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The Roman mosaic in the Nymphaeum of Villa Giulia in Rome.Characterization of the deteriogen agents and preliminary experimentation of eco-sustainable products

Miriam Lamonaca[1]

[1]*Museo Nazionale Etrusco (ETRU) di Villa Giulia, Piazzale di Villa Giulia, 9 Rome.*

Abstract **- One of the current challenges facing restorers and conservators of cultural heritage is to ensure the right balance between treatment efficacy, long-term conservation and reduced environmental impact. Certain cases, such as that of the Nymphaeum of Villa Giulia in Rome, the three elements cross each other, sometimes making the balance very difficult. The Nymphaeum of Villa Giulia in Rome, designed by the architect Bartolomeo Ammannati in the mid-16th century, is characterized by a complex architectural apparatus and is enriched by refined decorations in stucco, stone materials and statuary. This beauty is placed in an open environment, continuously exposed to soiling and related degradation phenomena, such as darkening caused by accumulation of particulate matter. Therefore, as with all external monuments, it is strongly compromised by degradation phenomena closely connected to the context in which it is located and aggravated by the presence of water from the fountain. In particular, the presence of microflora (algal colonies and to a lesser extent moss), small plants, humidity and atmospheric particles are the caused of these phenomena. Since 2019, experimentation was started on two eco-sustainable products: an enzyme-based biocide and an essential oil-based preservative. Scientific analyses were conducted to evaluate the short and medium-term effectiveness of these products, and to develop a scheduled maintenance plan for long-term conservation. The experimental areas selected for this study were the Roman mosaic floor, in the center of the low fountain of the Nymphaeum.**

Keywords

Restoration – Conservation – Eco-Friendly – Cultural Heritage – Villa Giulia

I. INTRODUCTION

The Nymphaeum of Villa Giulia in Rome develops on several levels; in the center of the lower level, called "Fontana Bassa", there is part of a Roman mosaic floor, dating back to the 2nd century AD and coming from Statua, along the Via Aurelia. In 1923, during road works to improve vehicular traffic, a Roman domus from the imperial age was discovered, characterized by black and white floor mosaics. Unfortunately, since part of the land belonged to the property of the Pio Ospedale di Santo Spirito and the Ospedali Riuniti of Rome, it was not possible to excavate the entire area to bring the whole mosaic to light. Only in 1941 it was possible to detach the State-owned part of the mosaic and transport it to the Villa Giulia Museum.

The mosaic represents fantasy marine animals made with black tesserae on a white background. The part depicting Triton blowing a horn and holding a rudder, has been placed in the center of the low fountain of the nymphaeum, while the sea deer and the fish have been placed on the upper floor under the two loggias that frame the nymphaeum. The detachment of a mosaic is already a traumatic event in itself; this condition is aggravated by changing the natural adaptation of the work to the original context and of having placed it in an environment with particular climatic and exposure conditions which alter its balance and compromise its conservation. The mosaic is positioned in an open environment, near the fountain and therefore in the continuous presence of water and humidity. This element has clearly further favored the development of consistent biological patinas and dark surface deposits which also compromise the reading of the work. Furthermore, the pavement is not regular, and depressions are formed which cause stagnation of rainwater. In order to be able to study the state of conservation of the mosaic, a diagnostic campaign was performed to identify the biological species present on the mosaic. The identification of biological patina allowed to employ a new eco-friendly cleaning approach [1]. Over the past few decades, biological cleaning became an important component in the conservation of cultural heritage field [2]–[4]. Namely, the use of enzymes [5]–[7] and essential oils [8] has emerged as a novel strategy for achieving controlled and selective cleaning procedures. The aim of the work was to identify the time efficacy to remove the biological patina on the roman mosaic, using an enzyme matrix mixed with an essential oil blend with the aim of establishing a programmed maintenance protocol.

II MATERIALS AND METHODS

A Biological analyses

For a better evaluation of the effectiveness of bio-cleaning it was necessary to characterize the microorganisms present on the mosaic. Figure 1 shows the sampling areas, and Table 1 lists the biological patina present of the mosaic. The biological analyses were performed in collaboration with Tecno.el srl and the "Charles Darwin" Department of Biology and Biotechnology of the "Sapienza" University of Rome. The steps were:

Step 1: Three types of patinas, visually different, were selected.

Step 2: Small quantities of biological material were collected in correspondence of the three selected areas using a sterile swab and scalpel.

Step 3: Samples and swabs were processed. In order to facilitate the growth of the various species of microorganisms present, more quickly than in the environment, the samples were subjected to favorable thermo-hygrometric and nutritional conditions (culture examination).

Step 4: Among the isolates, the most abundant species were identified to the species level, by molecular analysis.

Figure 1. Sampling location.

The biological material, taken with scalpels and swabs, was immersed in Luria-Bertani (LB) medium rich soil, to encourage the growth of molds and bacteria. An aliquot of LB medium, after two minutes of stirring to allow the detachment of the cells, was then spread into Petri dishes containing the same type of medium. The plates were then incubated at 28°C for 10 days and colonies with different morphologies were isolated. In the case of withdrawals made with the swab, an additional Petri dish with LB medium was used, in which the cotton of the swab soaked in the residual medium was swiped. This action becomes necessary to allow the recovery of those cultivable colonies which do not go into solution during the stirring, but which remain adhered to the cotton lattice.

The investigation was further deepened with 16S DNA sequencing conducted on three selected colonies. This investigation consists in amplifying, by means of the PCR (Polymerase Chain Reaction) technique, the 16S region, a portion of the DNA which has strain-specific variable nucleotide sequences. The amplified region is then sequenced, and the portion of DNA obtained is compared with those present in the BLAST database. On the basis of the similarity percentage with the known sequences (>90%), it can be established which species the analysed isolate belongs to.

Table 1. Summary of different biological patina.

Samples	Description
N-01	Black patina on the travertine
N-02	Red patina on white marble tiles
N-03	Compact black patina on white marble tiles
N-04	Mosses on the interstice mortar

B *Cleaning treatment*

The first phase of experimentation concerned the use of eco-sustainable detergents to remove biological patina, based on stabilized enzymes (proteases), non-toxic for the operator and the environment. The new biocide represents the development of traditional enzymatic cleaning, made possible only thanks to the use of micro-nanotechnological matrices patented by Brenta S.r.l. In addition to a selective removal of the patinas, the enzymes in the matrix allow to overcome the limits of use of traditional enzymes, which are effective only at certain temperature and pH ranges.

The gel-supported enzymes and traditional free enzyme cleaning patches were compared to evaluate their efficacy. Table 2 reports the cleaning treatment for each investigated area. Four different areas of the mosaic have been selected (Figure 2). The first area involves small travertine slabs at the top left (red dots), the second area involves the white tiles near the lower left corner (yellow dots), the third area involves both the white tiles and the frame in green serpentine near the upper right corner (green dots) and the last area involves a travertine slab near the lower right corner (light blue dot). All the areas were not treated with protective film but left in equilibrium with the open environment. The samples area measured 10x10 cm.

Figure 2. Area treated.

Table 2. Summary of testing areas.

Area 1 (travertine)	Only traditional enzymes; Only gel-based enzymes; Traditional enzymes + essential oils; Gel-based enzymes + essential oils.
Area 2 (white marble tils)	Traditional enzymes + essential oils; Gel-based enzymes + essential oils.
Area 3 (white marble tils and serpentine)	Traditional enzymes + essential oils; Gel-based enzymes + essential oils.
Area 4 (travertine)	Untreated

To verify the effectiveness of the cleaning and preservation systems, observations were made in optical microscopy with natural and ultraviolet light and bioluminometer measurements.
Monitoring with bioluminometer (AccuPoint® Advanced Reader APM635, UK) and digital microscope (Dino-lite, AM4113T-FV2W, NL) was performed at six different times:

- T0: time zero, before treatment
- T3: after 3 months of treatment with essential oil
- T6: after 6 months of treatment with essential oil
- T9: after 9 months of treatment with essential oil
- T12: after 12 months of treatment with essential oil

Gel-based enzymes

The mixture, called NASER L01, is composed of an aqueous gel based on a cellulose derivative (Table 3).

Table 3. Composition of NASER L01 product.

Substance	Concentration
Zeolites	1 – 5%
Tripsina[1]	≤ 0,1 %

[1]Index Number: 647-010-00-7

The product was applied with a brush and left to act for 90 min at an external temperature of 15°C. The product applied not required continuous monitoring of temperature ad pH values, nor pay attention to the metal parts of the brush. Once the processing times had elapsed, the product was removed with nebulized water and a brush.

Traditional free enzymes

Preparation of free enzymes:

200 mg of protease enzyme was dissolved in 100 mL of demineralized water at pH 6.5. The solution obtained was heated in a bain-marie up to 30 °C and then stored inside a thermal bag to transport it from the laboratory to the construction site area.

Application:

The ambient temperature was around 10°C, while that of the marble surface was a few degrees lower, with a pH of 7. The surface was treated by heating with a low-pressure hot air diffuser until 30°C. At this point the enzymatic solution was applied with a brush, taking care to avoid any interaction between enzyme and part of the brush itself. The treated surface was covered with a film to prevent evaporation and continuous exposure to a jet of hot air, trying to stabilize the temperature around 30°C. The enzyme was left to rest for 10 minutes and then rinsed with demineralized water and a brush.

During the application of the enzymatic solution, a greater absorption by the substrate and a propagation by capillarity were found which does not allow a very limited cleaning.

Essential Oils

The second phase of the experiment concerned the use of essential oils as a preservative product, called REISAN, necessary to prolong the duration of the cleaning (Table 4). The product was composed by peppermint essential oil and had a milky liquid appearance, and it was tested both on the area treated with gel-based enzymes and on the area treated with traditional free enzymes (Figures 3-4).
The product was applied by spraying on the already clean and dry surface up to saturation.

Table 4. Composition / Information on ingredients

Substance	x = Conc. %
Peppermint essential oil (natural oil)	5 ≤ x < 6

Figure 3. Area treated with only gel-based enzymes and area treated with both gel-based enzymes and essential oils.

Figure 4. Area treated with only traditional enzymes in the matrix and area treated with both traditional enzymes and essential oils.

II. RESULTS AND DISCUSSIONS

The preliminary biological analyses allowed to characterize the microorganisms present on the mosaic.
Patina N-01 is brown, very adherent and uniformly spread over the travertine (Figure 5).
The analysis of the patina at high magnifications (50x and 225x) has shown an extreme irregularity of the spots, it appears mixed with the crystals constituting the substrate due to its penetration into the alveoli of the travertine, visibly degraded. Overall, the culture analysis showed a prevalence of bacterial colonies. In detail, 21 mold and 35 bacterial colonies have grown from the swab sample, 97 c. fungal and 185 c. bacteria from scalpel sampling.

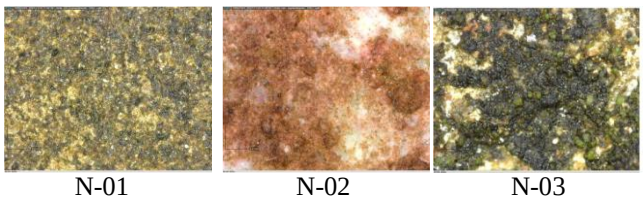
The abundance of bacterial colonies, despite their extremely small size, represents a considerable risk for the integrity of the Mosaic as bacteria are mainly responsible for the formation of biofilms. It is a set of bacterial cells adhered to the surface and incorporated in an adhesive polysaccharide matrix, secreted by the cells themselves.

The wide diffusion of the alterations on the travertine slabs (already evident macroscopically) not only produces

chromatic damage (dark gray spots) but, in combination with the presence of fungi, can trigger much more serious damages of a chemical nature, with the production of various metabolites, and mechanical, through the pressure exerted by the anchoring structures. From the analysis of the bacterial colonies, it has been seen that the most representative one can be traced back to a species. Therefore, it was decided to proceed with the molecular sequencing of the isolate of the same. Based on the percentage of identity, it was verified that the strain belongs to the bacterial genus *Bacillus subtilis*.

Patina N-02 has a typical reddish color, adhered to the white marble pieces and located only in a few areas of the Mosaic. Observation of the patina at high magnifications (50x and 225x) showed that the stone surface has a very bright color, characterized by a light red background and green/dark brown spots with three-dimensional development and non-uniform diffusion. The dark concretions have a sub-circular shape and accentuate the roughness of the substrate. The superficial inhomogeneity, due to the very nature of the mosaic (bedding mortar between tesserae of different shapes and sizes), seems to have accelerated the degradation process, above all in terms of irreversibility (Figure *5*). The cultural investigation highlighted a prevalence of bacterial colonies compared to fungi only in the swab collection. In detail, the N-02_T sample led to the growth of 67 bacterial colonies out of 129 c. total, while sample N-02_B has slightly higher numbers, with 74 fungal and 70 bacterial colonies.

The patina 03 is characterized by a black color with a rough appearance and is difficult to remove. Observation of the patina at high magnifications (50x and 225x) highlighted circular spots, black and with sharp edges, aggregated together to form patches visible to the naked eye and mixed with small bright green protrusions, probable growth principles bryophytic (Figure *5*). The biological culture analysis showed a very high number of bacterial colonies compared to the other samples, both for the swab collection (22 fungal colonies and 879 bacterial), and for that with scalpel (53 mycotic and 349 bacterial c.). In this area, the already strongly altered and inhomogeneous substrate is characterized by presence of mainly bacterial biofilm, favored by the particular microclimatic niche. As seen for patina 01, the presence of this matrix gives the microorganisms strength adhesion to the substrate, accumulation of substances and protection from external factors, as well as a shape resistance to traditional cleaning products.



N-01 N-02 N-03

Figure 5. Digital microscope observations of patina N-01, patina N-02 and patina N-03 at 225x.

The diagnostic morphological characters of sample 04 are those typical of the division of Bryophyta, class of Mosses (Figure 6). They are non-vascular plants, whose survival is strongly linked to the presence of water. A preliminary study there allows us to hypothesize that they belong to the *Tortula* genera (most likely *Tortula muralis*), *Bryum* and *Fissidens*. They have not yet been sequenced. The proliferation of mosses is linked to the presence of water.

Figure 6. Digital microscope observations of sample N-04 at 50x and 250x.

With the techniques in our possession and the common methods of extraction of DNA, however, it was not possible to identify the organism found in samples N-01 and N-02 and characterized by distinctive red colonies. Under a microscopy analysis it would not appear to have any characteristics typical of fungi or bacteria. It could belong to a red algal species, but the certainty could only be obtained following sequencing with appropriate molecular methods. As regards the fungi, they have been recognized in all the samples, with optical microscope observations, the typical structures of the *Aspergillus* genera (black colonies) (Figure 7) e *Penicillium* (white and green colonies).

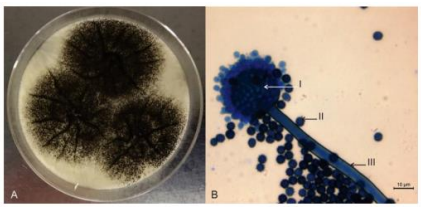


Figure 7. Black colonies belonging to Aspergillus spp. and (B) magnification of the structures.

Biological colonization is abundant in the N-03 patina, which corresponds to a highly disconnected area. In addition to the spaces between the mosaic tiles, a probable landslide has created gaps where particulate matter, rich in nutrients, and stagnant water accumulate. The samples that record the least total growth are those relating to patina N-02, most likely not because the biodegradation is less intense, but simply because, currently, not all species of microorganisms are cultivable in the laboratory. Recording overall, a prevalence of bacteria compared to fungi net above all in zones 03 and 01 (to a lesser extent). The patina N-02, on the other hand, presents an equal situation between bacteria and fungi.

The enzymes used had intrinsic specificity for the biological substrate, and further ability to catalyze a single type of reaction on the selected substrate (protease), therefore they did not affect the mosaic surface.

The bioluminometer analysis was employed to evaluate and monitor the efficacy of each cleaning treatment [9]. The bioluminometer gives us an index of the vitality of the microorganisms present and it shows on the display a value expressed in RLU (Relative Light Units) which is directly proportional to the concentration of ATP (Adenosine triphosphate) present in the sample.

The following graph (Figure 8) show the vitality of biodeteriogens in the untreatment area. It has been noted that the growth is influenced by seasons and the different cycles of vitality of the microorganisms throughout the year.

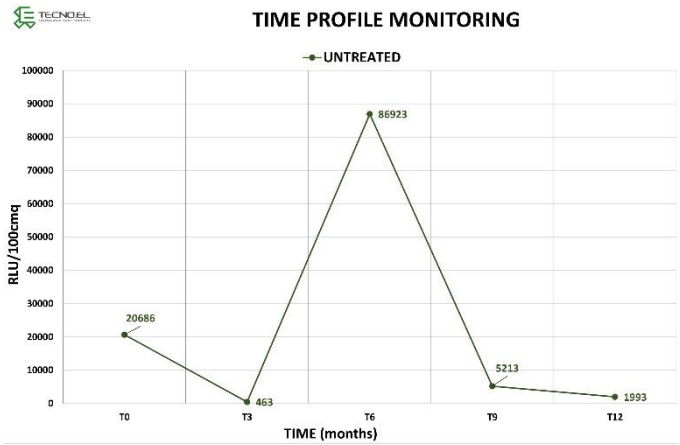


Figure 8. Cycles of vitality of the microorganisms. Untreatment area.

The areas treated only with gel-based enzymes and traditional free enzymes show a rather similar microbiological repopulation curve with a clear decrease up to the three-month reading, a positive peak at 6 months (summer), and subsequently a reduction up to 12 months (Figure 9) until 1164 and 0 RLU for traditional and gel-based enzymes respectively.

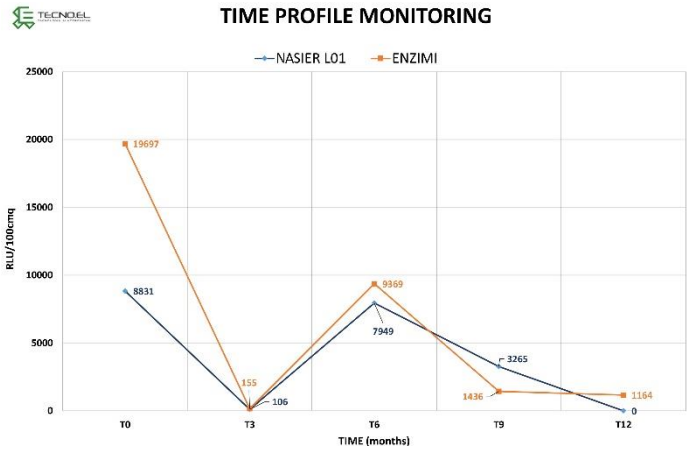


Figure 9. Microbiological repopulation curve. Areas treated with gel-based enzymes and traditional free enzymes (up to twelve months).

In the areas treated with essential oils, the repopulation curves showed different trends both due to the cleaning method and the location of the samples.

In Area 1 the repopulation curves overlapped (Figure 10). In Area 2 and Area 3 the repopulation curves showed the same trend for up to 3 months after the treatment, but afterwards there was a substantial difference between the two cleaning methods. In the case of the areas treated with gel-based enzymes, the microbiological repopulation showed rather low RLU values, only in Area 3 increased after 9 months. On the other hand, in the areas treated with traditional enzymes after 3 months the RLU values increased by more than double compared to gel-based enzymes, to then record a reduction after 6 months and a subsequent increase between 9 and 12 months (Figures 11-12).

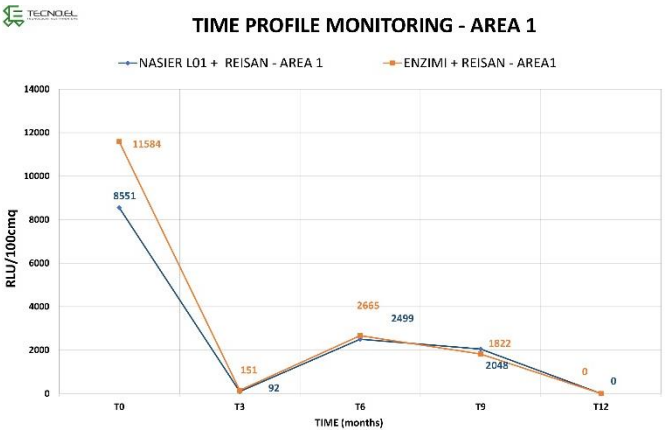


Figure 10. Microbiological repopulation curve. Area 1 (travertine) treated also with essential oil (up to twelve months).

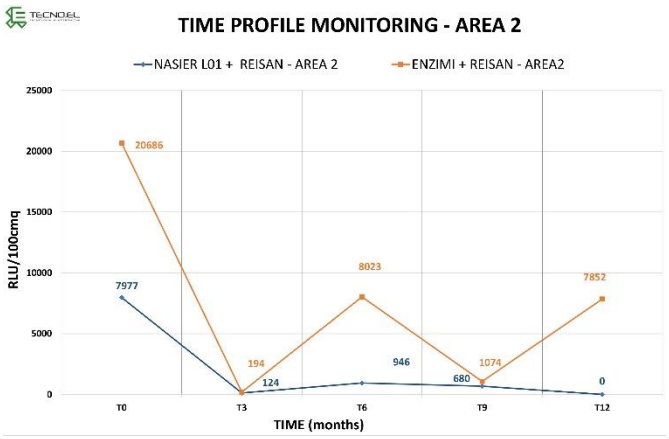


Figure 11. Microbiological repopulation curve. Area 2 (white marble tils) treated also with essential oil (up to twelve months).

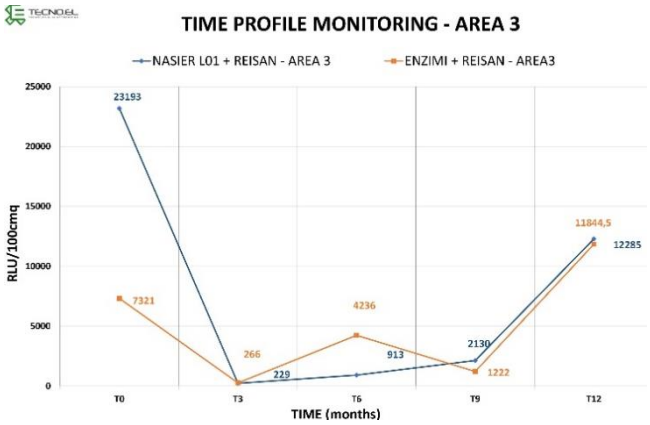


Figure 12. Microbiological repopulation curve. Area 3 (white marble tils and serpentine) treated also with essential oil (up to twelve months).

This result suggest that traditional free enzymes used in Area 2 and Area 3 are more susceptible to seasonality since a greater oscillation in the repopulation curve was recorded. The different trends of the curves may also depend on a different exposure of the samples and on the different constituent materials (Figure 2).

III. CONCLUSION

The application of traditional enzyme treatment results in difficulties due to the requirement of surface monitoring for temperature and pH throughout the entire reaction time. This difficulty of use increases for larger areas. The gel-based enzyme product exhibits the best antimicrobic effect after one year. However, its resulted susceptible to the seasonality. In area 1 the two cleaning methods were both effective, recording very similar results and growth trends. In area 2 and area 3 the essential oil-based preservative product has achieved the best results in combination with gel-based enzyme cleaning. In fact, during the summer season of the year, it has effectively reduced the proliferation of microorganisms and kept the microbial repopulation curve as flat as possible. The different effectiveness of essential oils, combined with cleaning with gel enzymes or traditional enzymes also depends on the location of the samples and the different exposure to degradation phenomena. These results suggest reapplying the product after 12 months, allowing the planning of a scheduled restoration and avoiding emergency interventions. The restoration of the mosaic made it possible to remove the biological patinas and block the biodegradation phenomena. Unfortunately, the particular microclimatic conditions of the site, immersed in a suggestive environment rich in aquatic vegetation, have destined the work to an easy and rapid biological recolonization.

The experimentation will be supplemented by colorimetric measurements, which will be detailed in a separate publication. Conducting colorimetric investigation to detect potential chromatic variation resulting from the treatment requires an extended monitoring period.

The decision to conduct a pilot restoration using low environmental impact products reflects a shared strategy. This approach stands in contrast to the practice of interventions and, in particular, with the past cleaning works of the Mosaic itself. The small scale of the work and the environment in which it is situated provides an opportunity for employing products from the green line into programmed maintenance protocols. This product serves as an excellent alternative to traditional chemical products, thanks to their non-toxicity nature for humans and the environment, absence of residues, high selectivity, and ease of application.

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