Synthesis and characterization of new materials for artefact bioremediation

Radu Claudiu FIERASCU[1] | Rodica Mariana ION[2] | Irina FIERASCU[3]

[1] The National Institute for Research & Development in Chemistry and Petrochemistry – ICECHIM Bucharest, Romania | [2] The National Institute for Research & Development in Chemistry and Petrochemistry – ICECHIM Bucharest, Romania | [3] The National Institute for Research & Development in Chemistry and Petrochemistry – ICECHIM Bucharest, Romania

Abstract: The influence of the environment on the artefacts often translates (in absence of protecting measures) into their damage trough physical, chemical or biological processes. Most artefacts (regardless of their nature) are affected by the action of fungi. Previously it was shown that the most widespread fungi are those of *Aspergillus sp.* and *Penicillium sp*. Developing new bioremediation recipes based on synthesized chemical substances (with low or absent toxicity for humans and to the rest of the environment) can remove the shortcomings of the currently used methods (gamma or UV irradiation, etc.).
This paper presents the synthesis and characterization of new materials (hydroxyapatite and its derivatives, hydroxides of alkaline earth metals). Characterization of the synthesized materials was performed using state-of-the-art analytical techniques (XRD, XRF, FTIR, thermal analysis). Characterization in terms of antifungal action was performed using the diluted inoculum technique on culture media. The obtained results allow us to hope for an alternative method for the removal of biodegradation, using selected synthesized materials.

Keywords: Materials, synthesis, characterization, artefacts, bioremediation.

Introduction

All the artefacts, regardless of their nature, can be affected by what are known as *environmental fungi* (ACOEM 2011). This action (also known as *biodeterioration)* may lead to irreparable damage to the artefacts, trough physical, chemical or biological processes, resulting both in economic loss and cultural and artistic loss (KEOPANNHA 2008).
Fungal infestation can affect limestone, marble or other stones (making theme discolored and powdery on the surface) (KUMAR & KUMAR 1999). Speaking of artefacts, the fungi most often attack paper (manufactured paper being more susceptible because its content in starch, glue, vegetable proteins, dyestuffs, pigments, and other additives that could provide nutrients) (KEOPANNHA 2008). Even though less frequently, textiles are also affected, cotton being more susceptible than silk (ARANYANAK 1995).
Fungi breed most successfully on damp walls and can move from there to infest artefacts (FLORIAN 2002). Besides its direct damage, moulds affect the artefacts through and indirect route: it attracts mould feeding insects, and thus exposes cultural materials to further infestations by other biodeteriogens (PINNIGER 1989).

Developing new bioremediation recipes based on synthesized chemical substances (with low or absent toxicity for humans and to the rest of the environment) can remove the shortcomings of the currently used methods (gamma or UV irradiation, etc.) (TIANO 2002; URZI & DeLEO 2010).
Lin e al. (LIN et al. 2008) presented the antibacterial effect of partially and totally strontium substituted hydroxyapatite. Based on these data, our group has synthesized and analytical characterized (trough energy dispersive X-ray fluorescence - EDXRF, X-ray diffraction - XRD, Fourier transform infrared spectroscopy - FTIR, thermal analysis) a series of strontium and barium substituted hydroxyapatite. The efficiency of the synthesized materials was evaluated by diluted inoculums technique on culture media.
Our group previously demonstrated that the most widespread fungi are belonging to *Aspergillus sp., Penicillium sp.* and, in lesser extent, *Mucor Sp.*, as well as some synthesized materials for the remediation of biodeterioration (FIERASCU et al. 2013).

Experimental

Materials

The strontium substituted materials were synthesized as follows: hydroxyapatite (HAP) was obtained as follows: 0.25 mol $Ca(NO_3)_2 \bullet 4H_2O$ (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.
SrCaHAP powder was prepared by the same recipe, by using a mixture of $Ca(NO_3)_2 \bullet 4H_2O$ (0.125 mol) and $SrCl_2 \bullet 6H_2O$ (0.125 mol, Merck KGaA, Germany) to obtain a Ca/Sr solution instead of the Ca solution used for HAP synthesis.
For the SrHAP synthesis, 0.25 mol $SrCl_2 \bullet 6H_2O$ were dissolved in distilled water and the previously presented procedure was followed. In order to obtain BaHAP, $BaCl_2$ (Merck KGaA, Germany) was used instead of $SrCl_2$ in the upper recipe.
In order to avoid the possible damage to real artefacts, *simulated artefacts* were used, as previously reported (FIERASCU et al. 2013).

Methods

Energy dispersive X-ray fluorescence analyses (EDXRF) were performed using a PW4025 MiniPal 2 spectrometer (PAnalytical). X-ray diffractions (XRD) were obtained by the use of a DRON UM1 diffractometer, operating at 32 kV and 25 mA, using Co Kα radiation (1.79021 Å). FTIR analyses were performed on a FT-IR GX (Perkin Elmer) spectrometer. Thermal analyses were performed on a TGA/SDTA 851 (Mettler Toledo). All the obtained results were processed using a dedicated data analysis software (Origin 8.0).

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 culture media used was solid Sabouraud (SS) (produced by INCDMI Cantacuzino, Bucharest, Romania).

Results and discussions

Analytical characterization

The materials synthesized were characterized by EDXRF (Fig. 1 and 2), XRD (Fig. 3 and 4), FTIR (Fig. 5 and 6) and thermal analysis (Fig. 7 and 8).

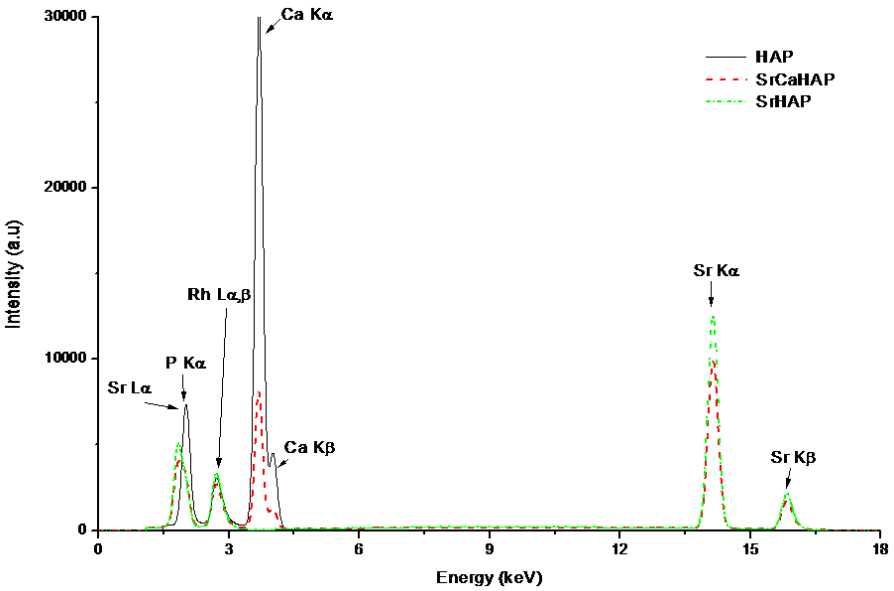

Fig. 1 – EDXRF results for HAP/SrCaHAP/SrHAP

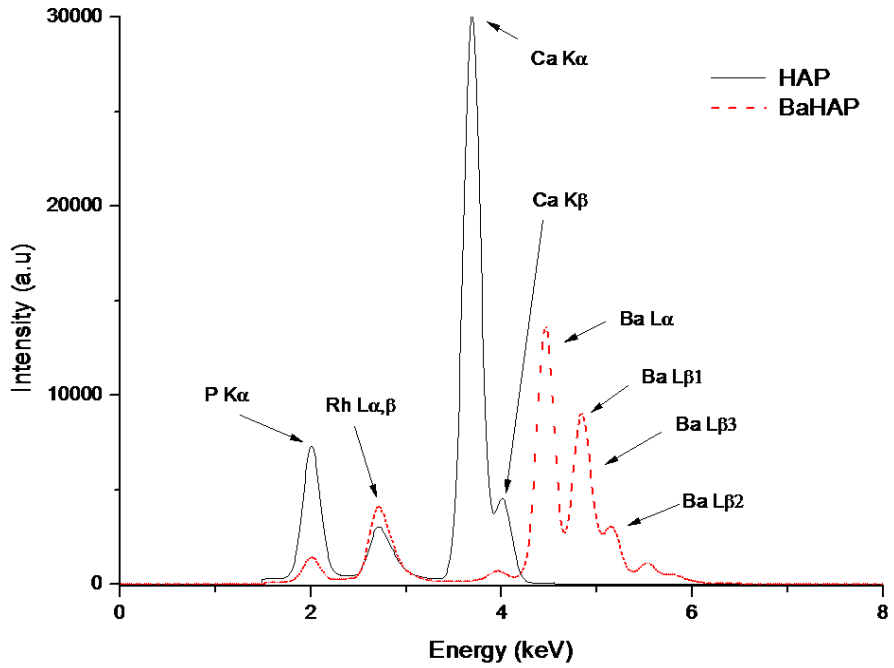

Fig. 2 – EDXRF results for HAP/BaHAP

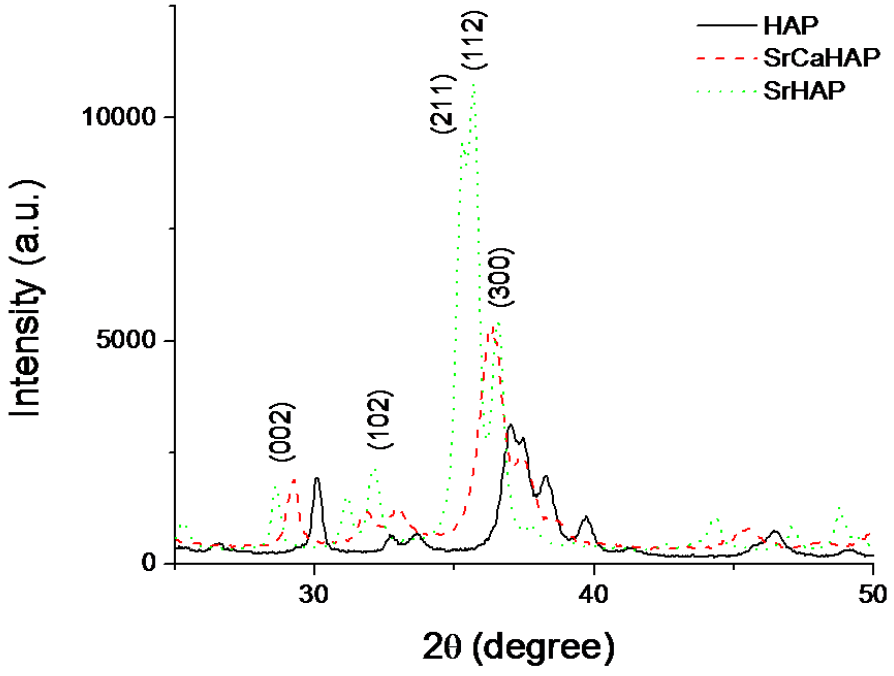

Fig. 3 – XRD results for HAP/SrCaHAP/SrHAP

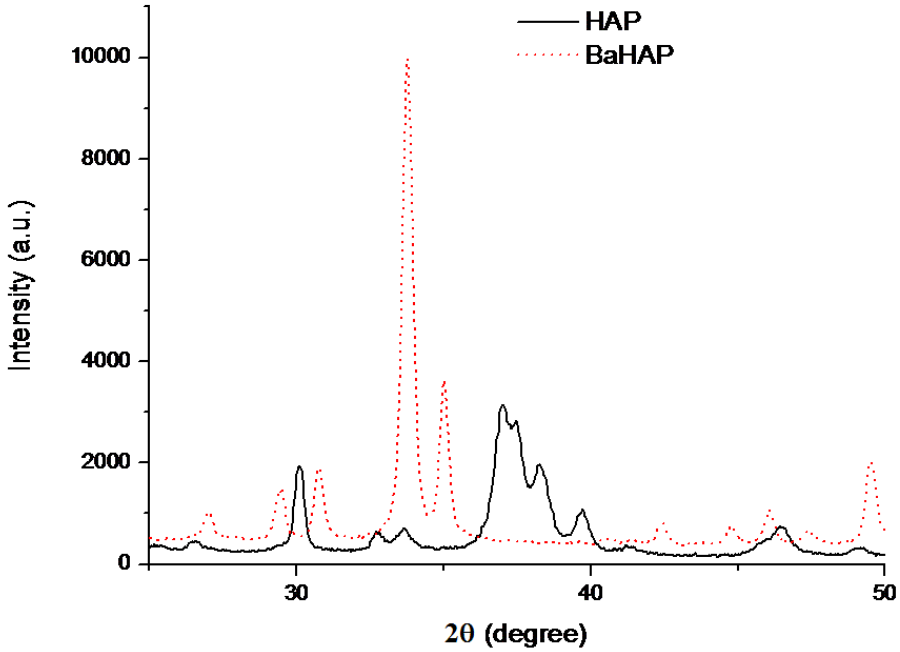

Fig. 4 – XRD results for HAP/BaHAP

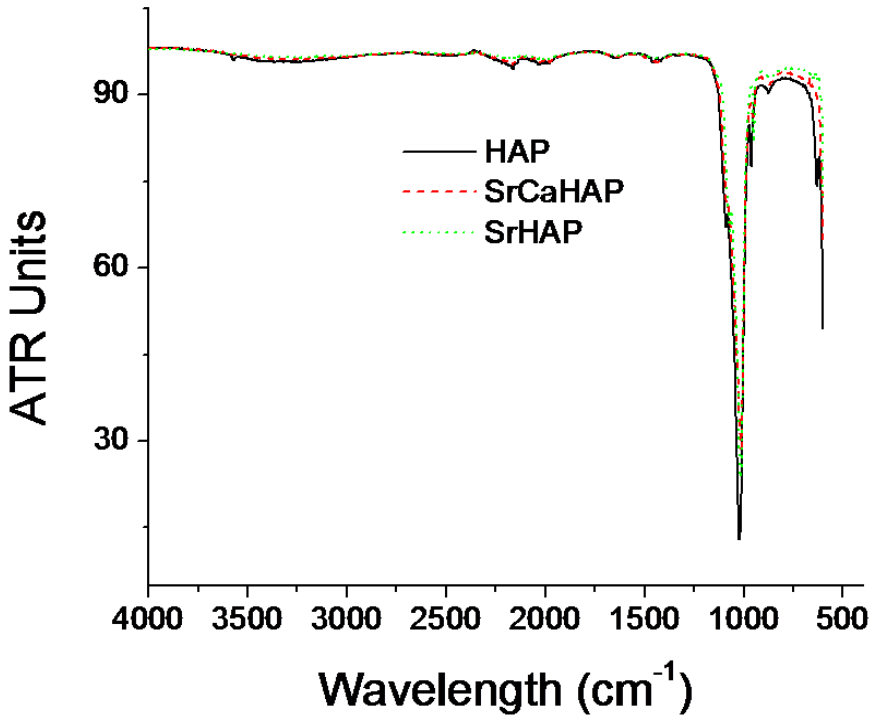

Fig. 5 – FTIR results for HAP/SrCaHAP/SrHAP

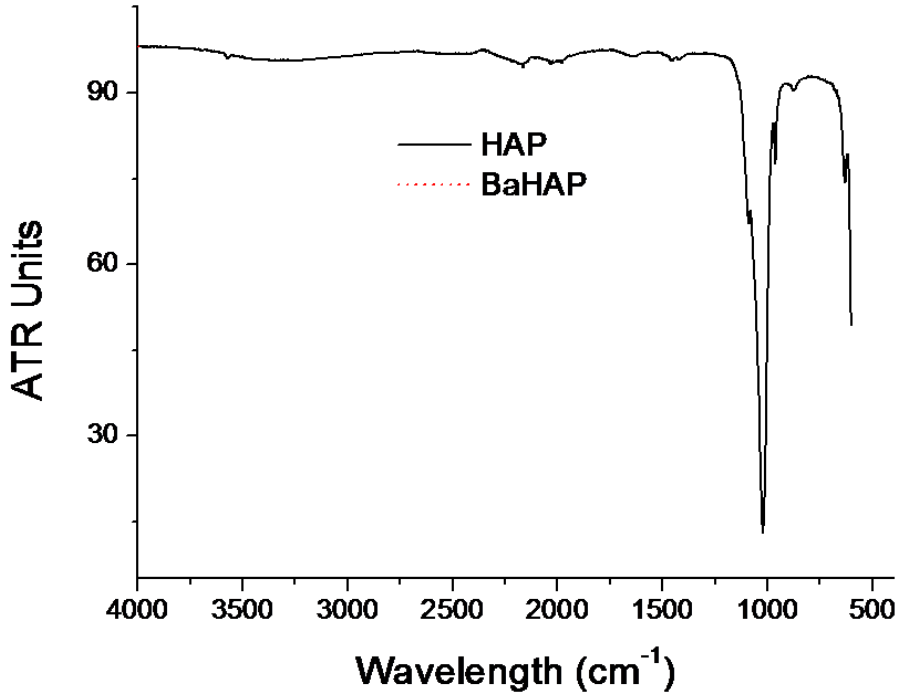

Fig. 6 – FTIR results for HAP/BaHAP

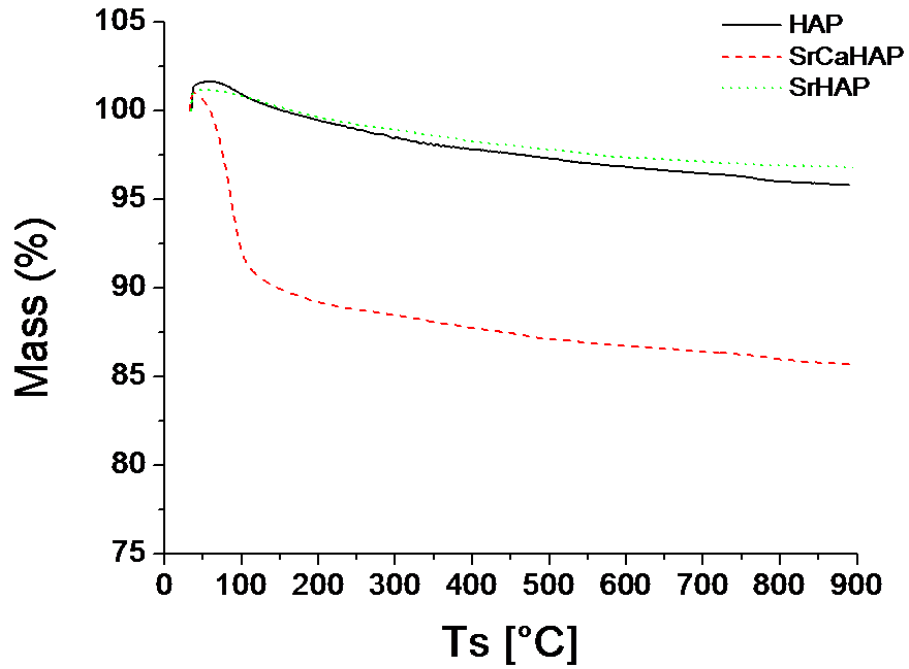

Fig. 7 – Thermogravimetric analysis results for HAP/SrCaHAP/SrHAP

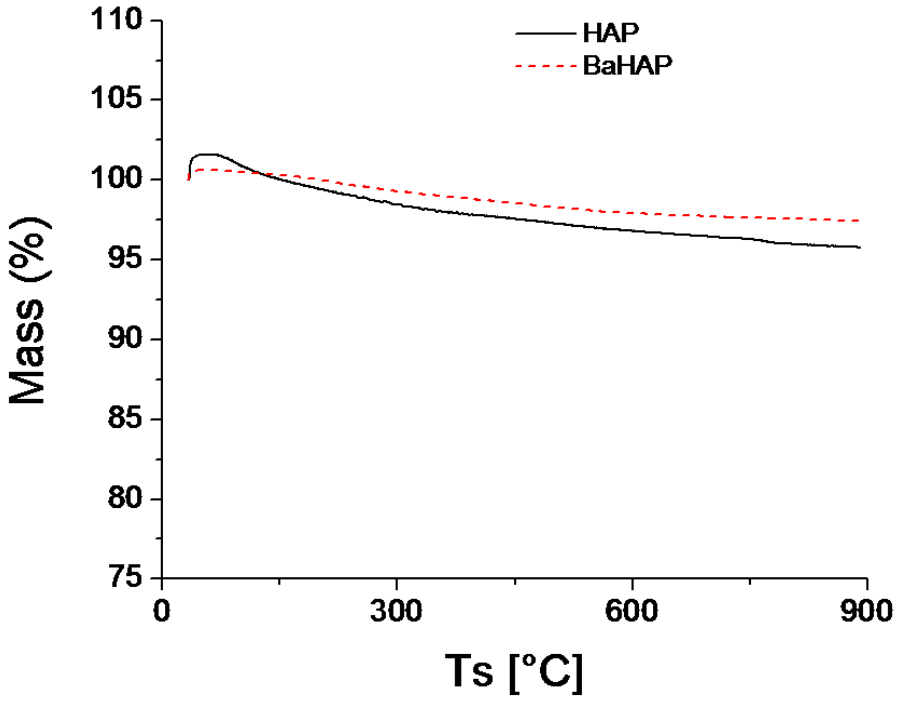

Fig. 8 – Thermogravimetric analysis results for HAP/BaHAP

EDXRF results show the lack of impurities in the synthesized materials. The FTIR spectrum presents strong bands, as follows: bands around 600 cm^{-1}, 955 cm^{-1} and 1020 cm^{-1} are characteristics for PO_4^{3-}. The observation of the asymmetric P-O stretching vibration at 955 cm^{-1} is a distinguishable peak, together with the peaks around 600 cm^{-1}, correspond to the triply degenerate bending vibrations of PO_4^{3-} in hydroxyapatite, similar to other data presented by the literature (MURUGAN R. & RAMAKRISHNA 2004, NEJATI et al. 2009). The fact that all FTIR spectra are identical suggests that all the compounds present the same basic structure, respectively the isomorphic substitution on the hydroxyapatite structure.
The XRD results, similar to the literature (NEJATI et al 2009, LIN et al. 2008) prove the successful isomorphic substitution, a fact not yet mentioned in the literature data, as well as the small dimensions of the synthesized materials (in the nanometric range). Thermogravimetric analysis performed in air 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.
The analytical results presented above prove the successful synthesis of the hydroxyapatite derivatives.

Antifungal activity

Characterization in terms of antifungal action was performed using the diluted inoculums technique on culture media. The treatment was performed by pulverizing the simulated artifacts samples with the synthesized materials suspended in isopropyl alcohol. After a period of 15 days, in which the samples were kept for 15 days in a dark and humid environment (favorable for the fungi growth) (FIERASCU et al. 2013), samples were collected in order to determine the efficiency of the treatment. The samples were inoculated at the surface of solid Sabouraud medium in Petri dishes. The plates were incubated at 28 °C for seven days

(except for blank sample, incubated for 120 h and sample D, incubated for 240 h). All the experiments were carried out in triplicate. Representative results are presented in Fig. 9.

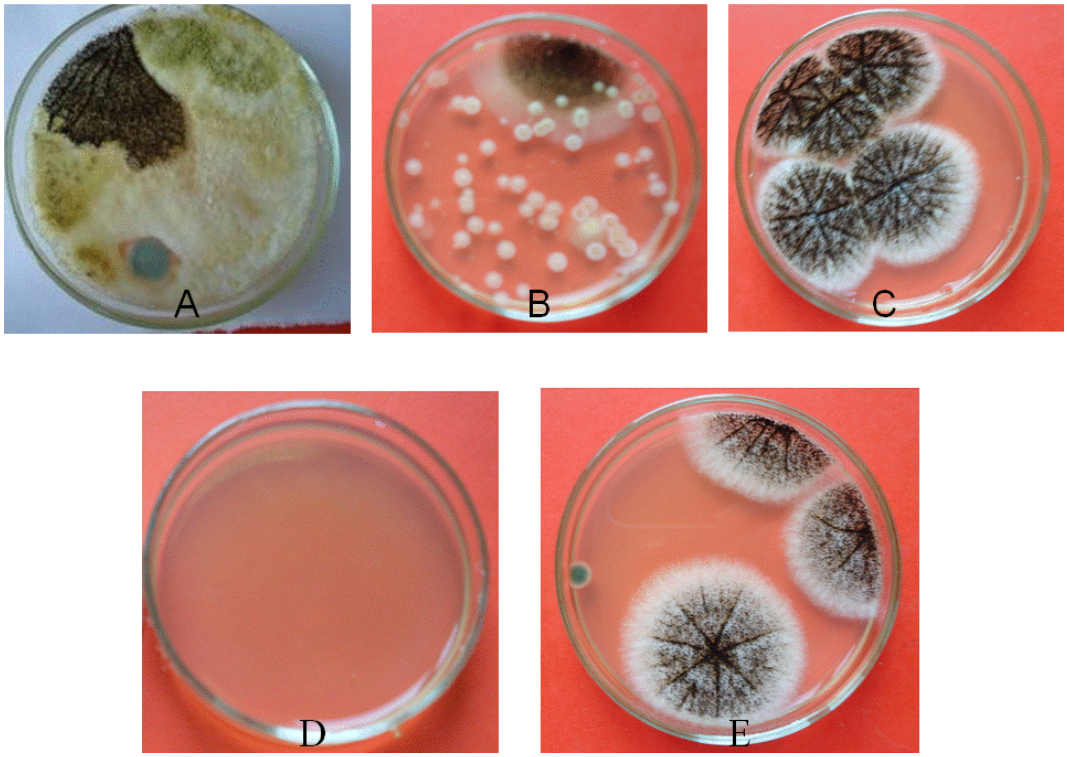

Fig. 9 – Results for the fungal growth after treatment: a – control (blank sample), b - HAP, c - SrCaHAP, d - SrHAP, e - BaHAP

The incubation of the blank sample was stopped after 120 hours due to the extensive growth of fungal colonies. Sample D (sample after treatment with SrHAP) was kept for 240 hours in order evaluate the time necessary for fungal colonies to appear. After that period, no colonies were identified. The incubated samples prove the fact that alcoholic suspension of SrHAP is a very efficient antifungal agent. Nevertheless, all synthesized materials present some antifungal activity, compared with the blank (control) sample. The antifungal activity of the synthesized materials can be described as follows (higher antifungal activity to lower antifungal activity): SrHAP > HAP > BaHAP > SrCaHAP.

Conclusions

Artefacts in general and especially paper artefacts are subjected to the fungal attack. The classical preventive measures (special conditions for their storage) can often prove to be expensive and/or inapplicable.

The present paper describes a study regarding the use of synthesized materials for the protection of paper artefacts against biodeterioration. The obtained results prove to be superior to those obtained when using natural extracts or other chemical compounds, previously reported by our group (HAP-barium hydroxide mixture) (DUMITRIU et al. 2010). The efficiency of the synthesized materials can be described as follows (higher efficiency to lower efficiency): SrHAP > HAP > BaHAP > SrCaHAP > control.

The obtained results allow us to hope for an alternative method for the removal of biodegradation, using selected synthesized materials.

Acknowledgements

This work was supported by a grant of the Romanian National Authority for Scientific Research, CNCS – UEFISCDI, project number PN-II-RU-PD-2011-3-0023. The authors wish to thank their colleagues Contantin Radovici, Sanda Doncea and Simona Pop for their analytic help.

References

ACOEM (2011), American College of Occupational and Environmental Medicine, *Adverse human health effects associated with molds in the indoor environment,* available online at: www.acoem.org/AdverseHumanHealthEffects_Molds.aspx

ARANYANAK C. (1995), *Microscopical Study of Fungal Growth on Paper and Textiles*. In: Chiraporn Aranyanak and Chalit Singhasir (ed.), *Biodeterioration of Cultural Property 3: Proceedings of the 3rd International Conference*, Bangkok, Thailand, 83

DUMITRIU I., FIERASCU R.C., ION R.M., BUNGHEZ R.I., COROBEA M.C. (2010), *Nanotechnology applied in archaeometry: restoration and conservation,* SPIE Proceedings, 7821, 78211I

FIERASCU I., DIMA R., ION R.M., FIERASCU R.C. (2013), *New approach for the remediation of biodeteriorated mobile and immobile cultural artefacts*, European Journal of Science and Theology, 9(2), *in press*

FLORIAN M.L. (2002), *Fungal Facts: solving fungal problems in heritage collections*, Archetype Publications, London

KEOPANNHA V. (2008), *Museum Collections and Biodeterioration in Laos*, Master Thesis, Gothenburg University, available online at: www.globalstudies.gu.se/digitalAssets/1170/1170137_Diss_Vanpheng.pdf

KUMAR R., KUMAR A. (1999), *Biodeterioration of Stone in Tropical Environments: An Overview,* The Getty Conservation Institute, Malibu

LIN Y., YANG Z., CHENG J., WANG L. (2008), *Synthesis, characterization and antibacterial property of strontium half and totally substituted hydroxyapatite nanoparticles,* Journal of Wuhan University of Technology-Mater. Sci. Ed., 23(4), 475-479

MURUGAN R., RAMAKRISHNA S. (2004), *Bioresorbable composite bone paste using polysaccharide based nano hydroxyapatite*, Biomaterials, 25, 3829–3835.

NEJATI E., FIROUZDOR V., ESLAMINEJAD M.B., BAGHERI F. (2009), *Needle-like nano hydroxyapatite/poly(L-lactide acid) composite scaffold for bone tissue engineering application*, Materials Science and Engineering C, 29, 942–949

PINNIGER D. (1989), *Insect Pests in Museums,* Institute of Archaeology Publications, London

TIANO P. (2002), *Biodegradation of cultural heritage: Decay mechanisms and control methods,* 9th ARIADNE Workshop "Historic Material and their Diagnostic", ARCCHIP, Prague, 22 to 28 April 2002, available online at: http://www.arcchip.cz/w09/w09_tiano.pdf.

URZI C., DE LEO F. (2010), *Biodeterioration of Cultural Heritage in Italy: State of Art,* available online at: http://www.arcchip.cz/w08/w08_de_leo.pdf

Imprint:
Proceedings of the 17th International Conference on Cultural Heritage and New Technologies 2012 (CHNT 17, 2012)
Vienna 2013
http://www.chnt.at/proceedings-chnt-17/
ISBN 978-3-200-03281-1
Editor/Publisher**:** Museen der Stadt Wien – Stadtarchäologie
Editorial Team: Wolfgang Börner, Susanne Uhlirz