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Scientific paper
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Remediation of biodegradation using synthesized nano - and micromaterials

The artefacts in general are subject to degradation processes that require physical intervention techniques and/or chemicals to minimize destructive effects. One of the most sensitive types of artefacts are the painting (both mural or on conventional support). The methods used for the prevention/removal of biodegradation must be able to prevent microbial contamination, or remove microorganisms already developed. The most common contaminant fungi (moulds) are Aspergillus sp. and Penicillium sp., species that shows a greater tolerance to environmental factors. They are versatile species, which requires relatively little moisture, compared with bacteria.

To obtain remediation of the biodegradation of simulated artefacts presented in the study, we used a mixture of hydroxyapatite - nano-shaped barium hydroxide dispersed in isopropyl alcohol.

***Key words**: artefacts, restoration, conservation, nanomaterials, biodegradation*

INTRODUCTION

From all the artefacts, paintings are probably the most beautiful way of expressing human creativity. Since ancient times, man has felt the need to express themselves through drawing. Mural paintings were part of human life since ancient times (it deserves mentioning in this context, the mural paintings from Lascaux cave, southern France, or the caves in Tassili Mountains, Sahara desert). Over time, painting support was diversified, so today the paintings appear on almost any type of support.

When speaking of paintings, the microorganisms involved are bacteria and fungi [5]. The literature provides numerous reports both on bacterials species (*Bacillus, Pseudomonas, Enterobacter, Proteus, Escherichia, Micrococcus, Serratia, Aeromonas*) and fungi (*Rhizopus arrhenius, Aspergillus niger, A. ustus, Penicillium citrinum, Chaetomium globosum, Alternaria altanata* and so on) isolated from paintings and other monuments [10, 13, 8, 11, 1, 2, 12, 14, 6, 9].

Among the most serious consequences of biodeterioration are foul smell, viscosity loss, discolouration, visible surface growth and others, leading to very serious economic and aesthetic loss [11].

A series of factors (relative humidity over 70%, temperature over 15°C, a neutral to acid pH, presence of organic nutritive sources) can transform any environment in a favourable environment for the growth and reproduction of mould species [7,25].

The extent of contamination with fungal species is easily explained by the fact that fungus produces spores easily dispersed by air currents (Vukojevic and Grbic, 2010). Mould is everywhere in nature, but it only develops in favourable conditions. Changes in relative humidity can affect fungal growth. If the relative humidity is too low, biodeterioration is minimized, but will predispose the artefact to mechanical damage. If the relative humidity is high, mechanical damage is kept to a minimum, but the growth of biological organisms is encouraged, especially disastrous for paper artefacts [15].

MATERIAL AND METHODS

Our group previously reported the potential use of some *simulated artefacts* as models for real, infested artefacts, in order to avoid further damage that the experiments could produce [4].

To obtain nano-shaped barium hydroxide, a commercial available barium hydroxide was used (Merck KGaA, Germany). Hydroxyapatite was obtained from $Ca(NO_3)_2 \times 4H_2O$ and $(NH_4)_2HPO_4$ commercial reagents (Merck KGaA, Germany). To synthesise $Sr(OH)_2$, $Sr(NO_3)_2$ (Merck KGaA, Germany) and NaOH (Merck KGaA, Germany) commercial reagents were used.

The synthesized materials were characterized through energy dispersive X-ray fluorescence (EDXRF, using a PW4025 MiniPal 2 spectrometer – PAnalytical), X-ray diffraction (XRD, using a DRON UM1 diffractometer, operating at 32 kV and 25 mA, Co Kα radiation - 1.79021 Å), thermal analysis (performed on a TGA/SDTA 851 Mettler Toledo) and Dynamic Light Scattering (DLS - Nano ZS -Red

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badge). To determine the efficiency of the treatment, we used the diluted inoculums technique.

RESULTS AND DISCUSSIONS

Hydroxyapatite (HAP) was obtained as follows: 0.25 mol $Ca(NO_3)_2 \times 4H_2O$ (Merck KGaA, Germany) were dissolved in 250 ml distilled water; 0.25 mol of $(NH_4)_2HPO_4$ (Merck KGaA, Germany) were dissolved in 250 ml distilled water; the calcium containing solution was put into a flask and heated to the temperature of 80 °C. The phosphorus containing solution (with the pH adjusted to 10 with NH_4OH – Chimreactiv, Romania) was added into the calcium containing solution under vigorous stirring. The reaction was performed at 80 °C for 3 h, with the pH constantly kept at 10. After the reaction, the deposited mixtures were washed with distilled water, filtered, and rinsed with ethanol (Merck KGaA, Germany). The ethanol-containing gel was dried in a vacuum oven at 45 °C.

For the synthesis of $Sr(OH)_2$, adequate amounts of $Sr(NO_3)_2$ (Merck KGaA, Germany) and NaOH (Merck KGaA, Germany) were separately dissolved in water in order to obtain solutions of 0.7, respectively 0.3 M. The high concentration of the strontium salt was needed to reach a high saturation, an important requirement for the production of $Sr(OH)_2$ nanoparticles [3]. The solution was heated to the synthesis temperature (60 °C); the reaction took place by dropping NaOH solution into the solution containning strontium salt, under vigorous stirring, keeping the temperature at a constant value of 60°C ± 1°C. When precipitation of $Sr(OH)_2$ was complete, to stirring was continued for another 60 min (for aging). Aqueous suspension of $Sr(OH)_2$ was cooled to room temperature and washed with deionized water three times. Finally, the solution of $Sr(OH)_2$ was ultrasonated for 30 minutes to further reduce particle size.

The barium hydroxide was obtained through calcination at 1000°C from commercial reagent and then the oxide was transformed in hydroxide by refluxing in the presence of water and solvent, under inert atmosphere.

The characterization of the synthesized materials through EDXRF and XRD (figures 1 and 2) confirms the successful synthesis and the lack of impurities in the analyzed samples.

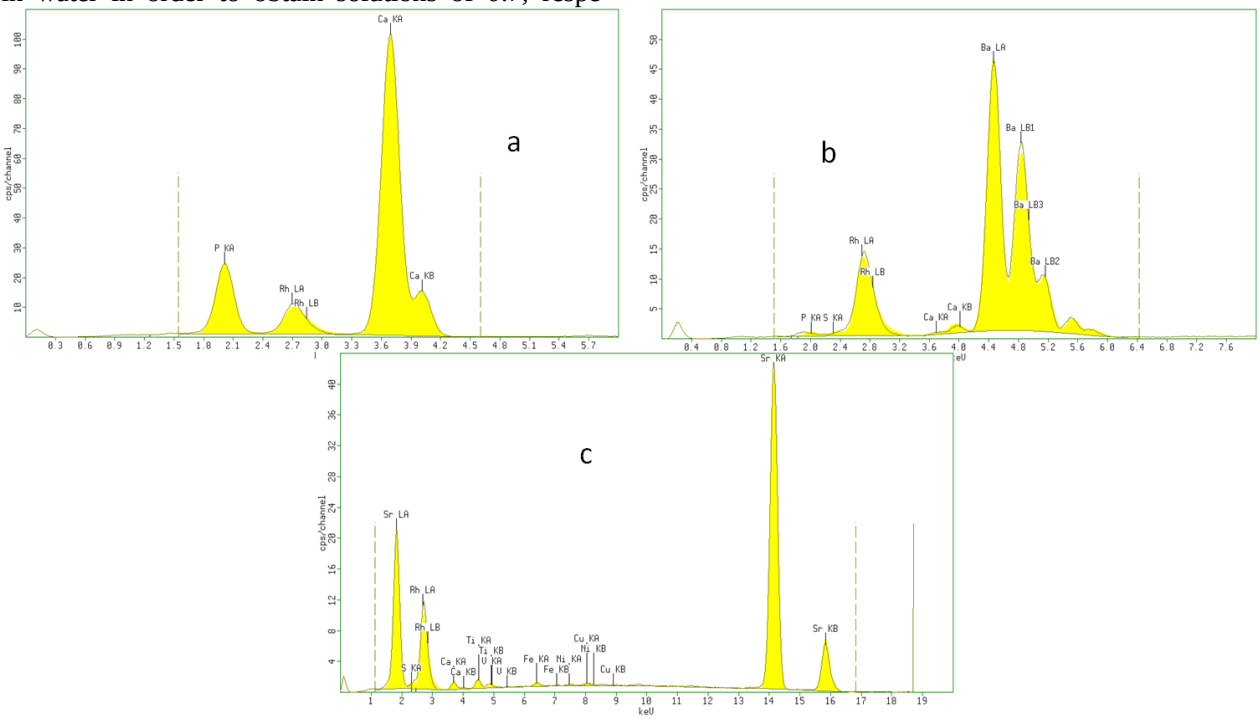

Figure 1 - EDXRF results: a) HAP, b) $Ba(OH)_2$, c) $Sr(OH)_2$

Thermogravimetric analysis, performed in air (figure 3), shows the endothermic effects accompanying the loss of water through evaporation and dehydration. The absence of other peaks indicates that the synthesized compounds are homogeneous and pure in composition.

In order to determine the sizes of the synthesized materials, DLS analyses were performed. The results are presented in figure 4. The results indicates that the materials are well in the nanometric scale, except $Sr(OH)_2$ which tends to agglomerate in larger clusters, in the micrometric range.

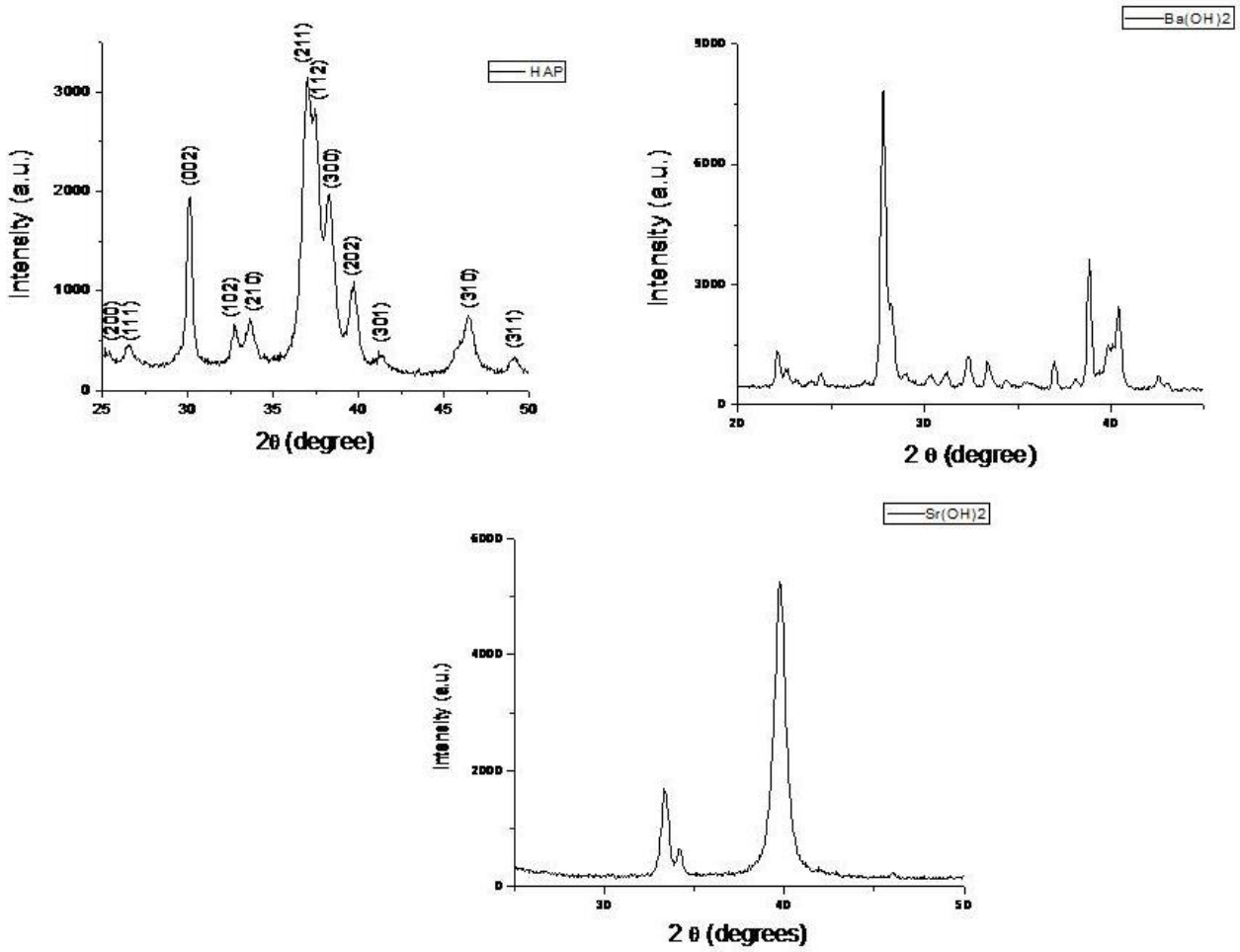

Figure 2 - XRD results of the synthesized materials

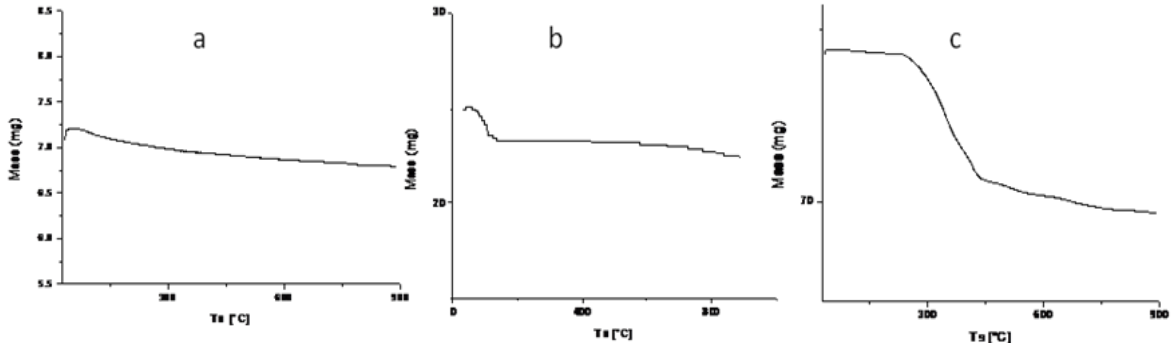

Figure 3 - Thermogravimetric results: a) HAP, b) $Ba(OH)_2$, c) $Sr(OH)_2$

Characterization in terms of antifungal action was performed using the diluted inoculums technique on culture media. The *simulated artefacts* were treated with the materials suspended in isopropyl alcohol and the artefacts kept for 15 days in conditions favourable to the development of fungal colonies (dark and humid environment). After that period, samples were collected from the artefacts and inoculated at the surface of solid Sabouraud medium in Petri dishes. The plates were incubated at 28 °C for seven days, except blank sample (incubated for 96 hours). All the experiments were carried out in triplicate. Representative results are presented in figure 5.

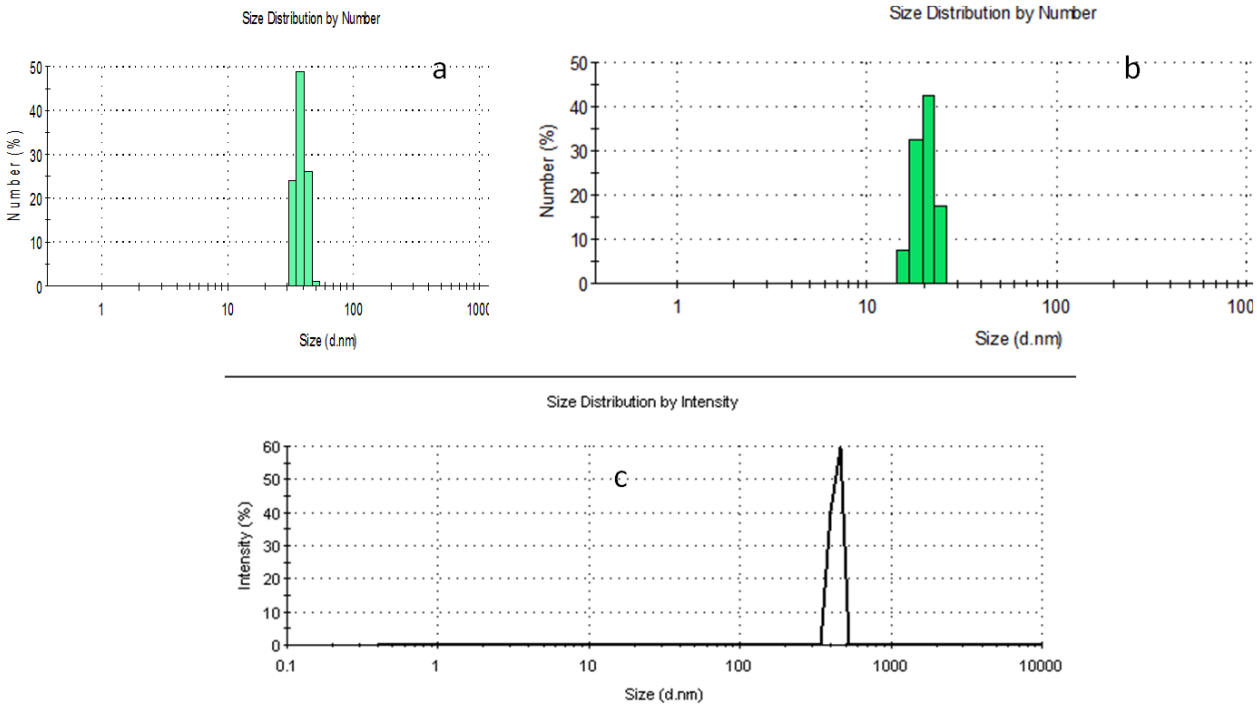

Figure 4 - DLS results: a) HAP, b) $Ba(OH)_2$, c) $Sr(OH)_2$

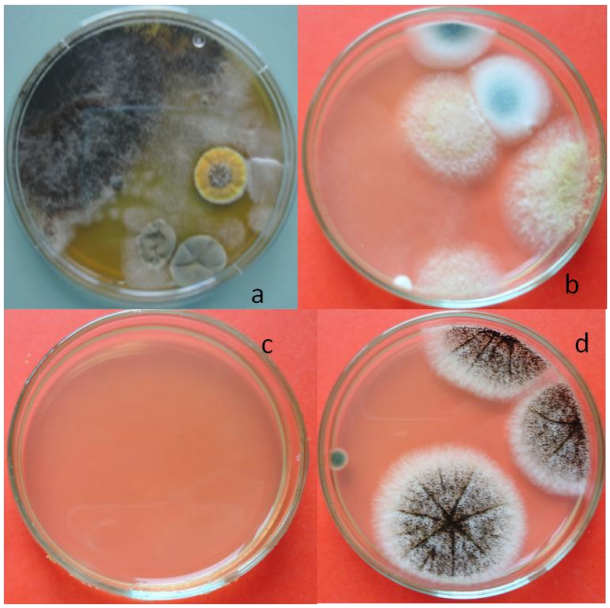

Figure 5 - Results after treatment: a) Blank sample, b) HAP, c) $Ba(OH)_2$, d) $Sr(OH)_2$

All the materials offers better results than the blank (untreated sample), but as can be seen from figure 5, best results are obtained with $Ba(OH)_2$.

CONCLUSIONS

All the artefacts (especially paintings and paper artefacts) are subject to (bio)degradation processes that require different types of intervention. Biological decontamination was effective in this study when using reagents with nanometer particle size. Barium hydroxide offered the best results as antifungal agent. The preliminary results presented encourage use to believe that a novel, less toxic or even non-toxic method for the treatment of biodeteriorated artefacts is to be developed.

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IZVOD

REMEDIJACIJA BIORAZGRADNJOM KORIŠĆENJEM SINTETIZOVANIH NANO I MIKROMATERIJALA

Predmeti u celini podležu procesima degradacije koje zahtevaju fizičke interventne tehnike i / ili hemikalije da bi se smanjili destruktivni efekti.

Metode koje se koriste za sprečavanje/uklanjanje biorazgradnje moraju biti u stanju da spreči mikrobiološku kontaminaciju, ili uklanjanje mikroorganizama koji su već razvijeni. Najčešći zagađivači među gljivicama (plesni) su Aspergillus sp. i Penicillium sp., vrste koje pokazuje veću toleranciju na faktore sredine. Oni predstavljaju raznovrsne vrste i zahtevaju relativno malo vlage, u poređenju sa bakterijam.

Da bi se dobila remedijacija biorazgradljivošću korišćenih predmeta , data u radu, korišćena je mešavina hidroksiapatita – nano u obliku barijum hidroksid dispergovanog u izopropil alkoholu.

Ključne reči: *predmeti, restauracije, konzervacije, nanomaterijali, biodegradacija*

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