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Study of the antifungal effects of quaternary ammonium salt for the preservation of inked paper

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Biological degradation of documentary heritage is a serious issue faced by libraries, museums and archives as it can lead to paper becoming brittle, fragile and discoloured, resulting in a loss of information. This study investigates the impact of didecyldimethylammonium bromide (DDAB) on the properties of pure or inked paper and the growth of different types of fungi. Three fungi species known for their ability to cause paper biodeterioration in libraries and museums, *Aspergillus clavatus* Desm, *Penicillium paneum* Frisvald and *Ulocladium chartarum* Preuss, were selected for the DDAB salt antifungal activity test. The study measured the pH and degree of polymerization (DP), monitored colour changes and conducted scanning electron microscopy analyses (SEM) to confirm that DDAB can be used as a biocide for fungi on pure or inked paper. The study also investigated the antifungal effect of DDAB on paper treated with different types of ink, including black (iron-gall ink) and brown (copper ink). Didecyldimethylammonium bromide (DDAB) can be used as a biocide for cleaning and preserving paper documents, with active concentrations of 0.7 and 1%.

Keywords: fungi, paper, quaternary ammonium salt, biodeterioration, ink

INTRODUCTION

One of the main factors responsible for the deterioration and aging of paper documents is biological and microbiological contamination, which is common in library, museums and archival collections. This is due to many external factors, such as improper storage, transportation and exhibition conditions when the premises do not provide a suitable microclimate (humidity, temperature, airflow and light), and others [1–4]. Regular microbiological controls of the contamination and microclimate of rooms and objects, as well as preventive measures, i.e. regular cleaning of rooms, shelves and documents, can reduce or prevent the biodegradation of documentary heritage [5–9].

Paper as a material is sensitive to the action of biological organisms, especially harmful fungi. Moreover, fungi not only destroy paper documents, but also harm humans. Microbial spores infect not only documents but also the environment surrounding them. Microbiological contamination by fungi can cause various allergic reactions and skin infections in the human body [10]. Physical and chemical methods are used to disinfect fungal damage. Chemical treatment usually involves liquid biocides or gas fumigation. The most commonly used chemicals as antifungal agents in preservation practice are phenol, formaldehyde, and titanium dioxide and ethylene oxide gas derivatives [11, 12]. Phenol and formaldehyde derivatives and ethylene oxide gas are most commonly used for preservation. Quaternary ammonium compounds (quarts) are used not only as surfactants but also as disinfectants with a broad spectrum of antimicrobial activity in food, textile and medical industries [13]. Recently,

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didecyldimethylammonium and benzalkonium chlorides have been proposed as biocides for restoration of historical buildings and disinfection of paper-based items [14–17]. In addition, quaternary ammonium compounds are ionic chemicals that have the properties of both disinfectants and surfactants. However, these compounds can sensitize and irritate human skin and mucous membranes, so it is necessary to use the lowest effective concentrations [18]. It is known that the most effective concentration of quarts against fungi is about 1% or less in disinfectant solutions [16]. Therefore, the search for new substances and methods that are non-toxic to humans, but inhibit the growth of fungi is an important and relevant task now.

The aim of this study is to evaluate the antifungal properties of didecyldimethylammonium bromide as a biocide against *A. clavatus*, *P. paneum* and *U. chartarum* for the prevention of damaged ink paper. Historical recipes of iron gall (black ink) and copper (brown ink), popular from the Middle Ages to the beginning of the 20th century, were selected for the study.

EXPERIMENTAL

The filter paper was chosen for its high pure cellulose content (ROTILABO type 1 A, 99% w/w, pH 6.74) and its well-known chemical, physical and structural characteristics. Additionally, it is additive-free and serves as a reference point for other experimental works since it is widely used for laboratory testing.

Three fungal strains, *Aspergillus clavatus* Desm (*A. clavatus*), *Penicillium paneum* Frisvald (*P. paneum*) and *Ulocladium chartarum* Preuss (*U. chartarum*), were selected due to their frequent occurrence in documents and works of art in libraries and archives.

Different ink solutions were prepared according to historical writing ink samples [19, 20]. For the brown ink recipe, 7.68 g of chopped nuts were added to 66.8 ml of distilled water and left to swell for 4 days. Then, 2.52 g of copper sulfate ($CuSO_4 \times 5H_2O$), 0.2 g of sodium chloride (NaCl), 2 ml of 10% acetic acid (CH_3COOH) and 0.316 g of potassium sulfate alum ($KAl(SO_4)_2\ 12H_2O$) were added to the mixture. The mixture was stored for 2 weeks in a closed container, then it was filtered and was ready for use. The pH value of the ink solution is 2.17.

The black ink recipe was the following: 100 ml of distilled water mixed with 2.34 g of tannin and 0.77 g of gallic acid ($(HO)_3C_6H_2COOH$). Then the mixture was prepared from 3.0 g of iron (II) sulfate ($FeSO_4 \times 7H_2O$), 1.0 g of crushed gum arabic, 0.7 g of 10% hydrochloric acid (HCl) solution and 0.1 g of phenol (C_6H_5OH). The resulting mixture was well mixed and kept for a few days in a closed container. The pH value of the ink solution is 1.71. The filter paper samples were immersed in the black (iron) or brown (copper) historical ink solution and dried at room temperature.

Didecyldimethylammonium bromide (DDAB, E-Farma, as product Bromosept 50 is 50% solution in 2-propanol (30%)) was used for the fungi growth test on pure and inked paper samples. Paper samples were soaked in appropriate concentration solutions and dried at room temperature. One part of the treated paper samples was artificially aged in an oven for 500 h at 90°C, and the other was used for the antifungal test performance.

The fungus cultures were inoculated on malt extract agar petri dishes (MycoLabs) and incubated in a thermostat at 26 ± 2°C temperature. Malt extract agar is an acidic culture medium designed to cultivate fungi, including yeasts and molds. From the cultures grown for 7 days aqueous suspensions of 10^4 in ml conidia were prepared, 0.1 ml of every suspension was spotted and spread in a petri dish on malt extract agar in order to form a lawn. The paper disks (5 cm) were sterilized for 6 h in an autoclave at 121°C and impregnated with a solution of 0.1, 0.5 0.7 and 1% concentration of didecyl dimethylammonium bromide (pH 6.6). The impact of biocide on the growth of fungus was evaluated on the 3rd, 6th and 10th days of the growth. Fungi growth and spreading on the paper sample were evaluated visually.

Before and after the treatment, paper pH measurements were performed according to the cold aqueous extracts standard ISO 6588-1 (2005) [21]. For the measurement, 0.1 g paper was weight, cut and placed in a glass bottle, then 5 ml of distilled water was added. The measurements were performed in triplicate using a Toledo MP220 Mettler pH meter.

The viscosity-based degree of polymerization (DP) was determined using the standard ISO 5351 (2004) [22]. The paper was dissolved in a dilute solution of 0.5 mol/l copper ethylenediamine (CED). The measurements are performed at 25 ± 0.1°C. The output of the viscometric results is the physical parameter describing intrinsic viscosity [η]. The intrinsic viscosity was calculated using the Martin's formula from the specific viscosity (obtained from the efflux time of solvent and polymer solution) and the concentration c (g/100 ml) of dry paper. The equation gives the average viscometric degree of polymerization DP: $[\eta] = K^a$, where K is 1.33 ml/g, [DP] is the average degree of polymerization, and *a* is 0.905.

For colorimetric measurements the pure, inked and treated with DDAB paper samples were aged for 100 h at 90°C, and colour CIELab coordinates $L^\star$, $a^\star$ and $b^\star$ were determined using the Spektroton OKBA equipment before and after the aging treatment. $L^\star$ represents lightness with values running from 0 (black) to 100 (white). On the axis $a^\star$, positive values indicate the amount of red, while negative values indicate the amount of green. On the axis $b^\star$, yellow is positive, and blue is negative [23]. ΔE values (ΔE between the two points) were recorded after pre-aging, treatment with DDAB and post-treatment aging. To illustrate colour changes the total colour difference ΔE_{Lab} was calculated using the Adams Nikerson equation

$$\Delta E = \sqrt{(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2},$$

where ΔE_{Lab} is the total colour difference, ΔL^* is the lightness difference, Δa^* (red/green) and Δb^* (yellow/blue) are the difference of colour component.

Fungi damaged and treated with DDAB paper samples were examined by SEM analysis to detect the adhesion of fungal species on the cellulose surface. Before the samples were washed with distilled water for several minutes and rinsed with 70% ethanol to remove cells [24]. The treated paper samples were cut into 2 × 2 mm strips and mounted on specimen stabs, and the surface morphology of the treated paper samples was studied using a Hitachi TM3000.

RESULTS AND DISCUSSION

Before starting to evaluate the antifungal activity of DDAB, the pH, degree of polymerization (DP) and colour changes value of the pure, inked and DDAB treated paper were determinated. The pH and DP of the paper are important parameters that cannot only reveal the condition of the paper medium, but also help to choose the right conservation or restoration procedure that ensures the longevity of the paper. Cellulose destruction is usually characterized by the degree of polymerization (DP) by monitoring the shortening of cellulose chains. The longer the cellulose chain, the higher the molecular weight of the cellulose, as well as the degree of polymerization. As the degree of polymerization decreases, the paper's mechanical strength and resistance to destruction during aging decrease. The pH and DP values of pure paper and treated with DDAB samples before and after 500 h thermal aging at 90°C accelerating aging are presented in Table 1.

The pH data show that the pH of the samples of pure and treated with DDAB paper are quite similar, and artificial aging only decreased by less than 10%. Whereas the pH of the paper samples treated with brown and black inks showed that in both cases, the pH of the ink treated samples was significantly lower than the pure paper values, e.g. pH 3.6 and 3.4 for brown and black ink samples, respectively. The pH values of the inked and treated with 1% DDAB samples and after accelerated aging are shown in Table 1. The results of the study revealed that after the treatment with 1% DDAB solution, the pH values of both brown and black ink samples increased slightly, due to pH of the DDAB 1% solution (pH is 6.6). During aging the pH of both pure and inked paper decreases slightly.

The degree of polymerization (DP) of a pure paper sample, which was thermally aged for 20 days at 105°C, showed a slight decrease. However, after the immersion in the brown and black ink solution, the degree of polymerization (DP) decreased and an extensive decomposition of the paper fibres was observed. The DP of the paper samples treated with brown ink decreased from 1153 (pure paper) to 998, while with black ink up to 923. From the results presented in Table 1, it can be seen that the use of DDAB for the treatment of pure and inked paper samples with 0.7 and 1.0% solutions has no significant effect on the DP of the paper and it slightly decreases

Table 1. **The pH and degree polymerization (DP) of pure paper and paper treated with DDAB samples**

Paper treated	pH	pH after aging	DP	DP after aging
Pure paper	6.90	6.32	1153	1120
with 0.7% DDAB	6.97	6.62	1071	1053
with 1% DDAB	7.01	6.72	1060	1047
brown ink	4.2	3.8	998	843
black ink	3.4	3.1	923	735
with brown ink and 1% DDAB	3.9	3.7	988	812
with black ink and 1% DDAB	3.8	3.7	915	722

after artificial aging (less than 3%). Thus, the change in DP value was influenced more by ink treatment and artificial aging, but not by treatment with 1% DDAB solution.

The colorimetric analysis was performed to verify the possible visual effects of the treatment with DDAD solution on pure paper and on coloured paper. To convert the visual human sense into quantitative data, UV/Vis reflectance spectroscopy can be used. The colour change values (ΔE) for the paper and the coloured samples after 100 h of thermal decomposition at 90°C are shown in Table 2.

Table 2. **The total colour difference Δ*E* of paper samples**

Sample	(Δ*E*)
Paper (reference)	10.8
Paper treated with 1% DDAB	13.2
Black inked paper (reference)	3.2
Black inked paper treated with 1% DDAB	5.0
Brown inked paper (reference)	2.3
Brown inked paper treated with 1% DDAB	3.3

The total colour change (ΔE) is considered perceptible to the human eye if it is greater than one. An optimal treatment would have an initial colour change of less than one, meaning that the treatment does not change the appearance of the object. The DDAB solution treatment causes less initial colour change in paper than in inked paper. It is known that the bromide ion, due to its action as a peroxide decomposer, can stabilize colour changes in inked paper [25]. This is why ammonium bromide salt was chosen for the antifungal properties test.

Fungal growth was examined after 3, 6 and 10 days of fungi growth (Table 3, Fig. 1). All concentration treatments showed some inhibition of fungal growth. When using the lowest concentration of 0.1%, a tendency of irregular colony development was observed, whereas 100% inhibition of fungal growth was found for all species at 0.7 and 1.0% solution concentrations.

It was found that the treatment with DDAB solutions of 0.5, 0.7 and 1.0% concentration (as active concentration) fully inhibited fungi growth on the paper surface after 3 days. The results show that DDAB has a fungicidal activity for the fungal strains – *A. clavatus*, *P. paneum* and *U. chartarum*.

Therefore, a solution with a DDAB concentration of 1% was used for the further antifungal tests on inked paper. Thus, black (iron gall) and brown (cooper) model inks were made according to the following historical recipes. Paper samples were immersed in the black or brown ink solution, dried and treated with 1% DDAB solution for the fungal growth test. Fungal growth was tested after 10 days, and the results are shown in Table 4. Fungal growth was determined daily by visual inspection, and the growth diameter of each colony was measured. The images of the samples damaged by *Penicillium paneum* Frisvald after 10 days are shown in Fig. 2.

The sample treated with 1% DDAB solution after 10 days of *P. paneum* growth is damaged partially, while not treated – fully overgrown. The same behaviour of *A. clavatus* and *U. chartarum* fungi was observed on brown or black inked paper. By visual observation, we can see that the brown ink samples treated with DDAB solution are less damaged. This can be explained by the different ink composition and lower pH of the black ink paper, as fungi tend to grow in a more acidic medium [26]. Another reason for the resistance could be the copper ions used in ink production, which can inhibit the growth of the fungus [27].

Table 3. **The antifungal effect of DDAB for fungi growth on paper**

DDAB Conc. (%)	*A. clavatus*			*P. paneum*			*U. chartarum*		
	3 days	6 days	10 days	3 days	6 days	10 days	3 days	6 days	10 days
0.0	–	–	–	–	–	–	–	–	–
0.1	+	±	–	+	±	±	+	±	±
0.5	+	±	+	+	+	+	+	+	+
0.7	+	+	+	+	+	+	+	+	+
1.0	+	+	+	+	+	+	+	+	+

+ Paper and medium sterile, **–** paper and medium fully overgrown, ± paper sterile, medium overgrown.

Fig. 1. Paper treated with DDAB solution (from left to right: 0.1, 0.5 and 0.7%) after 3 days and infected with *P. paneum* conidia

Table 4. **Antifungal effect of 1% DDAB solution for fungi growth on inked paper (after 10 days)**

Sample / Fungi	Paper	Paper + brown ink	Paper + black ink	Paper + brown ink + 1% DDAB	Paper + black ink + 1% DDAB
A. clavatus	–	–	–	±	±
P. paneum	–	–	–	±	±
U. chartarum	–	–	–	±	±

+ Paper and medium sterile, – paper and medium fully overgrown, ± paper sterile, medium overgrown.

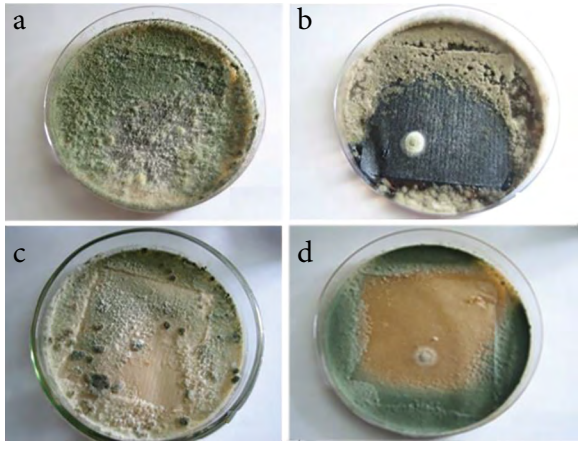

Fig. 2. *P. paneum* fungi growth (after 10 days) on the treated paper samples with: (a) black ink, (b) black ink and 1% DDAB solution; (c) brown ink, (d) brown 1% DDAB solution

Microscopic analysis of the paper was also performed using a scanning electron microscope (SEM). The morphology of the paper surface was examined on all paper samples treated with 1% DDAB solution and infected with fungi. At first, fungi infected samples in Petri dishes were washed with 70% ethyl alcohol for disinfection. After drying at room temperature, SEM analysis was performed to evaluate fungal colony growth and paper damage. SEM images of the paper samples treated with black ink are shown in Figs 3–5a, b.

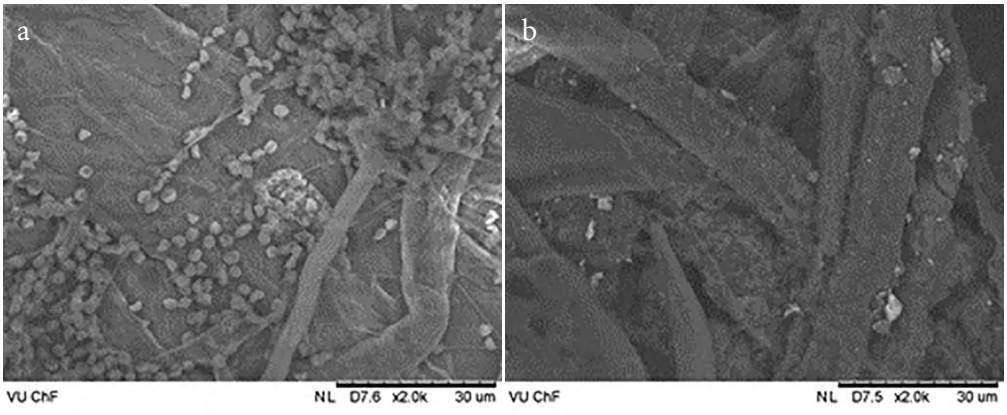

Fig. 3. SEM images of the paper with black ink: a) infected with *A. clavatus*, b) infected with *A. clavatus* and treated with 1% DDAB solution

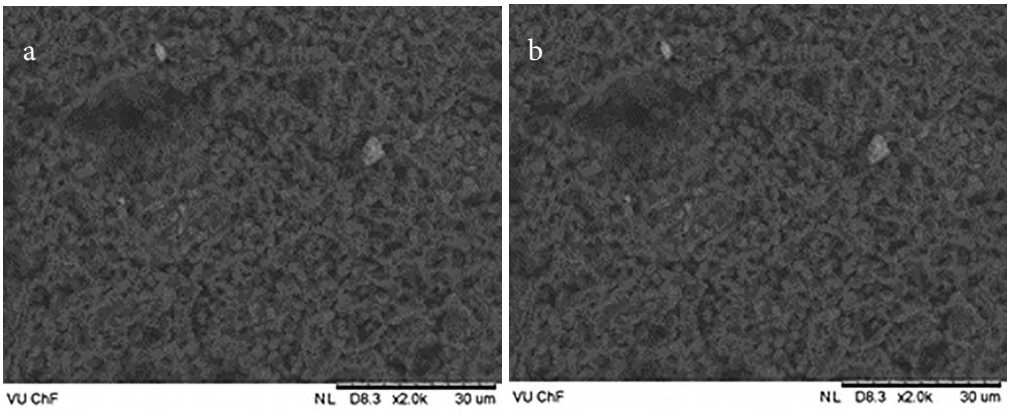

Fig. 4. SEM images of the paper treated with black ink: a) infected with *P. paneum*, b) infected with *P. paneum* and treated with 1% DDAB solution

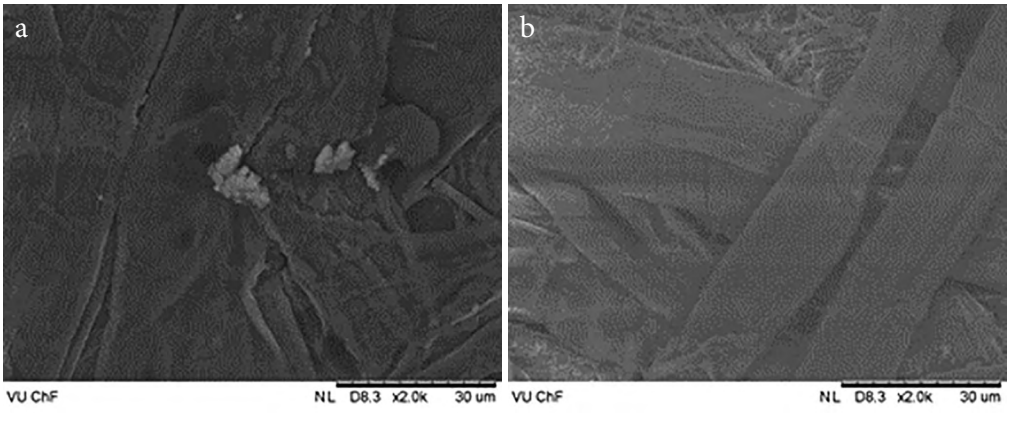

Fig. 5. SEM images of the paper treated with black ink: a) infected with *U. chartarum*, b) infected with *U. chartarum* and treated with 1% DDAB solution

The results of scanning electron microscopy (SEM) demonstrated distinct fungal morphology on the surface of both the control inked paper and the paper treated with DDAB solution. As shown in Figs 3a, 4a and 5a, a large amount of fungal *A. clavatus*, *P. paneum* and *U. chartarum* sporangia and hyphae were found on the surface of black inked paper samples. However, the quantity of fungal sporangia and hyphae on the paper treated with 1% DDAB solution was significantly reduced (Figs 3b, 4b and 5b). SEM images prove that DDAB acts as a biocide and has an antifungal activity against *A. clavatus*, *P. paneum* and *U. chartarum*.

The SEM images (Fig. 6) and visual observations on camera pictures (Fig. 2) show that the brown ink sample treated with DDAB solution is less damaged by *P. paneum* than the black ink sample.

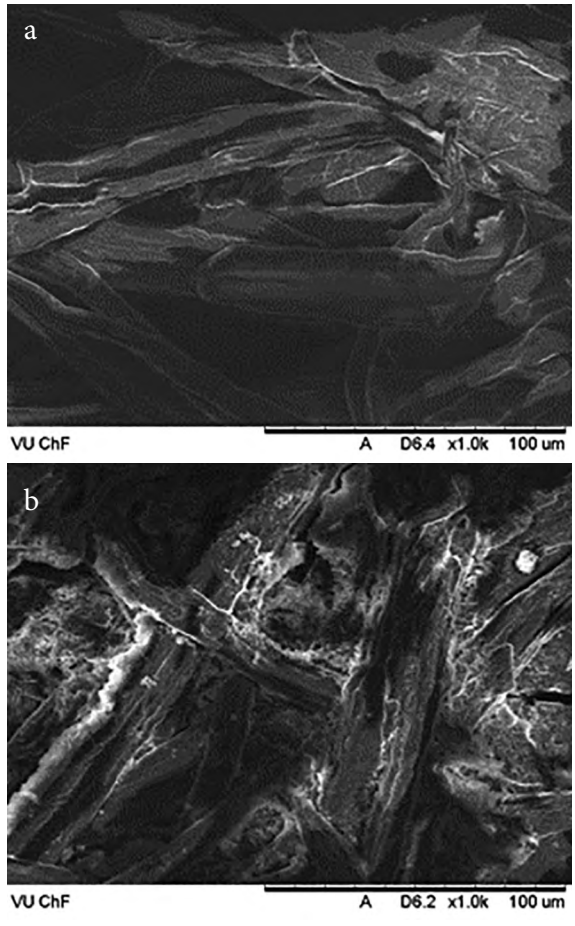

Fig. 6. SEM images of the paper treated with *P. paneum* and 1% DDAB solution: a) brown inked, b) black inked

CONCLUSIONS

Prior to conducting the fungicidal activity test, a study was performed to investigate the impact of didecyldimethylammonium bromide (DDAB) on the pH, degree of polymerization (DP), and optical properties of both pure and inked paper. To investigate the antifungal properties of DDAB on damaged paper, three species of fungi commonly found in libraries, archives and museums were used: *Aspergillus clavatus* Desm (*A. clavatus*), *Penicillium paneum* Frisvald (*P. paneum*) and *Ulocladium chartarum* Preuss (*U. chartarum*). The effectiveness of DDAB in aqueous solutions at various concentrations (0.1–1%) differed depending on the type of fungus, paper and ink components. The study results support the use of DDAB as a biocide for cleaning and preservation of paper documents, with active concentrations of 0.7 and 1%. Further analysis, including SEM and visual observations, revealed differences in fungal growth based on paper colour, ink type (brown vs black) and pH. Understanding the nature of ink components could help explain why certain paper and ink combinations promote or inhibit fungal growth.

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TYRIMAS APIE KETVIRTINIŲ AMONIO DRUSKŲ PRIEŠGRYBELINĮ VEIKIMĄ RAŠYTINIAM POPIERIUI IŠSAUGOTI

S a n t r a u k a

Biologinis dokumentų paveldo degradavimas yra rimta problema, su kuria susiduria bibliotekos, muziejai ir archyvai, nes gali sukelti popieriaus pažeidimą, dėl kurio jis tampa trapus, bespalvis ar taškuotas. Tai kelia grėsmę informacijos praradimui. Šio tyrimo tikslas yra ištirti didecildimetilamonio bromido (DDAB) poveikį rašytiniam popieriui ir skirtingų rūšių grybelių ant popieriaus augimui. Priešgrybelinio aktyvumo testui buvo pasirinktos trys grybelių rūšys, žinomos dėl savo gebėjimo sukelti popieriaus biodegradaciją bibliotekose, archyvuose bei muziejuose: *Aspergillus clavatus* Desm, *Penicillium paneum* Frisvald ir *Ulocladium chartarum* Preuss. pH ir polimerizacijos laipsnio (DP) matavimai, spalvų pokyčių stebėjimas ir skenavimo elektroninės mikroskopijos analizės (SEM) duomenys patvirtina, kad DDAB gali būti naudojamas kaip biocidas pelėsio grybelio augimui stabdyti ant gryno ir rašytinio popieriaus. Nustatytos aktyviosios DDAB koncentracijos yra 0,7 % ir 1 %. Taip pat buvo ištirtas DDAB priešgrybelinis poveikis, naudojant popieriaus pavyzdžius su skirtingos sudėties rašalais: juoda (geležies rūgšties rašalas) ir ruda (vario rašalas).