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Hyperspectral approach for the assessment of biocide treatments on archeological walls

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***Abstract* –In this study, a prototypal hyper-spectral imaging device was used in combination with ASD FieldSpec spectrometer for evaluating the efficacy and durability of a biocide treatment applied on selected areas covered mainly by lichens of a wall of *Macellum*, in Pompeii. These techniques were used for monitor the phototrophs health for a period of 7 months. The results were compared to bioluminescence measurements of the ATP, used as reference. The spectra showed significant characteristic signals of photosynthetic pigments and fresh organic matter (lignin, starch, cellulose, pectin).**

I. INTRODUCTION

The outdoor archaeological monuments and remains, being exposed to atmospheric agents, often present biological colonization on their surface, and they may induce serious damages. Indeed, the main biodeteriogens, such as phototrophic microorganisms (green algae and cyanobacteria), lichens (endolithic and epilithic), bryophytes and the plants with their roots, may exert physical-mechanical and chemical degradations.

The hyperspectral remote sensing systems are routinely used for identifying crops and vegetation and to assess their conditions [1, 2]. The vascular plants and bryophytes present a well distinguishable sharp absorption edge around 700 nm, known as the chlorophyll edge [3, 4], that can be observed as well only in the light and yellow lichens [5]. The presence of high amounts of other pigments (e.g. carotenoids, xanthophylls, anthocyanins, etc.) may induce a shift of this absorption edge to smaller wavelengths [3]. Discrimination between different foliose and crustose lichens was made taking into account their spectral properties, color and chlorophyll content [6], performing as well attempts for distinguishing between the rock substrate and organisms [3, 6, 7]. The spectral curves of the stone lichens present a reflectance increment between 700 nm – 1350 nm, resting salient at higher wavelengths. Three broad absorption features around 1730 nm. 2100 nm abd 2300 nm were attributed to the presence of cellulose in lichens [6], while spectral features for the stone substrate was possible to be identified in the range 2000 nm – 2400 nm, where important hydroxyl absorption is present for minerals [6-8].

The hyperspectral imaging approach is here proposed to evaluate the vitality aspect ratio of microorganism colonizing stone surfaces before and after biocide treatment, in order to assess the effectiveness and durability of the applied products avoiding the limitations related to the sampling [9]. The variation in signal intensity for the photosynthetic pigments, fresh organic matter and ergosterol features were monitored in the ASD spectra and the results were compared with the ones obtained by vitality assessment through bioluminescence measurements.

II. MATERIALS AND METHODS

A wall on the East side of the *Macellum* in Pompeii was taken as case study (Fig.1) for testing of hyperspectral imaging technique to assessing the effectiveness of the biocide treatment by Biotin R (produced by CTS, Italy).

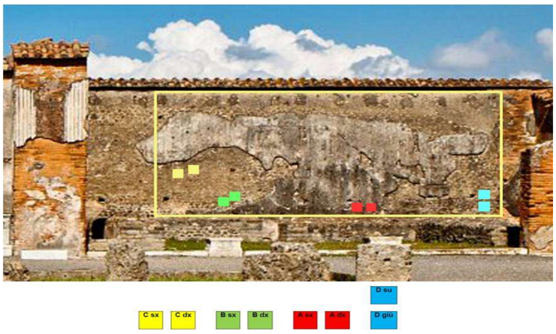


Fig. 1. The selected areas on a wall of Macellum in the archaeological site of Pompeii.

Biotin R (5% v/v) was used for the *in situ* application on colonized stone (Sarno limestone and Tuff) and mortar 4 areas were treated (Asx, Bsx, C sx and Dgiù), and 4 corresponding areas on the right hand side (Adx, Bdx, Cdx) and the uppermost one (Dsu) were left untreated as references (Fig. 1). The chosen surfaces were analyzed before (t0) and after application of the treatment (t1, after 3 months; and t2, after 7 months), by adenosine triphosphate (ATP) measurements (3M^{TM} Clean-Trace Surface ATP) and with a ASD FieldSpec spectrometer. Photographic documentation was taken through a Dino-Lite Digital Microscope Premier and a Lumix camera before and after the treatment. Table 1 shows the schedule for the control measurements.

Table 1. Schedule of the control measurements on selected areas, before (t0) and after (t1, t2) treatment.

Code	Month-Year	Time elapsed after treatment
t0	April 2016	-
t1	July 2016	3 months
t2	November 2016	7 months

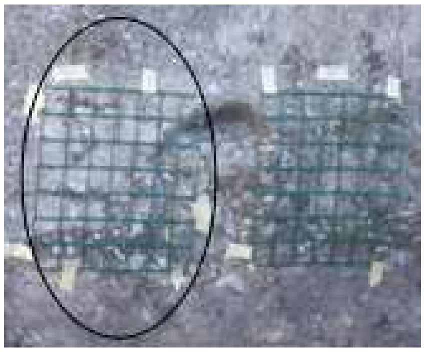
The results obtained by the previous analysis were related with the hyperspectral images acquired by the prototypal hyperspectral imaging device (called SIM-GA), produced by Leonardo Company SpA, Italy), is a modular push-broom avionic hyperspectral imager, originally designed for applications from mobile platforms (aircraft). The prototypal hyperspectral spectrometer covers the spectral range between 400 and 2500 nm, since it consists of two electro-optical heads: VNIR region (400-1000 nm) with 512 spectral bands and 1024 spatial pixels; SWIR region (1000-2500 nm) with 256 spectral bands and 320 spatial pixels.

The average sampling intervals are 1.2 nm (VNIR) and 5.8 nm (SWIR), respectively.

In this case study, the instrument was arranged on a tripod with a PC-driven rotating stage synchronized with the acquisition system. The rotation axis lied horizontally, parallel to the ground surface, and the images were acquired by vertically sweeping the scene.

III. RESULTS

Fig. 1 shows the selected areas on a wall from archaeological site of Pompeii, *Macellum* wall. Each area is composed of 49 sub-areas (Fig. 2), delimited by grill-masks used for a precise monitoring. Different species were found on the selected areas, some of these species were common to all zones (e.g. *Verrucaria nigrescens*) and some of them were present only in one zone (*Verrucaria fuscella* - zone D). The main biological growth on the selected area was divided in: black patinas (L3), lichens with different pigmentations (L1 – *Aspicilia* sp., white gray; L2 – *Caloplaca inconnexa* and L7 – *Caloplaca saxicola*, yellow; L4 – *Verrucaria nigrescens*, brown, L5 – *Verrucaria* lecideoides, black; L6 – *Caloplaca calybeaea*, grey; L8 – *Verrucaria fuscella*, sylver grey) and mosses (M1 – *Tortula muralis*, M2 – *Rhynchostegiella tenella* and M3 – *Grimmia pulvinata*), and an example of their distribution in an investigated area is presented in Fig. 2..



Zona A - sx

	1	2	3	4	5	6	7
A	L1 L4	L3 L4	L1 L3 L4	L3 L4	L1 L3	L1 L3	L1 L3
B	L1 L3	L1 L3 L4	L1 L3	L3 L5	L3	L5	L1
C	L3	L4	L3	L3	L1 L3	L1 L4 L5 M1	L1 L4 M2
D	L3	L3 L4 L5	L3 L4	L3	L1 L4	L1 M1	L1 M2
E	L3 L4	L3 L4	L1 L3 L4 L5	L1 L4 L5	L1 L5 M2	L1 L5 M2	L1 L4 M1
F	L1 L3	L1 L4 L5	L1 L4 L5	L1 L4 L5	L1 L4 L5	L1 L4 L5	L1 L3
G	L1 L4	L1 L4 L5	L1 L4 L5	L1 L4 L5	L1 L4 L5	L1 L4 L5	L1 L3

Fig. 3. The treated (Asx) and not treated (Adx) areas in the chosen zone called A (up) and the biodiversity distribution in the treated area Asx.(down)

The microscopic observation (Fig. 4) revealed yellowing or whitening of some lichens and mosses, as a result of the damage occurred at photosynthetic apparatus.

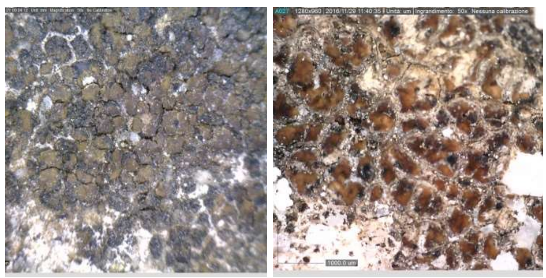


Fig. 4. Verrucaria nigrescens lichen, before (left) and after (right) the biocide treatment.

After 7 months from the biocide application, the ATP measurements revealed that biological activity almost inexistent on treated areas Asx, Bsx and Dgiù, with values <100 RLU (relative light units)/cm^2, while the highest values were registered for the Csx area: 1547.21 RLU/cm^2

on mortar and 617.62 RLU/cm^2 on stone surfaces (Fig. 5). The control areas showed higher ATP values in the autumnal season, as the summer extreme conditions induced the quiescence period for the analyzed species.

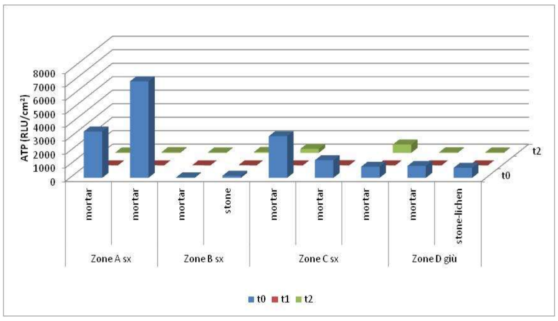


Fig.5. ATP measurements before and after biocide treatment on the treated areas.

The spectroscopic analysis performed by ASD FieldSpec spectrometer has allowed to identify several features for the biological colonization that help in the assessing of their vitality after the biocide treatment. The characteristic peaks before the treatment for the photosynthetic pigments (~ 500 nm, ~ 680 nm), were very evident in all the investigated areas where phototrophic organisms were present. In the case of all lichens, these are characteristic peaks for the photobiont (algal photobiont), and they disappeared in the 3 and 7 month spectra. If the photobiont is represented by cyanobacteria, the characteristic peaks at ~ 620 nm for the phycobilines should be considered. This absorption feature was also observed on the black patina areas (L3), which is mainly composed by cyanobacteria.

In Fig. 6 is showed the map of Chlorophyll a (680 nm) on the wall under test obtained by the hyperspectral system at t0.

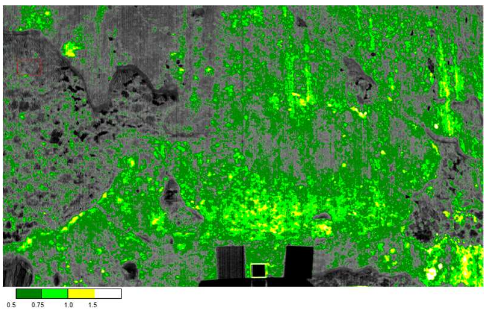


Fig.6. Map for 2nd derivate of the Chlorophyll a (682 nm) in terms of coloring at positive value thresholds

A characteristic absorption signal at ~1620-1680 nm was observed especially in the black lichens which was attributed to the presence of chitin and ergosterol present in the mycobiont [10]. This signal disappeared after 7 months as well.

In the Fig. 7 it is shown an example of the spectra taken from Dsx area, in a sub-area entirely covered by one lichen species - L8. The characteristic peaks for photosynthetic pigments (chlorophyll a and carotenoids) are more intense for bryophytes with respect to the lichens, and they were not observed after 3 and 7 months from the biocide treatment. Changes at the 1730 nm, 2100 nm and 2300 nm, characteristic for the presence of fresh organic matter, was observed, these peaks disappearing after 7 months.

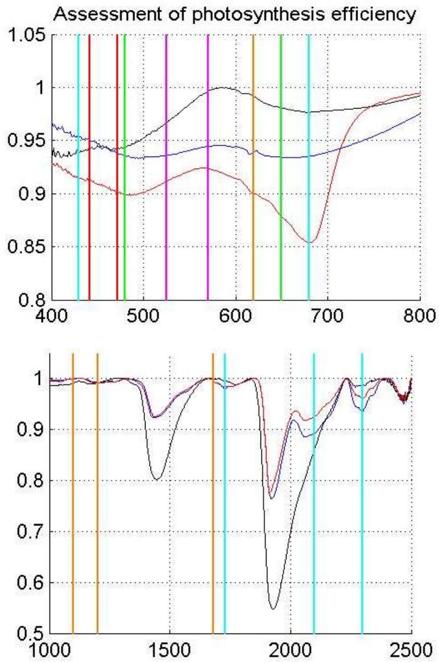


Fig.7. Spectra of the grey-silver lichen - L8 (Verrucaria fuscella) before treatment (red line), after 3 months (blue line) and after 7 months (black line).

IV. CONCLUSIONS

The spectra of the phototrophic organisms were acquired without significant interference of the stone substrata present on the selected areas (mortar, Sarno limestone, Tuff). The spectra of the investigated areas were changed after the treatment revealing serious damages at the photosynthetic apparatus, as the characteristic peacks of chlorophyll a (~ 680 nm), phycobilines (~ 620 nm) and carotenoids (~ 500 nm) were significantly reduced or disappeared after 7 months from the treatment. The vitality of the phototrophic microorganisms was drastically reduced after the biocide use, as it was detected by bioluminescence measurements of the ATP molecule, being in agreement with the hyperspectral measurements. Moreover, the signal reduction at ~1620-1680 nm for the principal metabolites of the fungal component after 3 and

7 months led to conclude that the used biocide is effective not only against the photobiont, but against the mycobiont as well, which is considered more resistant to chemical and environmental stress.

Damages on the photosynthetic apparatus were also observed by hyperspectral imaging measurements that could be recommended for non-invasive diagnostics campaigns of archaeological monuments.

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