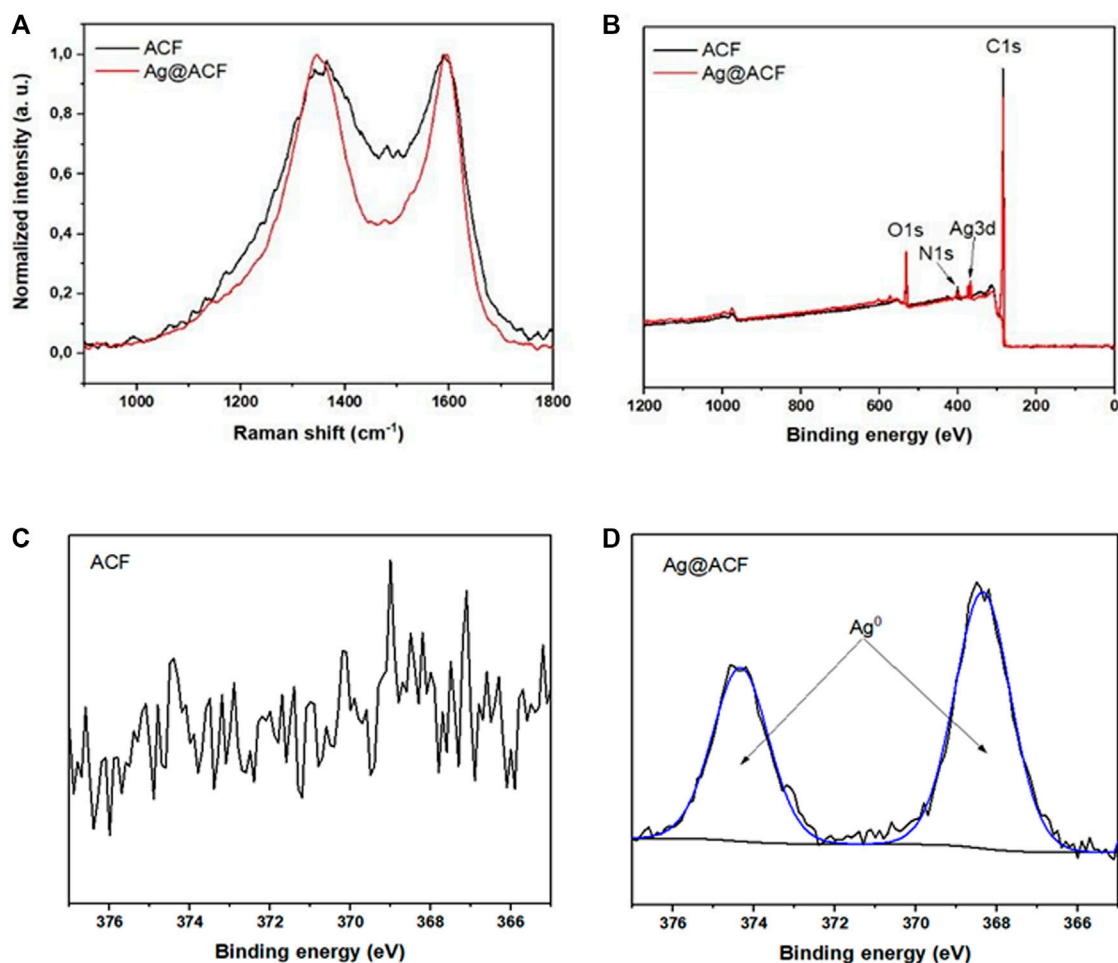


FIGURE 3
Spectral analyses: (A) Raman spectrum of ACF and Ag@ACF; (B) XPS spectra of ACF and Ag@ACF; (C) High resolution spectrum of ACF; and (D) Ag@ACF samples for Ag3d.

TABLE 1 Quantification of the atomic percentage of species found on the ACF and Ag@ACF surface.

	C1s (%)	O1s (%)	N1s (%)	Ag3d (%)
ACF	91.8	5.00	3.17	0
Ag@ACF	90.7	7.74	1.04	0.53

the culture medium, resulting in a layer of organic matter, which may have prevented the visualization of Ag particles.

3.5 Toxicity analysis of Ag@ACF-filtred water samples

3.5.1 Cytotoxicity evaluation for Vero cells

Cell viability after 24 h of exposure in DMEM medium treated with Ag@ACF for 2 h was classified as grade 0 (non-cytotoxic) and in DMEM medium treated with Ag@ACF for 24 h as grade 2 (mild). After 48 h of exposure at both treatment times, cell viability was

classified as grade 1 (mild) with 87% viability when exposed to the 2-h treatment and 85% viability when exposed to the 24-h treatment (Figure 7).

3.5.2 Toxicity evaluation for *Drosophila melanogaster*

D. melanogaster flies fed water filtered through Ag@ACF for 2 (Figure 8A) and 24 h (Figure 8B) showed survival rates of 96.67% and 100%, respectively, allowing to classify the water sample as non-toxic.

3.5.3 Toxicity evaluation for *Raphidocelis subcapitata*

Within 2 h of treatment, the mean algal growth rate obtained at 50% (-0.02 days^{-1}) and 100% (0.10 days^{-1}) were significantly lower ($p < 0.05$) compared to that obtained for the control group (0.59 days^{-1}) (Figure 9A). We observed a similar effect for the 24-h treatment, where the mean values of microalgae growth rate at 50% and 100% (-0.05 days^{-1} and 0.04 days^{-1} , respectively) were significantly lower ($p < 0.05$) that recorded in the control treatment (Figure 9B).

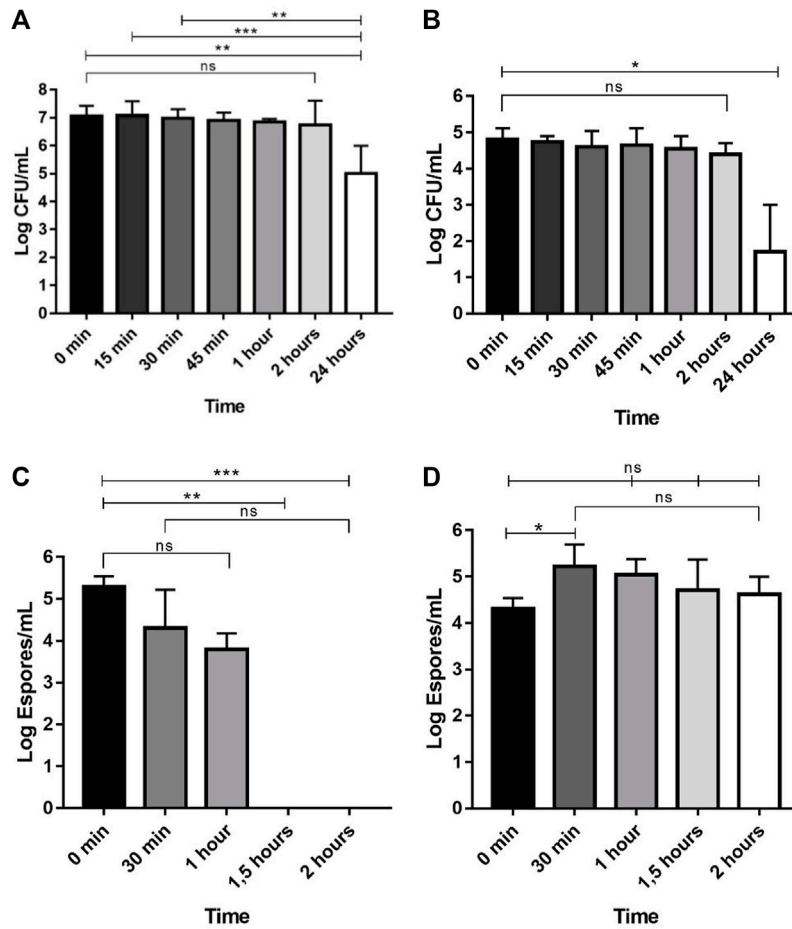


FIGURE 4

Antimicrobial activity of (A) *E. coli*; (B) *C. albicans*; (C) *A. niger*; (D) *P. funiculosus* after treatment with Ag@ACF filtration at different times. The graphs show the median and range data. Data were analyzed by ANOVA using the Kruskal–Wallis test, followed by Dunn’s post-hoc multiple comparison test. (n.s. = not significant; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$).

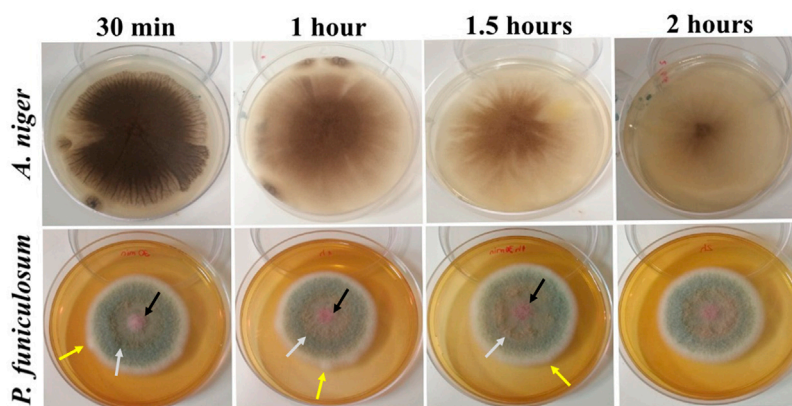


FIGURE 5

Fungal growth after treatment with Ag@ACF filtration from 30 min to 2 h. Ag@ACF filtration showed a strong antifungal effect for *A. niger*, with effective growth reduction over time. *P. funiculosus* had a slight reduction effect with a pink central area and diffuse high to flat growth (black and gray arrows) over treatment time and a slight growth reduction of the outer margin (yellow arrow).

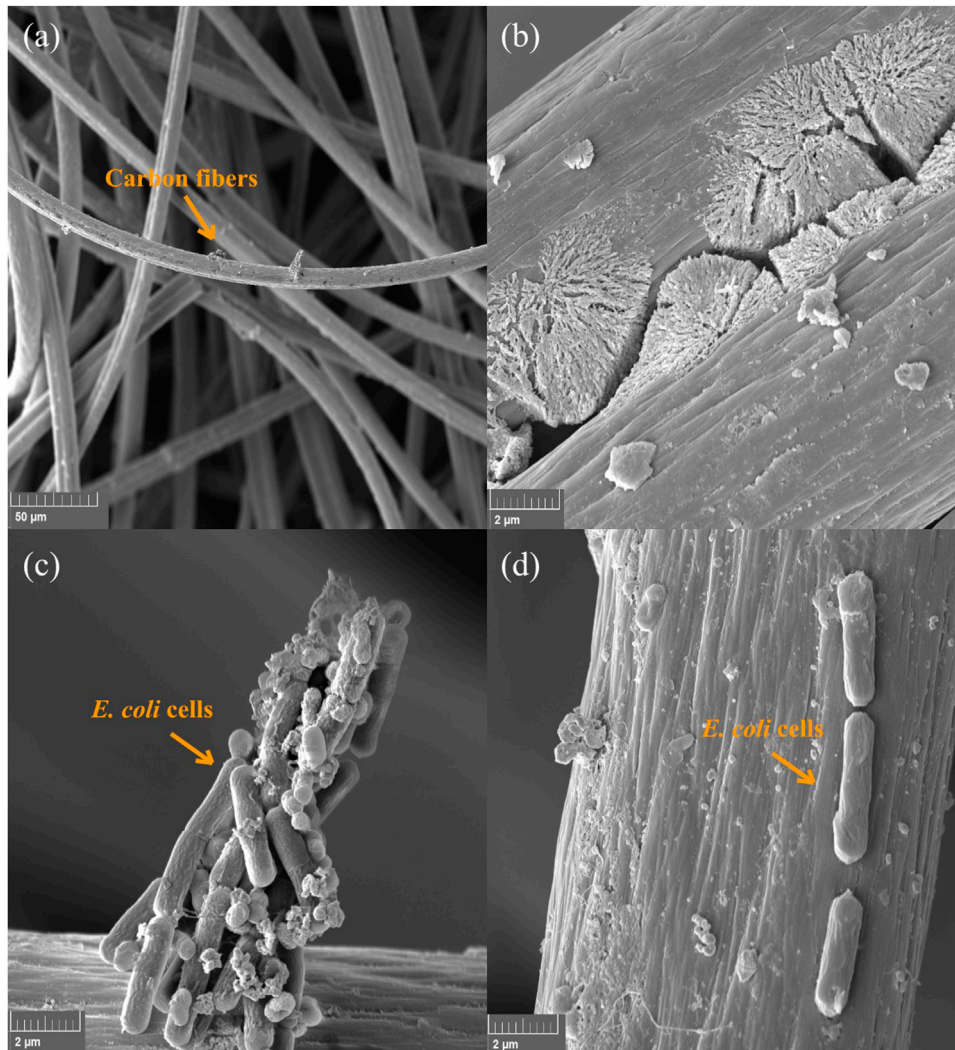


FIGURE 6

FEG-SEM micrographs at 1kx and 20kx magnification. In (A) Carbon fibers (1.00 kx); (B) Matrix possibly formed by the reaction between the carbon fibers and the culture medium (20.0 kx); (C) *E. coli* cells without morphological changes (20.0 kx); (D) *E. coli* cells retained unchanged in the formed matrix (20.0 kx).

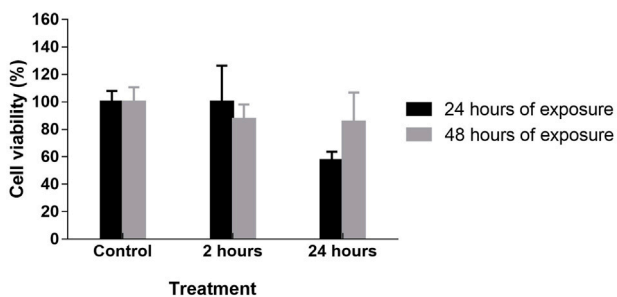


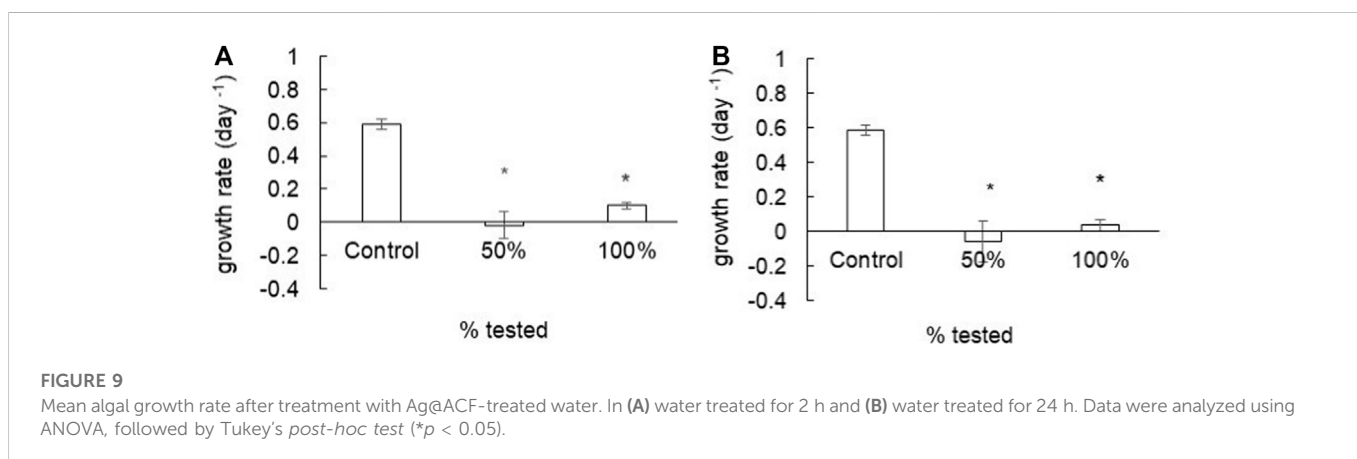
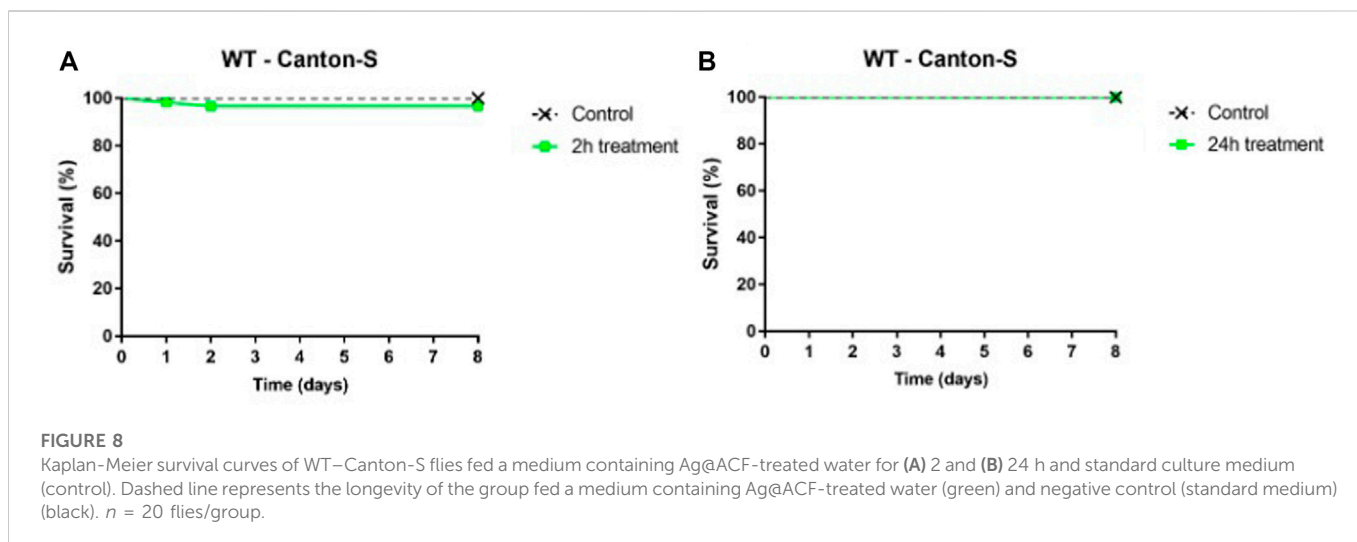
FIGURE 7

Analysis of Ag@ACF cytotoxicity expressed by cell viability percentage of Vero cells, DMEM treated for 2 and 24 h. Cytotoxicity assessed by MTT assay and cell viability evaluation verified after 24 and 48 h of exposure in Ag@ACF-treated DMEM medium.

4 Discussion

This study incorporated Ag particles into PAN-based activated carbon nanofibers to improve particle adsorption and microbial reduction. The analyses performed showed a heterogeneous Ag impregnation distribution along the fibers, which may have positively influenced the antimicrobial activity, since the NPs dispersed on the material's surface might be more accessible to microorganisms (Khare et al., 2014; Arakawa et al., 2019).

Ag@ACF filtration of microbial suspension for 24 h showed time-dependent reduction in *E. coli* and *C. albicans* with microbial reduction of 97.8% and 100%, respectively. *A. niger* fungal filtration showed a 99.4 and 99.8% reduction after 1.5 and 2 h filtration. No growth inhibition was detected for *P. funiculosus* over the analyzed times. A similar result was observed in a study carried out by Fujioka et al. (2019) which evaluated the removal of an industrial scale sand



filter in a drinking water treatment plant and observed a microbial reduction of 95.2%–99.3%. Similarly, a filtration of *E. coli* suspension with a ceramic filter in the absence and presence of colloidal silver obtained 97.8 and 100% reduction, respectively (Oyanedel-Craver and Smith, 2008).

Our study aimed at the impregnation of silver in ACF as an antimicrobial material during the filtration process. Silver nanoparticles have demonstrated high antimicrobial efficacy against different microbial species (Cavassin et al., 2015) and demonstrated action in altering efflux pumps, alteration, and accumulation in membranes, causing rupture and leakage of intracellular content, in addition to internal damage when in contact with the genetic material (DNA) (Bruna et al., 2021). However, the antimicrobial and cytotoxic action of AgNP may be dependent on the nanoparticle size (Jeong et al., 2014). Antimicrobial activity in different sizes of Ag nanoparticles was also observed by Oya et al. (1996) on activated carbon fibers containing 100–200 nm Ag particles for water filtration. Zhang et al. (2015) observed antimicrobial activity on multiple species, such as *S. aureus*, *E. coli*, and *C. albicans* of activated carbon fiber impregnated with Ag particles ranging from 20–40 nm. Bacterial inhibition of *E. coli* was observed in ACF covered by different concentrations of Ag and average particles ranging from 12.13 to 15.52 nm (Yoon et al., 2008). Inhibitory effect was also observed for *A. niger* after contact with nanocomposite films

dispersed with AgNPs with a mean diameter of 8.6 nm (Pinto et al., 2013). No studies have been found so far that have studied the contact between *P. funiculosum* and AgNP.

Although Ag@ACF filtration was ineffective in inhibiting *P. funiculosum*, we observed colony morphological changes with variation in colony pigmentation and outer margin, suggesting possible Ag@ACF-related alterations. We found no previous research on the Ag mechanism of action on *P. funiculosum* and its possible consequences on pigmentation. Future studies are needed to verify its causes and other effects on the fungus phenotype.

Its potential application in water treatment was also evaluated by toxicity tests using different *in vitro* and *in vivo* models representative of different biological complexities. First, we performed cytotoxicity tests on Vero cells, widely applied to evaluate the effects of chemicals and toxins (Ammerman et al., 2008). Next on *D. melanogaster*, a fast-living, reliable, robust, economically viable model organism that has been successfully studied to investigate the toxicity of nanomaterials, including AgNPs (Alaraby et al., 2015). And lastly on *R. subcapitata*, a primary producer present in freshwater and a reference species for studying metal toxicity (Expósito et al., 2017; Kleiven et al., 2019).

Water filtered for 24 and 48 h were non-cytotoxic and slightly cytotoxic for Vero cells, respectively. Pannerselvam et al., 2021

obtained an IC₅₀ of 20 mg/L for AgNPs (70–90 nm) and IC₅₀ of 5 mg/L for AgNO₃. Quintero-Quiroz et al. (2019) reported a median lethal concentration (LC₅₀) of 19.11 mg/L after a 24-h exposure of Vero and NH3T3 cells to AgNPs (diameter ~10.30 nm). These authors observed viability above 80% at concentrations below 5 µg/ml. Thus, it appears that IC₅₀ and LC₅₀ obtained with smaller sized AgNPs are higher than the concentrations of 0.25 and 0.96 mg/L evaluated in the present study. Differences in concentrations and toxic effects may be related to different AgNPs preparation methods, particle size, and exposure time (Saleh et al., 2020).

Water samples filtered for 2 and 24 h by Ag@ACF showed no toxic effect for *D. melanogaster*. Previous studies reported *D. melanogaster* toxicity only at higher concentrations than those used in the present study. Acute toxic effect on flies occurred at a concentration of 20 mg/L (29 ± 4 nm), at which 50% of the flies tested showed developmental difficulties during pupae release, did not complete the development cycle, and presented changes in body pigmentation (Panacek et al., 2011). At the concentration of 100 mg/L, 97% of the larvae died and no pupae formed. However, Panacek et al., 2011 prepared AgNPs via microencapsulation using mannitol as the encapsulating agent, which may have increased its ingestion by flies. Similar result was reported by Ahamed et al. (2010), in which AgNPs at 50–100 mg/L (10.91 ± 4.58 nm) induced the heat shock protein 70 expression, oxidative stress and apoptosis in *D. melanogaster* cells.

Results showed a toxic effect for *R. subcapitata* with significant reduction in the average growth rate by 50% and 100%. Among the chemical elements detected in the treated water analysis, Cu, Fe, Mg, P, Se, and Zn can be considered nutrients for *R. subcapitata*, depending on the concentration. In concentrations above those considered essential, however, these elements can be toxic for the organism. According to the literature, the 96-h EC₅₀ value of Cu for *R. subcapitata* is between 0.071 and 0.137 mg/L (Oliveira-Filho et al., 2004), 96-h EC₅₀ of Zn for the species is 0.179 mg/L (Rodgher et al., 2020), and the limits of trophic state levels for P can range from 0.306 to 77.02 mg/L (eutrophic or hypereutrophic environment) (Tito and Luna, 2020; Cunha et al., 2021). The concentrations of the chemical elements detected in the samples were below those considered toxic to microalgae; thus, even with the presence of elements that favor microalgae development, mainly P, the presence of Ag at concentrations above the tolerable level would result in a toxic effect.

Wang et al. (2012) evaluated the toxicity of three commercial AgNPs and AgNO₃ materials in different aquatic organisms including *R. subcapitata* and obtained EC₅₀ values of 0.29 µM, 8.31 µM, 37.14 µM and 196.55 µM in tests with AgNO₃ (free ions), DIS-nAg (dispersant-prepared AgNPs–15 nm), PVP-nAg (coated AgNPs–80 nm) and Bare-nAg (uncoated AgNPs–35 nm), respectively. These results pointed to an inverse correlation between particle size and its toxic effect, showing that the smaller the particle size, the greater the observed toxic effect. Moreover, both AgNPs and Ag ions affect organisms, with the latter having the greatest effect. Kleiven et al., 2019 observed similar effects, obtaining greater growth reduction for *R. subcapitata* on exposure to 0.008 and 0.025 mg/L AgNO₃. These studies show that both metallic and ionic Ag act on organisms; however, Ag speciation is considered more relevant for toxicity than total metal concentration (Köser et al., 2017). Research also indicates that Ag ions contribute greatly to the toxicity of *R. subcapitata*, which may be caused by the high ion accumulation capacity of microalgae, since the bioconcentration of ions in phytoplankton is higher than in zooplankton or fish (Fortin et al., 2001).

Despite the promising antimicrobial activity, ICP spectrometer analysis of the Ag@ACF-treated water (Supplementary Table S1) identified a limitation in the study regarding Ag deposition above the limit values established by WHO (0.1 mg/L) and CONAMA (0.01 mg/L), suggesting Ag detachment from the surface of the material. Since we used the filter unit for dynamic filtration with continuous circulation from 2 to 24 h, this movement may have contributed to greater Ag detachment and, consequently, to the toxicity of *R. subcapitata*.

Hence, new methods to impregnate Ag into felt should be analyzed in future studies to mitigate this limitation. Future evaluation of different methods aimed at improving the characteristics, effect and availability of Ag to pathogenic microorganisms should be conducted to improve the material and reduce its metal release. AgNPs optimized by chemical reduction (Quintero-Quiroz et al., 2019) and by variations in physicochemical characteristics (Pannerselvam et al., 2021) showed good antimicrobial effect and are potential alternatives to be considered.

5 Conclusion

Silver nanoparticles were successfully incorporated into textile carbon fibers using a cheap and electroless method.

The production of textile PAN is five times less expensive than special acrylic fiber and for this reason, it could be a cheaper option.

Microbial suspensions filtered for 24 h through textile carbon fibers decorated with nanoparticles of Ag (Ag@ACF) showed time-dependent reduction for *E. coli* and *C. albicans*, with reductions of 97.8% and 100%, respectively. *A. niger* fungal suspension filtered with Ag@ACF showed a 99.4 and 99.8% cell count reduction after 1.5 and 2 h. Filtration by Ag@ACF did not inhibited *P. funiculosus* suspension after 1.5 and 2 h filtration. Water filtered with Ag@ACF for 24 and 48 h were non-cytotoxic and slightly cytotoxic for Vero cells, respectively.

Water samples filtered for 2 and 24 h with Ag@ACF showed no toxic effect for *D. melanogaster*, although showed some degree of toxicity was observed for *R. subcapitata*. Textile carbon fibers decorated with nanoparticles of Ag (Ag@ACF) is a promising material for water treatment.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material, further inquiries can be directed to the corresponding author.

Author contributions

IM: conceptualization, methodology, investigation, resources, formal analysis, writing - original draft. AS: methodology, investigation, resources, formal analysis, writing - original draft. GL: conceptualization, methodology, investigation, resources. MC: methodology, investigation, resources, formal analysis. SR: methodology, investigation, resources, formal analysis. AR-S:

methodology, investigation, resources. MB: methodology, investigation, resources. JM: methodology, investigation, resources. CK-I: methodology, conceptualization, supervision, writing review and editing.

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Conflict of interest

Author JM was employed by company JMHP Consultoria em Materiais e Informática LDTA.

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Supplementary material

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fenvs.2022.1100583/full#supplementary-material>

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