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Original article

Prolonged antibacterial action of CuO-coated cotton fabric in tropical climate

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ABSTRACT The paper reports the results of a large-scale testing of antibacterial textiles with extremely stable and long-lasting copper oxide coating. Using disk diffusion method, ICP-OES and specific *lux* biosensors it was shown that the coating does not leach copper ions into the environment. Laboratory experiments performed according to the ISO 20743 protocol showed high antibacterial activity of the produced coating, up to complete growth suppression for some strains. The long-term field tests were carried out in a tropical climate, at the Climate test station "Hoa Lac" (Hanoi city, Vietnam). The number of microorganisms on the textile materials remained within the range of 1–3% in comparison with the control sample for the entire duration of the field exposure (12 months).

KEYWORDS climate test, composite materials, metal oxide nanoparticles, ultrasonic cavitation, antimicrobial activity, field testing.

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1. Introduction

Woven fabrics effectively absorb sweat and other body exudate, retain moisture and warmth. Textiles which are worn close to the skin offer an ideal environment for microbial growth. With the problem of antibiotic-resistant bacteria being an ever-increasing concern and the rising awareness in public and personal hygiene, textiles with antimicrobial properties are becoming attractive for both the manufacturers and researchers. An emerging trend in textile finishing is the use of metal oxide nanoparticles (NP) [1].

Copper compounds have found numerous applications as bactericide, algaecide, fungicide, nematocide, molluscicide, and anti-fouling agent [2]. They are especially attractive for pretreatment of fabrics intended for the use in warm tropical climate with high biodiversity, because they exhibit both bactericidal and fungicidal properties simultaneously. In previous work related to textile testing in tropical climate, it was shown that fungi are the more prominent type of microorganism in these conditions and make a significant contribution to the fabric deterioration [3].

To be used in textile production, the chemical compounds should be safe for the final consumer. Copper ions are known for their toxicity and associated health risks [4], which requires use of copper compounds that do not leach copper ions, do not dissolve, do not penetrate the skin barrier, etc. In this regard, copper oxide is one of the suitable copper compounds. Compared to the other commonly used antibacterial agents, CuO has low cytotoxicity to human hepatocellular carcinoma (HepG2) cells [5]. Recent studies have shown that CuO nanoparticles (NPs) do not translocate though the skin barrier [6]. In a recent paper [7], a detailed study of the cytotoxic effects of fabrics treated with CuO nanoparticles was conducted. Human dermal fibroblast (HDF) cell viability above 95 % was observed for all the tested fabrics. Hence, the risk of NP-mediated damage to human cells is minimal and the NP-coated fabrics could be used safely. Moreover, CuO-loaded wound dressings were found to improve and accelerate wound healing, and no histological differences were found between open wounds treated with conventional dressings and CuO-treated dressings [8].

While the human skin does not experience any negative effects upon contact with copper oxide nanoparticles, microorganisms are extremely susceptible to it. A significant bactericidal effect was reported even for fabric coated with 1wt.% of CuO nanoparticles [9, 10]. Several studies (primarily short-term laboratory experiments with individual strains of microorganisms) have shown that impregnation or coating of fabrics with CuO nanoparticles endows them with broad-spectrum antimicrobial properties [11, 12]. It is suggested that in case of CuO NPs the cellular damage to the microorganisms is caused by the hydroxyl radicals produced on its surface [12, 13].

As previously mentioned, the textile coating must not release any ions into the surrounding media. The long-term stability of bactericidal materials is crucial for their application [14–16]. In order to produce long-lasting stable coating, various ways of immobilizing the metal oxide nanoparticles in organic or inorganic matrices have been actively studied in recent years [17].

One of the possible approaches to production of stable fabric coatings is the use of ultrasonic cavitation for the incorporation of nanoparticles into the textile fibres. This method ensures even distribution of the coating components, allows for large-scale production and provides stability of the coating for at least 20 washing cycles [18–20].

Though some studies of CuO-coated cotton fabrics have already been reported [9, 21], the vast majority of these studies were limited to testing with the model objects only, such as *E. coli* and *S. aureus* [22–29]. Data characterizing the performance of these composites in field conditions are very limited. The effect of the climate might be extremely significant, and it could also severely affect the speed of fabric deterioration [30]. Antibacterial activity strongly depends on the temperature [31], humidity [32], and insolation conditions as well. Tropical climate is characterized by high temperature and high humidity. Biological diversity of microorganisms occurring in a tropical climate is significantly higher compared to other climatic zones [33]. Thus, the materials intended for use in the tropical climate will be subjected to very harsh environmental conditions, and therefore these materials require special testing.

In this paper, we report the whole production cycle of the textiles coated with copper oxide nanoparticles and the results of their testing both according to the standard laboratory-scale procedures and in the long-term field tests in the tropical climate at the Climate test station “Hoa Lac” (Hanoi city, Vietnam).

2. Materials and methods

2.1. Preparation of the fabric samples

CuO nanoparticles were purchased from ROTH and used without any preliminary processing. Chemical purity of the nanopowder is $\geq$99 %. CuO particle size according to the manufacturer is 15–50 nm. Surface area (BET) is $\geq$15 m^2/g. White calico (100% cotton) with density of 140 g/m^2 (manufactured by IvanovoTextile ltd., Russia) was placed in a 1.25 g/L suspension of CuO nanoparticles in water at 20°C at 20 mm from the ultrasonic emitter and treated with ultrasound. The frequency of the ultrasound was 22 kHz, the power was 750 W. The fabric was moved along the ultrasonic emitter with the speed of 1.5 m/min. The sample was dried at 120°C for 3 h. The procedure for the immobilization of metal oxide nanoparticles on a substrate of fibrous material using high power ultrasound was reported earlier by Abramova et al. [19, 20, 34].

2.2. Study of the physical and mechanical characteristics of composite materials

For the quantitative characterization of the prepared coated textile samples, approximately 1 g of the coated cotton was ashed in a platinum crucible for 6 hours at 900°C in a resistance furnace under air.

Analysis of the elemental composition was performed by energy dispersive X–ray (EDX) spectroscopy using a high resolution scanning electron microscope (Carl Zeiss NVision40) equipped with an Oxford Instruments X-Max detector. The analysis was carried out at an accelerating voltage of 20 kV and the working distance of 11 mm. For calibration, standard cobalt reference sample was used.

The surface morphology of the obtained composite materials was studied by scanning electron microscopy (SEM). SEM images were obtained using a Prisma E microscope (Thermo Scientific, Czech Republic) with an accelerating voltage of 3.5 kV. The samples were preliminarily coated with 10 nm thick gold layer by a Q150R ES plus sputter coater (Quorum Technologies, UK).

The tensile strength of the samples was measured according to the TCVN 1754–1986 standard on a Zwick/Roell Z010TH Proline device (Germany).

Assessment of copper ions leaching into the aqueous media was performed by inductively coupled plasma optical emission spectroscopy (ICP-OES) using a Thermo Scientific iCAP XP (USA). To prepare the solutions for analysis, 10×10 mm fabric fragments were submerged in 1.5 mL of water for five days at 30°C. Then the fabric fragments were removed and the resulting solution was analysed.

2.3. *In vitro* evaluation of antibacterial activity of composite materials

The antibacterial activity of the obtained composite materials was tested *in vitro* using the following model objects: 1) opportunistic Gram-negative bacteria *Escherichia coli* strain BW25113 (the parent strain for the Keio Collection of single-gene knockouts) [35], the strain was kindly provided by Dr. Alexander Mironov (Engelhardt Institute of Molecular Biology, Russian Academy of Science, Moscow, Russia); 2) Gram-positive bacteria *Staphylococcus aureus*; 3) opportunistic human pathogen *Chromobacterium violaceum* strain CV12472 (a characteristic representative of the soil and water microbiome in tropical and subtropical climatic zones [36]), the strain was kindly provided by Dr. Leonid Chernin (The Hebrew University of Jerusalem, Rehovot, Israel); 4) rhizosphere *Pseudomonas chlororaphis* strain 449 [37], which was obtained from rhizosphere of maize (Ukraine) [38]; and strains isolated from fabric samples studied in 2020 in the field tests at the Climate test station "Hoa Lac" (Hanoi city, Vietnam) [3] which were the strains most commonly found on the surface of cotton fabric after exposure at the test station, namely 5) *Stenotrophomonas rhizophila*; 6) *Exiguobacterium indicum*; 7) *Brachybacterium paraconglomeratum*; 8) *Bacillus amyloliquefaciens*.

Two variations of the laboratory-scale experimental procedure were used to conduct preliminary tests of the antibacterial activity.

a. Evaluation of antibacterial activity of the composite materials on a solid nutrient medium

Antibacterial activity of the composite materials was tested using the disk diffusion test as described elsewhere [3]. Briefly, the fabric composite materials were sterilized, cut into 10×10 mm pieces and placed on the surface of the Petri dishes. After 24 hours of incubation at 30°C, the Petri dishes were examined for the presence of growth inhibition zones around the studied samples. The diameters of inhibition zones were measured. All the assays were carried out in triplicate.

b. Evaluation of antibacterial activity of composite materials based on ISO 20743.

An experimental protocol was developed on the basis of ISO 20743 [39]. The studied fabric (in three replicates) was cut to size of 6×15 cm and sprayed with microbial suspension (using the strains listed above). After adding the strain, the fabric was suspended in an airtight vessel with sterile water at the bottom to provide moisture and then incubated under humid conditions at 30°C for 28 days. At the "zero point" and after the contact period (28 days of incubation at 30°C) the microorganisms were counted. The antimicrobial effect of the fabric samples on microorganisms was assessed by analysing the growth of microorganisms on the surface of the samples and by comparing of number of surviving organisms, counted as the number of colonies forming unit (CFU), of CuO-coated cotton fabric samples with that of reference sample (without CuO coating, control).

2.4. Evaluation of the leaching of copper ions from cotton fabric with CuO coating into an aqueous solution using specific *lux* biosensor

Specific *lux* biosensor based on Gram-negative bacteria *E. coli* JM83 with hybrid plasmid pCopA'::lux was used to evaluate the rate of copper ions release from CuO-coated cotton fabric and to assess the degree of bacterial cell damage caused by these ions. The tests were carried out as described elsewhere [40, 41], with some modifications. Briefly, sterile fabric pieces (10×10 mm) (1, 2 or 3 pieces) were placed in 2 mL centrifuge tubes containing 1.5 mL of sterile mQ water. The tubes were placed on a vortex and then moved into a thermostat and incubated for 5 days at 30°C. After 5 days, the content of copper ions in the aqueous solution (with or without fabric pieces) was measured using the *lux* biosensor *E. coli* JM83 (pCopA'::lux). Copper sulfate (aqueous solutions of $CuSO_4$, 5, 10 and 50 μg/mL) was used as reference compound for the induction of bioluminescence in *lux* biosensors.

2.5. Field Tests

All field experiments were carried out at the Climate test station "Hoa Lac" (Hanoi city, Vietnam). The experiments were carried out in three repetitions. The coated textile was sterilized at 120°C for 30 minutes and cut into 5×10 cm pieces. The fabric samples were placed on the following test sites:

a. "Concrete site": the site is located in a field with no shading. The fabric samples were placed at a height of 1 m from the ground, with 3 cm distance between the samples.

b. "Mycological site": the site is located in a shaded wooded area. The fabric samples were placed in a closed cabinet with a roof at 0.5 m from the ground, with 3 cm distance between the samples.

2.6. Evaluation of microorganisms' growth on studied materials after various duration of field tests

The fabric samples were collected after 1, 3, 4, 6, 9 and 12 months of exposure at the test sites. Microorganisms from the collected samples were isolated and counted according to procedure described elsewhere [3].

2.7. Statistical Analysis

The statistical analysis was carried out using the IBM SPSS software v.26 (New York, NY, USA). Significant differences were determined using a one-way analysis of variance (ANOVA), followed by Tukey's HSD (Honestly Significant Difference) post hoc test. Differences were considered to be significant at $p \leq 0.05$.

3. Results and discussion

3.1. Production and characterization of coated textiles

Cotton fabric coated with CuO nanoparticles was prepared using ultrasonic treatment. Details of this procedure along with the study of the coating stability during washing can be found elsewhere [19, 20, 34]. The content of CuO NPs in the obtained fabric was determined gravimetrically and was found to be 5.25 g/m^2. The experiment was carried out in four repetitions and the standard deviation of this value was 10%. Chemical composition of the samples was also analysed by EDX (Fig. 1a). EDX mapping proved the loading of copper atoms to be 5 at.% showing a sufficiently uniform distribution of the CuO nanoparticles on the cotton fibres.

The surface morphology of the obtained samples is shown in Fig.1b. The role of surface texture for the antibacterial efficiency was demonstrated previously in numerous reports [42, 43]. Coating cotton textiles with TiO_2 was shown to create a hydrophobic surface, which additionally prevented bacterial adhesion [3, 44]. However, in the case of the CuO coating, the fabric demonstrated full wetting, which means that any observed antibacterial effect is associated with the action of CuO and not with hydrophobicity.

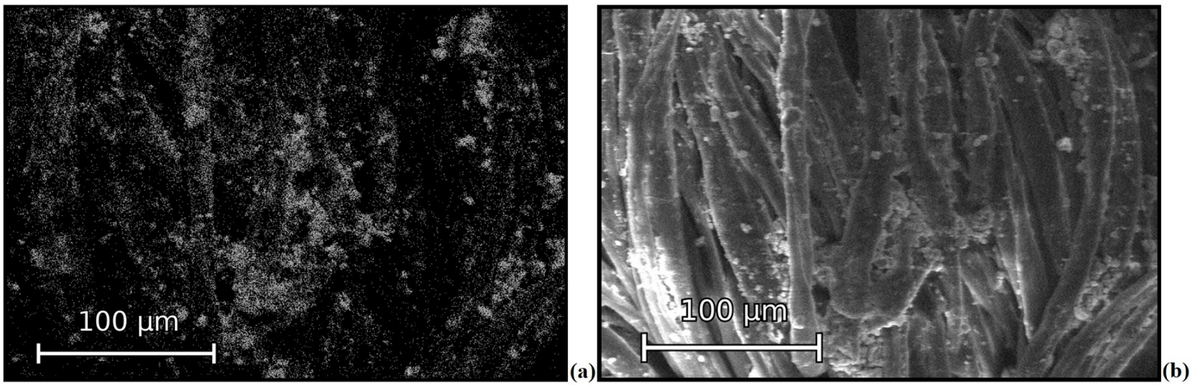

FIG. 1. EDX mapping (a) and SEM image (b) of fabric coated with CuO nanoparticles

3.2. Study of the antibacterial effect of CuO coating on solid nutrient medium

Preliminary laboratory-scale tests were carried out using the disk diffusion method. The results of *in vitro* tests of the CuO-coated fabrics on solid nutrient medium with bacteria of different taxonomic groups are presented in Table 1.

The experiments were carried out using both gram-positive (*S. aureus, E. indicum, B. paraconglomeratum*, and *B. amyloliquefaciens*) and gram-negative (*E. coli, C. violaceum, P. chlororaphis, and S. rhizophila*) bacteria. For the majority of the tested strains the growth inhibition zone is almost negligible after short incubation time. This means that CuO nanoparticles have very low diffusion rate in solid nutrient medium, which prevents their diffusion into the agar. The absence of an inhibition zone cannot be interpreted as the absence of an antibacterial effect, since it is only an indicator of low release rate and poor diffusion of the active component of the coating during short-term exposure of the test samples to bacteria.

The data presented in Table 1 confirm minor antimicrobial activity of CuO coating on gram-positive *B. amyloliquefaciens* and gram-negative *S. rhizophila* species after 24 h. This might evidence higher susceptibility of these bacteria to copper ions. It should be noted, however, that the presence of the growth inhibition zone does not necessarily imply that microorganisms have been killed – they might have only been prevented from growing.

3.3. Evaluation of the leaching of copper ions from cotton fabric with CuO coating into an aqueous solution

The disk diffusion method showed that CuO nanoparticles are strongly bound to the textile and do not leach copper ions into the solid nutrient medium after 24 h. To evaluate Cu^{2+} ions leaching over a longer period of time and in "harsher" conditions, the ICP-OES method was used. To prepare the solutions for analysis, 10*10 mm fabric piece was submerged in 1.5 mL of water for five days at 30°C. After five days the fabric was removed and the resulting solution was analysed. The concentration of copper ions in the solution was found to be 0.2 mg/L with relative standard deviation of 2%.

TABLE 1. Antibacterial activity of the textiles coated with CuO on solid medium

Bacterial strain	Diameters of inhibition zone (DIZ)[a], mm	
	the reference sample	fabric coated with CuO nanoparticles
E. coli	0 ± 0.1	0 ± 0.1
C. violaceum	0 ± 0.1	0 ± 0.1
P. chlororaphis	0 ± 0.1	0 ± 0.1
S. aureus	0 ± 0.1	0 ± 0.1
E. indicum	0 ± 0.1	0 ± 0.1
B. paraconglomeratum	0 ± 0.1	0 ± 0.1
B. amyloliquefaciens	0 ± 0.1	0.8 ± 0.1
S. rhizophila	0 ± 0.1	0.5± 0.1

[a]Diameter of the growth inhibition zone (DIZ) is calculated as the size of entire growth inhibition zone around the fabric sample minus the size of the fabric sample (which is 10 mm); all the assays were carried out in triplicate and the data were recorded as mean ± standard error.

In vitro studies using a specific *lux* biosensor were employed to elucidate whether defence systems responsible for protection against excess of copper ions of living bacterial cells were triggered in the presence of the coated fabric at such concentration of leached ions. In the case of *E.coli* JM83 strain used in this study, it is the induction of the *copA* gene (encoding the CopA protein, a member of the P-type ATPase cation transporter family) that indicate the activation of defense system of bacterial cells, since CopA is known to confer resistance to copper ions in *E. coli* as an ATP-dependent exporter of monovalent copper [45].

The solutions for analysis were prepared in the same way as for ICP-OES, by incubation of a fabric piece in 1.5 mL of water for five days at 30°C. Solutions with varied number of fabric pieces were prepared. The obtained data are presented in Fig. 2. It can be seen that aqueous solutions containing fabric coated with CuO nanoparticles do activate luminescence of *E. coli* pCopA *lux*-biosensor (induce expression from the P*copA* promoter). Specifically, fabric samples coated with CuO nanoparticles increase luminescence intensity of *lux*-biosensor by 5–10 times after 30 minutes of measurement compared to the control (aqueous solution without fabric samples) or aqueous solutions containing fabric samples without nanoparticles.

Thus, the obtained data indicate that copper ions are capable of leaching from CuO-coated cotton fabric into the aqueous medium to induce expression from the promoter of the *cop*A gene responsible for copper homeostasis in *E. coli.*

However, even the samples prepared with 3 pieces of the fabric coated with CuO nanoparticles (to achieve higher concentration of Cu^{2+} ions) had luminescence intensity of *E. coli* pCopA *lux*-biosensor 4.5–6 times lower (after 30 minutes of measurement) compared to that of the reference compound (5 μg/mL aqueous solution of $CuSO_4$). It means that the rate of the Cu^{2+} ions release from the CuO coating after 5 days of incubation is relatively low. This makes the ultrasonically treated coated fabrics suitable for prolonged use.

3.4. Determination of antimicrobial activity of CuO-coated fabric samples using ISO 20743 standard

The experiments described above provide evidence towards the slow leaching of Cu^{2+} ions in aqueous medium and in agar. However, the prepared fabric is not intended for use in aqueous medium (underwater use). Thus, the antibacterial activity of the CuO-coated textile was additionally evaluated in accordance with ISO 20743 standard, as presented in Table 2. The ISO 20743 (Textiles - Determination of antibacterial activity of textile products) is a commonly used test for evaluation of the antimicrobial efficiency. It is applicable to all textile products and allows for comparison between various antimicrobial treatments, as well as various treatment-levels on the same textile [39]. This approach includes applying aerosol of bacterial suspension onto the samples and then incubating these samples in a dry enclosed space.

The data obtained demonstrate that the CuO-coated fabric has high bactericidal activity compared to reference samples. Antibacterial effect of CuO coating was the highest against bacteria *B. amyloliquefaciens* (complete growth suppression), then against *B. paraconglomeratum* and *E. indicum* (4-log(10) CFU' reduction), and *S. rhizophila* (with a 3-log(10)

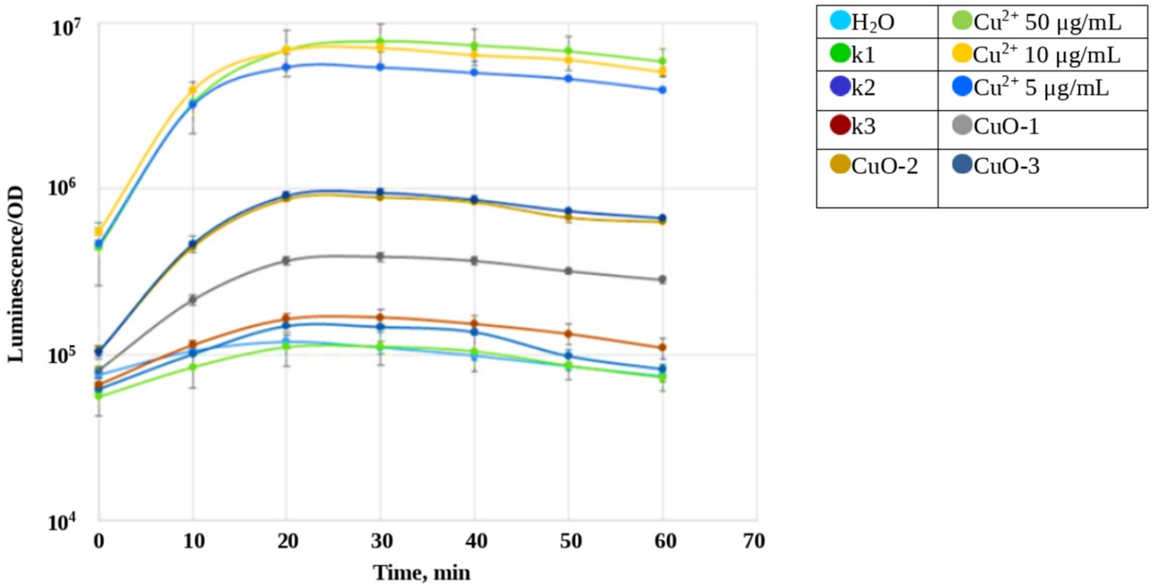

FIG. 2. Response of the biosensor strain *E. coli* JM83 (pCopA'::lux) to the presence of copper ions in aqueous environment. The curves show the change in the luminescence response of the sensor over time, which is expressed as the ratio of the average values of light emission (in RLU) measured at time t (every 10 min in the time interval 0–60 min) to its optical density (OD600) measured at 600 nm in the presence of various concentrations of $CuSO_4$ (5, 10, and 50 μg/mL) and aqueous solutions of the samples (k1, k2, and k3 - aqueous solutions with 1, 2 or 3 pieces of cotton fabric without CuO coating, and CuO-1, CuO-2, CuO-3 - aqueous solutions with 1, 2 or 3 pieces of CuO-coated cotton fabric, respectively). All values were means ± standard deviations (SD) for n = 3. $CuSO_4$ is a reference compound for the *E. coli* JM83 (pCopA'::lux) sensor and is used as a positive control to check activity (induction of bioluminescence) of the *lux* sensor strain.

CFU' reduction) after 28 days of incubation (Table 2). It must be noted that in the previously described experiments on solid nutrient medium, no growth inhibition zone was observed for *E. indicum* and *B. paraconglomeratum* compared to the uncoated sample of textile.

This highlights that the antibacterial efficiency rate depends strongly on the testing method and the conditions of bacterial cultivation. The long-term (28 days) experiments studying the antibacterial activity of the CuO-coated textile according to ISO 20743 standard confirm a high antibacterial potential of CuO nanoparticle coating compared to short-term laboratory experiments.

3.5. Field Tests

3.5.1. Determination of the antimicrobial activity of CuO-coated textiles after field tests in the tropical climate. Two types of conditions were used for field testing of the fabrics coated with CuO nanoparticles. Open concrete site had high degree of insolation and good air circulation; the samples were placed on a stand without any roof, shielding or coverage. The mycological test site was located in a forested area, and the samples were closer to the ground in shelves with doors and a roof. Presumably, the conditions at the mycological test site would promote faster leaching of the active coating component due to higher humidity at this site. The results of the field tests on these two test sites are presented in Table 3.

The data obtained show that the growth of microorganisms on the control samples of textile (original cotton fabric without a nanoparticle coating) was not suppressed and the number of colonies per sample increased over time during exposure to the tropical environment. On the other hand, the samples of textile coated with CuO nanoparticles have fewer colonies per sample compared to the reference samples, proving the inhibitory ability of the composite material. In general, composite materials had 30–100 times less CFU per sample than the control (Table 3). It should be noted that textile samples coated with CuO nanoparticles retain their antimicrobial activity during the entire period of field testing which indicates the microbiostatic effect of such coatings. No considerable differences in the long-term antibacterial activity of the samples on the two test sites were found. The number of CFU on the samples is comparable, though after 6 months of exposure the ratio of the average CFU value of microorganisms on the CuO-coated sample to the average CFU value on the reference sample became slightly higher for the samples placed on the concrete open site. The difference is small and should be interpreted with caution, but it might be suggested that the presence of the copper ions due the minor leaching occurring at the mycological test site invokes additional mechanisms of antibacterial action. Toxicity of copper ions to microorganisms, including toxicity to viruses, may occur through the displacement of essential metals from their native binding sites, from interference with oxidative phosphorylation and osmotic balance as well as from

TABLE 2. Results of laboratory-scale testing of CuO-coated textiles for antibacterial activity based on ISO 20743. The average value of CFU derived from three repetitions is given

Bacterial strain	Type of coating	Sample incubation time	
		"zero point"	After 28 days of incubation
		Colonies/Sample, CFU	
S. rhizophila	Reference sample	2.1×10^6	6.2×10^6([a]295%)
	CuO coating		3.4×10^3 ([a]0.2%; [b]0.05%)
B. paraconglomeratum	Reference sample	4.3×10^6	1.6×10^7 ([a]372%)
	CuO coating		9.4×10^2 ([a]0.02%;[b]0.005%)
B. amyloliquefaciens	Reference sample	5.8×10^5	2.4×10^6 ([a]413%)
	CuO coating		0
E. indicum	Reference sample	3.2×10^6	7.5×10^6 ([a]234%)
	CuO coating		2.0×102 ([a]0.006%; [b]0.003%)

[a] The ratio of the average CFU value for the CuO-coated sample after 28 days of incubation to the average CFU value of the sample at the "zero point" of the experiment

[b] The ratio of the average CFU value for the CuO-coated sample to the average CFU value of the reference sample after 28 days of incubation

TABLE 3. Results of the field tests of CuO-coated textiles at the Climate test station "Hoa Lac" (Hanoi city, Vietnam). The averaged value of CFU from three repetitions is given

Type of test site	Type of coating	Duration of exposure to a tropical environment				
		1 month	3 months	6 months	9 months	12 months
		Colonies/Sample, (CFU)				
Concrete open test site	Reference sample	1.6×10^6	3.3×10^7	4.3×10^7	8.2×10^7	4.2×10^8
	CuO	9.4×10^4 ([a]5.9%)	1.3×10^5 ([a]0.1%)	1.6×10^5 ([a]0.4%)	2.4×10^5 ([a]0.3%)	3.8×10^5 ([a]0.1%)
Mycological shelf test site	Reference sample	1.1×10^6	3.7×10^7	9.0×10^7	1.2×10^8	7.2×10^8
	CuO	3.4×10^4 ([a]3.1%)	9.4×10^4 ([a]0.3%)	1.0×10^5 ([a]0.1%)	1.2×10^5 ([a]0.1%)	2.5×10^5 ([a]0.04%)

[a] The ratio of the average CFU value of microorganisms on the the CuO-coated sample to the average CFU value on the reference sample;

alterations in the conformational structure of nucleic acids, membranes and proteins [2]. The experiment with specific *lux* biosensor described above shows that the amount of copper ions leached from the fibres is sufficient to have an input in the antibacterial activity of the textile.

As discussed previously, the antimicrobial properties of metal oxide nanoparticles are commonly attributed to the formation of reactive oxygen species (ROS) under UV-irradiation [2, 44–47]. The high efficiency of the coating on the shaded test site indicates that intense UV-irradiation is not necessary and it does not contribute significantly to the antibacterial performance.

Our research group previously reported similar case of tropical field tests for fabrics with TiO_2 and ZnO coatings, and these types of coatings were only able to decrease the number of CFU by 35–50 times in similar conditions [3]. This comparison shows higher efficiency of the CuO coating in tropical environment.

3.5.2. Determination of the types of microorganisms on the fabric surface after the field tests. The number of different types of microorganisms found on the samples after field testing is presented in Table 4. As can be seen from Table 4, the ratio of the microorganism types on the fabric coated with CuO nanoparticles did not change significantly over time on both of the test sites.

TABLE 4. Number of different types of microorganisms on the CuO-coated textile after different periods of exposure at the Climate test station "Hoa Lac" (Hanoi city, Vietnam). An average value from three repetitions is given

Time of exposure		Number of Species After														
		1 months			3 months			6 months			9 months			12 months		
Type of Microorganism		B	F	Y	B	F	Y	B	F	Y	B	F	Y	B	F	Y
Mycological shelf test site	Reference sample	3	5	2	4	6	2	4	7	2	5	7	2	5	7	2
		10			12			13			14			14		
	CuO	2	3	2	2	5	2	1	5	2	2	5	2	3	5	2
		7			9			8			9			10		
Concrete open test site	Reference sample	4	4	1	5	5	2	5	5	2	6	6	2	6	7	2
		9			12			12			14			15		
	CuO	2	4	2	2	4	2	2	3	1	2	4	1	3	4	1
		8			8			6			7			8		

B – Bacteria, Y – Yeast, F – Fungi

Yeasts were the least common, and the biggest variety of species was determined for fungi. The samples at both test sites were found to have very similar proportions and amounts of various microorganisms at 'zero point'. However, over time, slightly bigger variety of microorganisms was found in the samples collected from the mycological site. The difference is not very significant and can be explained by the difference in the environment conditions during the period of field testing. It is logical that greater variety of species was observed at the site with favourable conditions for microbial diversity such as high moisture and the absence of intense insolation. Fungi prevailed among identified types of microorganisms both on coated and uncoated textiles during the entire period of field testing. The mycelium species can significantly contribute to material deterioration in tropical conditions. Copper oxide nanoparticles were originally chosen because of their fungicidal properties, but it can be seen that while the number of organism decreases, the diversity is not affected as much.

3.6. Tensile strength evaluation

To estimate the deterioration of the textile samples, the tensile strength was evaluated according to the TCVN 1754-1986 protocol. The results of the measurements are summarized in Table 5 and visualized in Fig. 3.

TABLE 5. Tensile strength of the CuO-coated textiles after different periods of exposure at the Climate test station "Hoa Lac" (Hanoi city, Vietnam). Average values from three repetitions are given

Type of exposure	Type of coating	Duration of exposure to the tropical environment			
		0 (before the field test)	1 month	3 months	4 months
		Tensile strength, MPa			
Concrete open test site	Reference sample	23.1	17.4	14.1	13.2
	Fabric coated with CuO nanoparticles	27.1	15.5	12.2	10.5
Mycological shelf test site	Reference sample	23.1	21.3	17.3	16.0
	Fabric coated with CuO nanoparticles	27.1	16.0	15.4	14.9

The application of metal oxide nanoparticles was previously shown to improve the mechanical properties of a textile [48]. However this effect is not always observed even with similar fabric, coatings and coating techniques [3]. In the present work, it was found that application of CuO NPs coating initially increases the tensile strength by ∼17%.

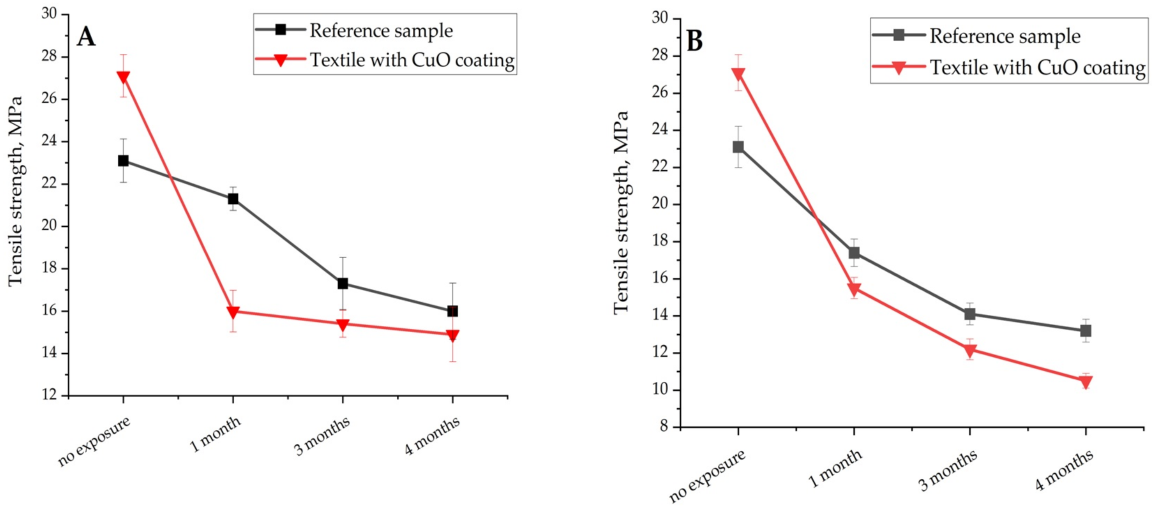

FIG. 3. Tensile strength of the fabric samples after different periods of exposure at the mycological shelf test site (A) and at the open concrete site (B). Standard deviation represents the average from three separate experiments

After exposure at the open concrete test site for one month, both the control sample and the coated sample showed a significant decrease in tensile strength. The decrease rate over the next three months was similar, but the sample coated with CuO nanoparticles had a slightly higher rate of degradation. This might be explained with high insolation at the test site, with CuO nanoparticles acting as photocatalysts. A more interesting and unexpected trend is observed in the case of the samples placed at the mycological test site: the CuO-coated fabric shows a major decrease in the tensile strength after the first month of exposure, while the control sample deteriorates more steadily over the course of 4 months. The reasons for this effect will be analysed in the further studies of the textiles coated with metal oxide nanoparticles.

4. Conclusions

Cotton fabric with CuO coating was produced using ultrasonic cavitation technique. It was shown that this method is quite effective for the immobilization of the nanoparticles. The release rate of copper ions was estimated by disk diffusion method, ICP-OES and using specific *lux* biosensors. Field tests of the coated fabrics carried out at the "Hoa Lac" test site (Hanoi city, Vietnam) showed that the coated fabrics reduced the number of microorganisms to 1–3% compared with the reference at both test sites. No significant difference cased by insolation degree or air humidity was observed. The types of microorganisms found on the textile samples after exposure in tropical climate were identified, with fungi being the most prevalent species despite the previously reported fungicidal activity of copper oxide.

The textile materials coated with CuO nanoparticles not only exhibit antimicrobial activity in short-term and long-term laboratory experiments, but also retain it for a long time (up to 12 months) during field tests in tropical climate. The data obtained on antimicrobial activity, analysis of the species found on the samples, and the mechanical characteristics of the fabrics coated with copper oxide nanoparticles in a tropical climate, after field testing both at open concrete and mycological sites, clearly demonstrate the potential for the use of this textile composite materials in a tropical environment.

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