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NON-DESTRUCTIVE TESTING OF STONE BIODETERIORATION AND BIOCLEANING EFFECTIVENESS

Sasso S.[1], Miller A.Z.[2, 3], Rogerio-Candelera M.A.[2], Cubero B.[2], Scrano L.[4], Bufo S.A.[1] and Saiz-Jimenez C.[2]

[1, 4] University of Basilicata, Via dell'Ateneo Lucano 10, 85100, Potenza, Italy.
[2] Instituto de Recursos Naturales y Agrobiologia, IRNAS-CSIC, Av. Reina Mercedes 10, 41012 Seville, Spain
[3] CEPGIST/CERENA, Instituto Superior Técnico, Universidade de Lisboa, Av. Rovisco Pais 1049 -001, Lisboa, Portugal

INTRODUCTION

The combination of several non-destructive techniques is compulsory in the field of cultural heritage in order to develop and design new and effective conservation strategies to prevent, control and minimize the causes of biodeterioration.
In this study Digital Image Analysis (DIA), Confocal Laser Scanning Microscopy (CLSM) coupled with chlorophyll α (chlα) quantification, were applied in order to evaluate a new procedure of stone cleaning consisting of the application of potential natural biocides: cells free culture filtrates (CFF) of *Trichoderna harzianum* and *Burkholderia gladioli*, and glycoalcaloids from spontaneous *Solanaceae* plants. These substance were tested on Hontoria limestone samples, a biosparitic limestone used in many Spanish monuments, previously inoculated with a multi-species phototrophic culture.

MATERIALS AND METHODS

1. Stone probes preparation: stone probes were washed with sterile water, dried, placed into glass Petri dishes and autoclaved.
2. Stone probes inoculation with 200 µL of the multi-species phototrophic culture (*Chlorella, Stichococcus*, *Leptolyngbya* and *Pleurocapsa*) and incubated for 20 days under laboratory conditions near a window for natural light/dark cycles.
3. Potential biocides preparation and application on stone probes: *Burkholderia gladioli* pv. *agaricicola* (*Bga*) ICMP11096, *Trichoderma harzianum* T-22 strain and glycoalcaloids obtained from unripe berries of *Solanum nigrum*.
4.Monitoring of biocides efficiency by Digital Image analysis (DIA), photosynthetic biomass quantification and Confocal Laser Scanning Microscopy (CLSM).

DIA was performed from photographs taken using a Canon IXUS 90 IS digital camera. The images obtained were processed by Principal Component Analysis (PCA), using the HyperCube v. 11.0 software (US Army Topographic Engineering Centre, Alexandria, Virginia, USA) The detected particles within the selected area were measured using ImageJ v. 1.47 software (National Institutes of Health, Bethesda, Maryland, USA).
Photosynthetic biomass quantification was performed through chlα extraction by adding the crushed stone probes into 10 ml of DMSO and incubating at 65 °C for 1 h. after filtration to remove stone particles absorbance of the extract was measured by using a JENWAY 6315 spectrophotometer. Chlα and pheophytin concentrations was calculated by the equation of Lorenzen (1967) using the extinction coefficient from Talling & Driver (1963).
CLSM FluoView FV1000 (Olympus) in fluorescence and reflection modes was used to observe the development of the phototrophic biofilm on Hontoria limestone.

RESULTS. All commented pictures show the evolution of the contamination and the efficiency of the treatments through instrumental analysis-

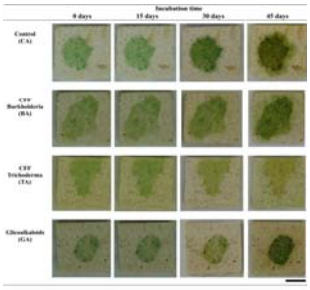

Figure 1

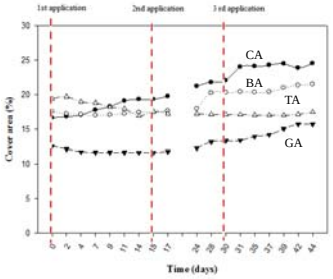

Figure 2

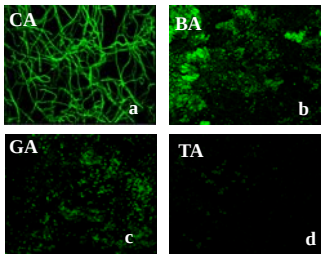

Figure 3

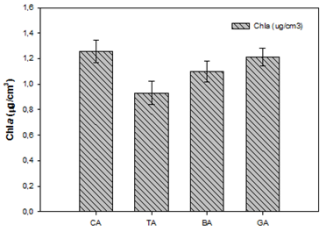

Figure 4

After 20 days of biofilm incubation on non-treated stone probes, a green mat was clearly visible over the upper surfaces.
Figure 1 shows the evolution in time of the green biofilms on representative stone probes of the control (CA) and stone probes treated with: CFF of *Bga* (BA); CFF of *Trichoderma harzianum* (TA); and Glycoalkaloids (GA). Visual inspection revealed that green stains tended to expand on the stone surfaces of CA probes in the course of time. The same trend was observed for replicates treated with BA and GA. In contrast, TA treatment revealed a decrease of green covered area and a colour change from light green to yellow after 45 days of incubation
Figure 2 shows green covered areas measured by Digital Image Analysis during the incubation time on the non-treated (CA) and treated stone probes with: CFF of BA; CFF of TA and GA. During the first 15 days of incubation, the photosynthetic colonization process on CA led to a gradual increase of the green mats on the surfaces whose growth reached a steady stage from 30 days-incubation. On the stone probes treated with BA, the green covered area remained stable during the first 30 days from 30 days-incubation until the end of the experiment. GA-based treatment resulted to be efficient in the first instance, as the photosynthetic biomass decreased in area, but after 30 days the biofilm slightly increased until the end of the experiment (45 days).TA-treated stone probes showed a slight decrease of the stone surface covered area immediately after the first biocidal application. This trend was also observed during the following treatment applications. Biocide efficiency was also assessed by CLSM technique.
Figure 3a shows the presence of abundant filamentous phototrophic microorganisms on the CA stone probes (control). Figures 3b, 3c, 3d display the different colonization of the stone after the treatments with BA, GA and TA . The lowest chlorophyll fluorescence intensity, both from filamentous or unicellular microorganisms, was obtained for TA-treated samples (Fig. 3d).
Photosynthetic biomass estimation (**Figure 4**), performed by means of chlorophyll α extraction, has confirmed results obtained by DIA and CLSM.

CONCLUSION

Digital Image Analysis (DIA), Confocal Laser Scanning Microscopy (CLSM) coupled with chlorophyll α (chlα) quantification have revealed that none of the treatments was efficient against all inoculated phototrophic species, probably due to different biocide resistance.
Among all treatments, the culture filtrates of *T. Harzianum* and the GLAs' extract showed higher biocidal efficiency than the *Bga* culture filtrate.
It seems evident that non-destructive analysis and ecological cleaning methods can represent a innovative strategy for the protection of our stone cultural heritage. Further work on the biocidal effectiveness and durability of secondary metabolites in the medium and long-term is needed for the design of effective and sustainable treatments for minimizing or eradicate stone biodeterioration.