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A NEW APPROACH FOR THE REMEDIATION OF BIODETERIORATED MOBILE AND IMMOBILE CULTURAL ARTEFACTS

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Abstract

Mould affects mobile and immobile cultural artefacts often causing irreparable damage. Fungi (particularly *Aspergillus sp.* that was found to be the most common and harmful species) affects a large variety of support materials, from buildings, ceramics, wood and paper to textile, paintings (mural or on canvas) and even metals. In order to preserve the cultural heritage objects, adequate conservation treatments are required. In the last decades, the main objective of the scientist working in this domain was the development of new treatments for the remediation of biodeterioration using either synthetic nanostructured, microstructured materials or natural extracts. The present paper objective is to show our own vision regarding the remediation of biodeteriorated mobile and immobile cultural artefacts. Based on our previous experience and on the results that will be presented the most promising synthetic materials are the hydroxides of alkaline earth metals as well as hydroxyapatite and its derivative compounds (the antibacterial and antifungal effect of hydroxyapatite being well-known). These preliminary results allow us to hope that in the near future some well defined methods involving the above materials will be applied for the remediation of artefacts biodeterioration.

Keywords: mould, biodeterioration, synthetic compounds, cultural heritage

1. Introduction

The cultural heritage is the legacy of a particular group or population, consisting of physical artefacts and intangible attributes contributing to the specific identity of the group; it is (or it should be) inherited from past generations, valued by present generations and maintained for the benefit of future generations. When speaking of cultural heritage, we could include tangible evidence (buildings, monuments, landscapes, books, artwork and other artefacts), intangible culture (folklore, traditions, language, etc.) and natural

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heritage (cultural landscapes and biodiversity) [1]. The interest regarding cultural heritage preservation is an important indicator of the degree of civilization and awareness of every country. At international level, UNESCO (United Nations Educational, Scientific and Cultural Organization) has among its objectives the conservation of the world cultural heritage. Currently, there are 962 world heritage sites (745 cultural, 188 natural and 29 mixed) in 157 countries [*World Heritage List*, online at <http://whc.unesco.org/en/list>]. Each of these sites is considered important for the international community.

Cultural heritage consists of almost all types of materials produced by nature and used by humans to perform various types of artefacts, from very simple single-component to complex structures integrating organic and inorganic materials. These artefacts, even if they are made of materials considered resistant (rock, metal), are influenced by environmental factors that can change their structure and composition. In addition, once introduced into the biosphere, they can be affected by biological mechanisms [P Tiano, *Biodegradation of Cultural Heritage: Decay Mechanisms and Control Methods*, online at http://www.arcchip.cz/w09/w09_tiano.pdf].

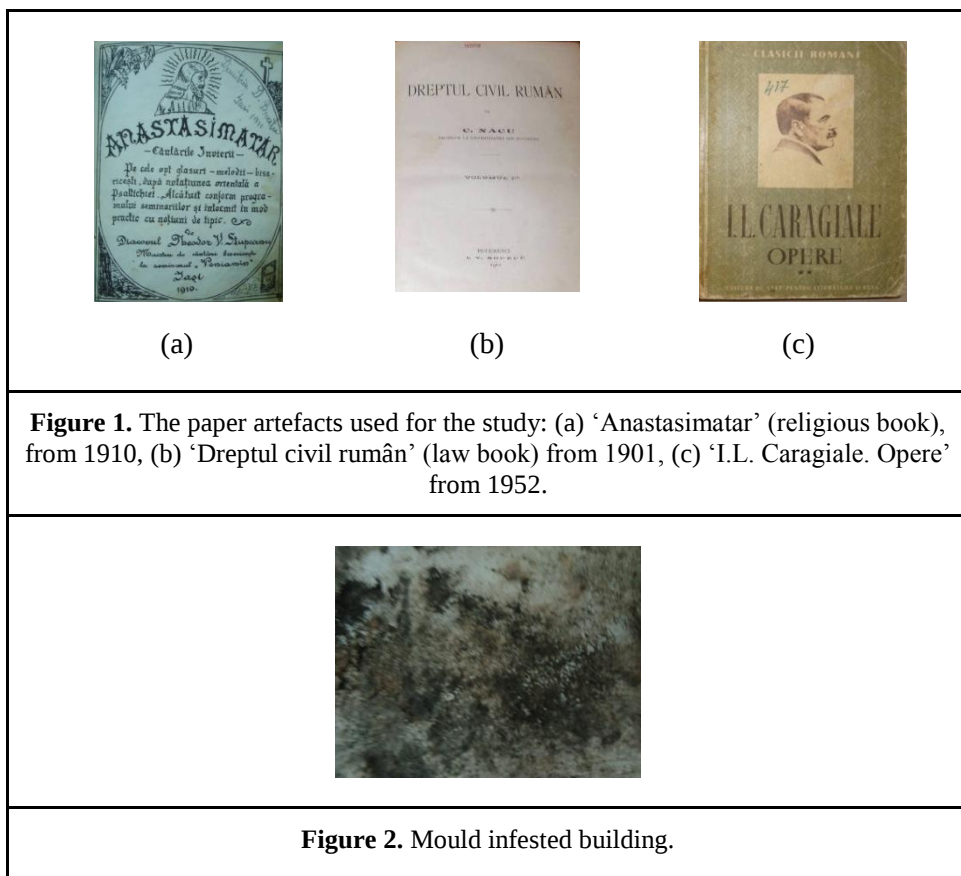
Among the many treats to the cultural heritage, in the present paper we will focus on biodeterioration. The artefacts colonization by harmful microorganisms is classified under the definition of *biodeterioration* [2]. The emergence of micro-organisms colonies and biodeterioration are often related to environmental conditions. The most important factors affecting microbial growth are the physical one (especially humidity, temperature and light) and the chemical nature of the substrate. Among biodeterioration producing colonies, many species of deuteromycetes (*Aspergillus* sp., *Fusarium* sp., *Penicillium* sp., *Stemphylium* sp., etc.) and ascomycetes (*Chaetomium* sp.) are frequently isolated from books, documents and printing [3]. Fungal infestation can lead to very rapid degradation of organic artefacts [4-6], but can also affect the ones of inorganic nature [7]. Fungi of the genus *Aspergillus* and *Penicillium* are among the most prevalent, being responsible for many of the observed effects of biodeterioration [4-7]. It must be noted that fungi grow on any material that can provide the necessary nutrients (organic matter). However, fungi can also grow on inorganic materials, obtaining nutrients from the material surface or air. The wide spread of fungi, their undesirable effects on artefacts and human health and the need to repel those represents the reason of the present research paper. In the following paragraphs we will present some approaches to the remediation of biodeteriorated artefacts, as well as our own vision on the presented topic, supported by some preliminary results.

2. Experimental

2.1. Materials

In order to probe the spread of fungi, paper artefacts from private collections (presented in Figure 1), buildings (Figure 2) and simulated artefacts

were used. The simulated artefacts represent cardboards kept for 6 months in wet and dark environment.



Hydroxyapatite (HA) used in the biological decontamination study was obtained from $\text{Ca}(\text{NO}_3)_2 \times 4\text{H}_2\text{O}$ and $(\text{NH}_4)_2\text{HPO}_4$ commercial reagents (Chimreactiv, Romania). Each of them was dissolved in distilled water. $\text{Ca}(\text{NO}_3)_2$ solution was added under vigorous stirring over $(\text{NH}_4)_2\text{HPO}_4$ solution until a milky precipitate was obtained. The resulting solution was refluxed for one hour and after that it was filtered. The filtrate obtained was dried in an oven and then calcined at 1000°C for an hour [8]. Nano-shaped barium hydroxide ($\text{Ba}(\text{OH})_2$) 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 [9].

2.2. Methods

In order to determine the biological contaminants present on the surface of the materials we used the diluted inoculums technique. For this type of analysis,

samples are collected and suspended in sterile distilled water. The samples are inoculated at the surface of a solid growth medium in Petri dishes; the liquid is dispersed evenly on the surface of the plate (using a Drigalski rod, through tilt/rotation motions of the plate). The plates are incubated at 28°C for several days. The used culture media are solid (SS) and liquid Sabouraud (LS) and gelose (G) (all produced by INCDMI Cantacuzino, Romania).

The synthesized materials were characterised through dynamic light scattering (DLS), using a Malvern NANO ZS (RED BADGE) apparatus, FTIR (FT-IR GX, Perkin Elmer) and energy-dispersive X-ray fluorescence (PW 4025 MiniPal 2). For the visualisation of the fungal growth, a Novex B-series trinocular microscope was used (at a 40x magnification).

3. Results and discussions

3.1. Fungal growth

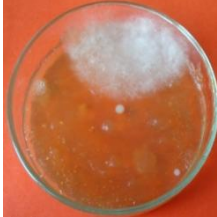
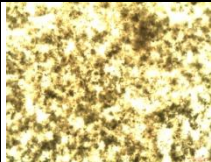
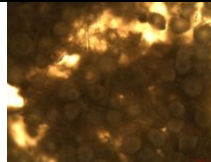
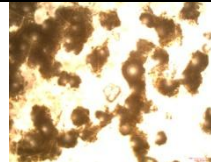
From the materials mentioned above, samples were collected and the diluted inoculums technique applied. Some representative results are presented in Figures 3-8.

		
Figure 3. Sample from book a in SS + glucose media, 192 h.	Figure 4. Sample from book b in LS + glucose media, 192 h.	Figure 5. Sample from book b in SS + glucose media, 192 h.
		
Figure 6. Sample from book c in LS + glucose media, 192 h.	Figure 7. Sample from book c in SS + glucose media, 192 h.	Figure 8. Sample from book b in G + glucose media, 192 h.

In Figures 9-12 are presented the results for samples collected from infected building. The results obtained using the ‘simulated’ artefacts are

presented in Figures 13-16. The fungi grown on the culture media were also visualised using an optical microscope (Figures 17-20).

The results presented above prove that, generally speaking, the same fungi (*Aspergillus Sp.*, *Penicillium Sp.* and *Mucor Sp.*) that naturally colonise the paper artefacts and buildings are found on the simulated artefact. Therefore, for future experiments, the use of solid Sabouraud media (eventually with added glucose) will be sufficient.

			
Figure 9. Sample from building in LS no added glucose, 96 h.	Figure 10. Sample from building in G + glucose, 96 h.	Figure 11. Sample from building in SS no added glucose, 96 h.	Figure 12. Sample from building in LS + glucose, 96 h.
			
Figure 13. Sample from simulated artefact in LS + glucose, 144 h.	Figure 14. Sample from simulated artefact in G no added glucose, 144 h.	Figure 15. Sample from simulated artefact in G + glucose, 144 h.	Figure 16. Sample from simulated artefact in SS + glucose, 144 h.
			
Figure 17. Blue mould (<i>Penicillium Sp.</i>)	Figure 18. Black mould (<i>Mucor Sp.</i>)	Figure 19. Green mould (<i>Aspergillus Sp.</i>)	Figure 20. White mould (<i>Aspergillus Sp.</i>)

3.2. Analytical characterization of synthesized materials

The synthesized materials were characterized through Fourier Transformed Infrared Spectroscopy (Figures 21 and 22) and EDXRF (Figures 23 and 24). The nanosize of the materials was evaluated using the DLS technique (Figure 25).

From the FTIR spectra it was possible to identify the materials synthesized, respectively hydroxyapatite and barium hydroxide [8, 9].

The DLS measurements (Figure 25) revealed the nanosize of the $\text{Ba}(\text{OH})_2$ materials, used as a mixture in combination with hydroxyapatite.

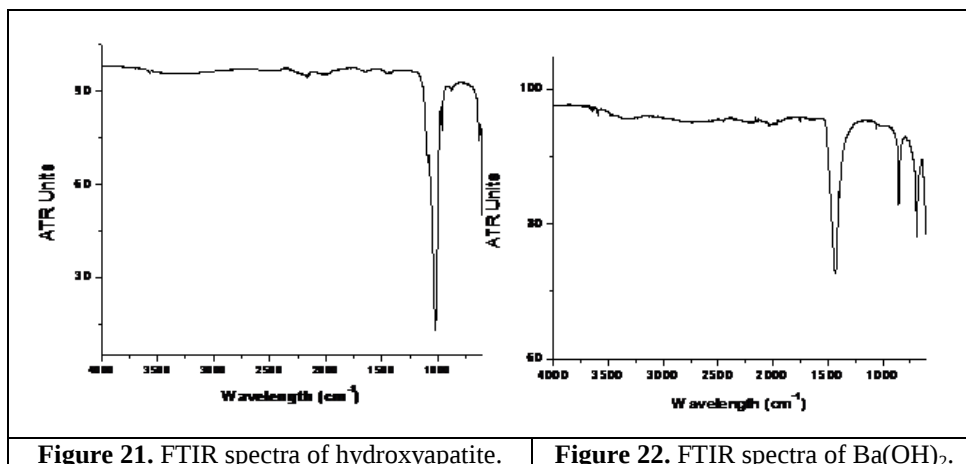


Figure 21. FTIR spectra of hydroxyapatite.

Figure 22. FTIR spectra of $\text{Ba}(\text{OH})_2$.

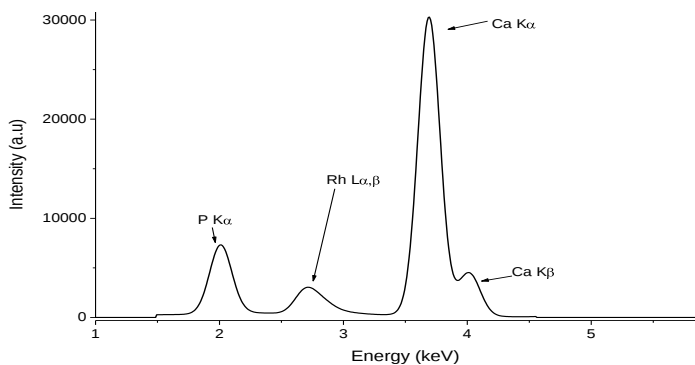


Figure 23. EDXRF spectrum of hydroxyapatite. Ca and P peaks are indicated. The Rh peak is a characteristic of the X-ray tube.

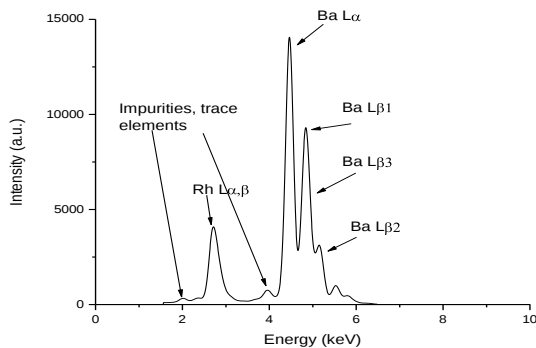


Figure 24. EDXRF spectrum of barium hydroxide.

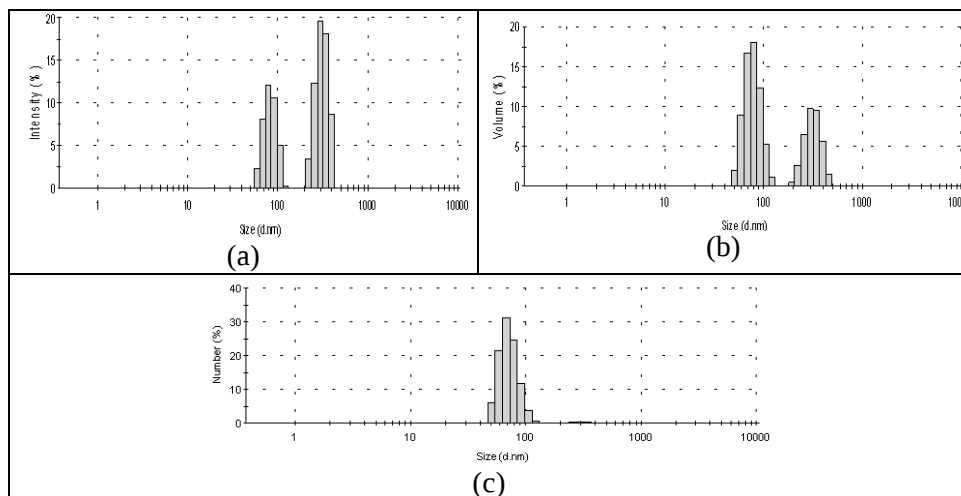


Figure 25. Particle size distribution for the mixture barium hydroxide and hydroxyapatite (by intensity, by volume and by number).

3.3. Experimental effects of treatment

After 15 days from the treatment with hydroxyapatite and barium hydroxide (pulverized as alcoholic suspension in isopropyl alcohol), in which the simulated artefacts were kept in the special conditions mentioned above, other samples were collected in order to determine the efficiency of the treatment. In Figure 26 is visible the growth of significant fewer colonies (1-2 colonies) of *Aspergillus* sp and *Penicillium* sp, as compared with those appearing before treatment (as shown in Figures 3-16).

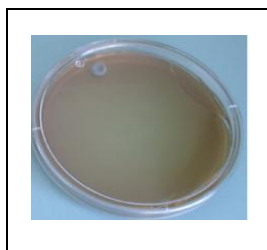


Figure 26. SS + glucose - 144 hours of incubation culture – after treatment.

4. Conclusions

The presented results gave information for bioremediation studies on simulated artefacts, thus allowing the protection of real artefacts. The study conducted on simulated artefacts demonstrated the efficiency of the biological decontamination when using barium hydroxide - hydroxyapatite mixture is used.

Our future works will focus on the optimization of this method, as well as on the discovery of new materials that can be successfully used for the restoration and/or conservation of historical artefacts.

Also, the use of natural extracts will be presented in our future papers, having as starting point the similar work carried out around the world: Verma *et al* [10] studied the antifungal activity against moulds growing on buildings of some essential oils, while in another study Afifi [11] proved the antifungal properties of plants extracts (*Anethum graveolens*, *Cymbopogon citrates*, *Juniperus oxycedrus*) and evaluated the growth inhibition of the stucco ornaments in the Ribate of Mostafa Pasha that belonged to the Ayyubid period.

The presented preliminary results encourage use to believe that a novel, less toxic or even non-toxic method for the treatment of biodeteriorated artefacts is to be developed.

Acknowledgement

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