BIOLOGICAL MACRO AND MICRO SYSTEMS CO-EXISTING IN THE "FOUNTAIN OF THE TWO DRAGONS", PALERMO

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Keywords: fountain; stone biodeterioration; micro and macro-organisms

1. Introduction

Outdoor fountains located in parks or urban environments are often constructed using different lithotypes and are generally equipped with a water supply and decorative elements made of metals or metal alloys [1-2]. Different biological systems, mainly related to the presence of water, may colonize these artifacts. The photosynthetic microflora, algae, cyanobacteria, lichens, mosses, vascular plants on stones, represent a complex ecosystem that develops depending on environmental conditions and the physical and chemical properties of the material. These initial populations can also promote the growth of other communities and can be involved in the biodeterioration of artifacts frequently enhanced by the indirect action of insects and other animals [3-5]. Biological decay and the intensity of biodeterioration processes are strongly influenced by water availability, and is determined by material-specific parameters, such as porosity and permeability, environmental conditions of the site and exposure of the object [6].

Within the framework of co-operation between the University of Palermo and the *Salvare Palermo* Foundation, the Fountain of the two Dragons in Palermo was identified as one of the monuments needing conservative restoration measures. In this scenario, a monitoring campaign was undertaken in order to obtain preliminary results on the conservation state and the biological systems colonizing the monument.

The Fountain of the two Dragons was executed in 1630 by the sculptor *Nunzio La Mattina*, according to the design of the architect *Mariano Smiriglio* [7]. The marble fountain consists of one big basin (elliptical in shape) and two statues featuring dragons, positioned on opposite sides; in the middle of the basin there is a large decorative pinecone sculpture (Figure 1A).

The two mythical sculptures are equipped with an appropriate water supply system (Figure 1B) and surrounded by a small semicircular space.

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Figure 1. (A) View of the Fountain of the two Dragons; (B) detail of the water flow device in the dragon statue on the left.

2. Evaluation of the state of conservation

The fountain is located in the old urban area of Palermo and a protective barrier was erected to protect the space. Moreover, behind the fountain there are some trees whose roots have caused the paving materials to break and come loose (Figure 2A); the presence of planted trees increases leaves, fibers, pollen, flying insects, bird feces on the monument (Figure 2D).

Figure 2. Deterioration signs. (A) Trees behind the fountain; (B) front of dragon with evidence of water leaching, rusty patina; (C) other patinas and dirt on dragon's wing; (D) vegetal remains on dragon statues or plants inside fissures and loss of materials due to the presence of metal brackets; (E) colored patinas on lower part of dragon; (F) flaking of material composing the rim of fountain.

The conservation state of both basin and dragons is characterized by deterioration processes. The basin is affected by discoloration phenomena, a widespread presence of leaves or other plant remains, encrustations, flaking (Figure 2F), different pigmented patinas and soiling. In addition, on the two statues there are several biodeteriogens which include plants and mosses, insects, patinas, and alterations, such as discoloration, rusty patinas, black crusts and dirty deposits, fissures, detachment of material. Moreover, mechanical stress due to the presence of brackets made of metal and pipes inside the structures is also evident (Figure 2C, E). Traces of past water leaching and the consequent formation of biofilm and incrustations due to metal corrosion are distinguishable on the front of the dragons' chests (Figure 2B).

In order to define an adequate conservative restoration, the identification of both macro and micro-biological deteriogens was performed through an integrated approach based on microscopy, *in vitro* culture, molecular biology investigation (PCR, sequence analysis).

3. Materials and methods

3.1 Sampling

In February 2014, biological sampling was performed on the fountain's decorative elements and basin surfaces. The biological samples were collected from areas affected by chromatic alterations, deposits, exfoliations, incrustation, biological or pigmented patinas. Sampling was performed by sterile swabs (Figure 3A, B, C), by scalpel (Figure 3D, E, F) or by direct sampling of specimens by means of tweezers.

The sampling in proximity of the coloured patinas was justified by the fact that microorganisms contribute both to chemical-physical alteration of the constituent materials and to the formation of pigmented patina on the surfaces. These micro-organisms are able to produce coloring pigments, such as chlorophyll, carotenoid, melanin, that may generate chromatic variations towards a yellow, orange, red, or even brown colour [5]. Table 1 contains a description of the collected samples.

Table 1. Samples collected with corresponding sampling area reported in Figure 3.

Sample	Sampling area		Figure 3
swab 11	base of pinecone	biological patina	A (star)
swab 13	dragon on right side	pigmented area	B (star)
swab 14	dragon on left side	pigmented area	C (star)
sample 15	basin edge	vegetal growth	D (circle)
sample 7	dragon on left side (back)	vegetal growth	E (circle)
sample 4	dragon on right side (wing)	larvae	F (green circle)
sample 5	dragon on right side (wing)	insects gastropods	F(grey circle) F(blue circle)

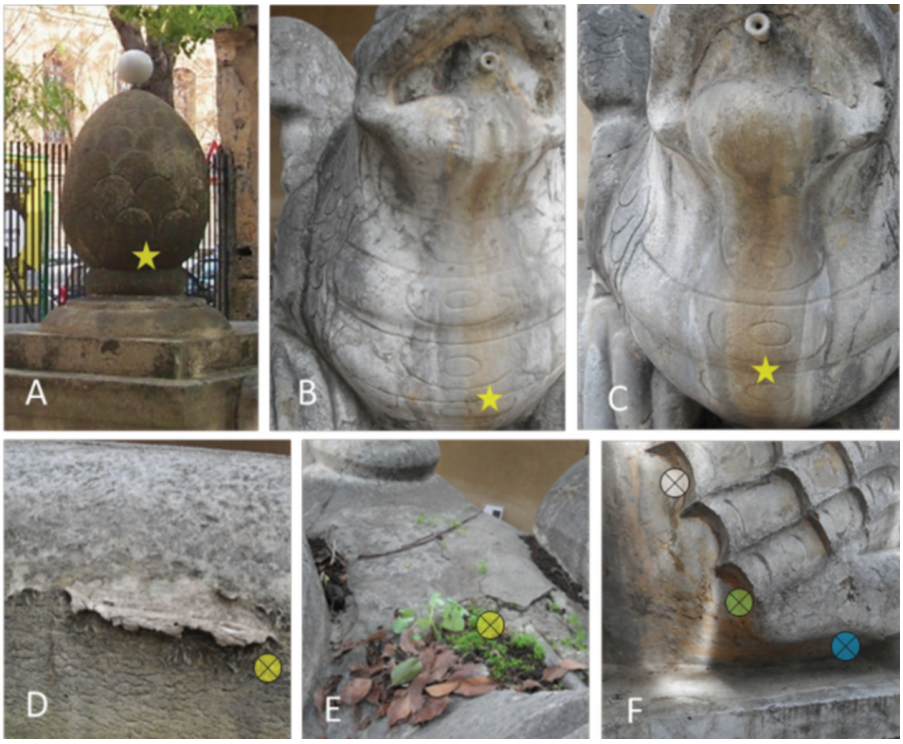

Figure 3. Sampling areas. (A-B-C) sampling on biological and pigmented patinas using sterile swabs (stars); (D-E-F) sampling of macro biological systems by scalpel (colored circles).

3.2 Identification of microorganisms

Samples collected by swabs were diluted in liquid culture media. Solid nutritive media (Nutrient Agar and Sabouraud, *Difco*) in Petri-plates were inoculated with 50 µl of the resulting suspensions. The plates were incubated at 30°C for 16 up to 36 hours. Isolated microorganisms were identified by morphological analysis and molecular biology investigation. Microbial colony morphology was observed by stereomicroscope (Wild Heerbrugg). The micro-morphology of the reproductive structures was identified by identification keys [8], after staining with Lugol's solution and optical microscope (Leica) observation.

Molecular biology investigation was performed through *ad hoc* protocols, including genomic DNA extraction by Genomic DNA Purification Kit (Fermentas) from isolated colonies or directly from biological patinas (biofilm). Genomic DNA extracted from microbial colonies was utilized as a template for *in vitro* amplification (Polymerase Chain Reaction) of target sequences [9-11]. Each PCR reaction mixture consisted of microbial genomic DNA; 1X Reaction Buffer; 10 µM Forward primer; 10 µM Reverse primer; 2.5 mM dNTP mix; 5 mM MgCl2; 2Us of Taq DNA polymerase (*Invitrogen*). PCR products were analyzed by electrophoresis on 2% agarose gel in TAE (Tris-HCl/acetate/EDTA) buffer, the sequencing was performed by Eurofins MWG-Operon, sequenc-

ing service (Germany) and sequence analysis by BLAST platform (NCBI-NIH–USA, EMBL-Germany) [12, 13].

3.3 Identification of macro-organisms

Macro-systems collected in the different areas of the Fountain of the two Dragons were observed by digital microscope (DinoLite) and stereomicroscope (Wild Heerbrugg); bryophytes were characterized by optical microscope (Leica, 40X), after appropriate preparation of the samples according to the taxa.

4. Results

4.1 Identification of microorganisms

Analysis of samples *11*, *14* and *15* revealed the presence of several bacterial and fungal colonies. The morphological analysis of the bacteria grown on nutritive media plates was carried out by direct microscopic observation, distinguishing pigmented bacterial colonies (e.g., yellow, red, pinkish) (Figure 4). In particular, pigmented bacteria belonging to the *Arthrobacter* genera were identified by molecular investigations (sample *11*). Reddish and pinkish bacteria were identified as *Arthrobacter* spp. and *Paracoccus* spp. (sample *13*). In the same sample, fungal colonies belonging to *Alternaria* spp. and *Phoma* spp. were revealed. *Arthrobacter* spp. and *Paracoccus* spp. were also revealed in sample *14*, as well as fungi *Alternaria* spp., *Fusarium* spp. and *Cladosporium* spp. (Figure 5).

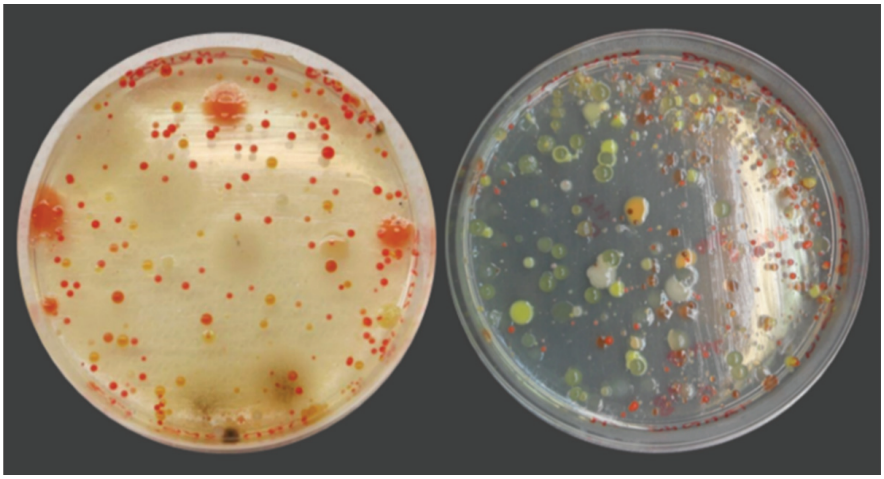

Figure 4. Bacterial colonies grown on agar plates.

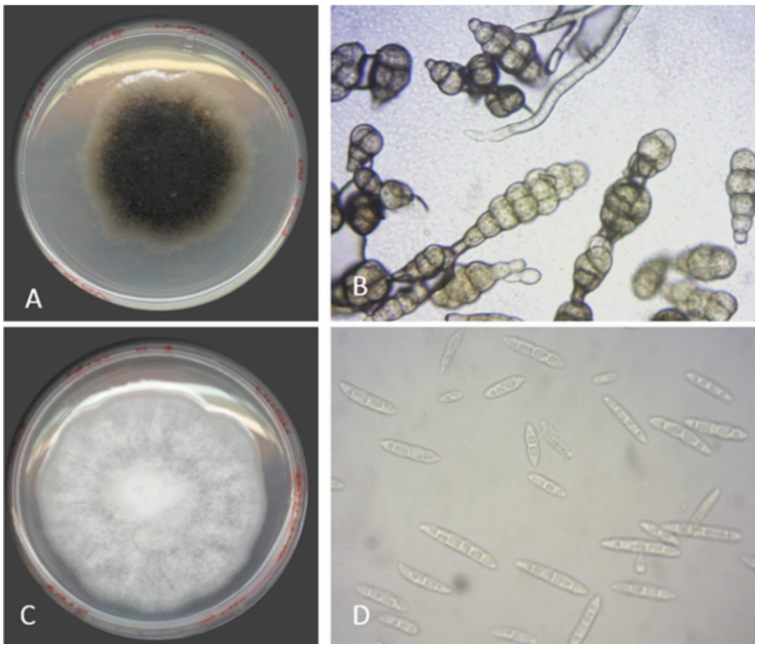

Figure 5. Morphological analysis of fungi colonies. Alternaria (A) and Fusarium (C) isolated on Sabouraud media and observed by optical microscope (40X), after staining with Lugol's solution (B-D).

Table 2. Microbial colonizers.

Samples *(swab)*	**Sampling areas**		**Microorganisms**
11	base of pinecone	biological patina	*Arthrobacter* spp.
13	dragon on right side	pigmented area	*Arthrobacter* spp. *Paracoccus* spp. *Alternaria* spp. *Phoma* spp.
14	dragon on left side	pigmented area	*Arthrobacter* spp. *Paracoccus* spp. *Alternaria* spp. *Fusarium* spp. *Cladosporium* spp.

Arthrobacter, *Alternaria*, *Fusarium* and *Cladosporium* represent the main microbial genera isolated from the fountain samples. These microorganisms are frequently found on stone materials and outdoor monuments and may be considered potential biodeteriogens, due to their metabolic products (e.g., organic acids, pigments) [14].

4.2 Identification of macro-organisms

Complex communities with the presence of plants and animals were observed in some samples (Table 3). In *sample 15* (from the basin edge for example), *a* Bryophyte was revealed by stereo and optical microscopy observations (Figure 6).

Aerial and sub-aerial parts were observed, seeds (Figure 7) of *Stellaria media* (weed plant belonging to *Caryophyllaceae*) were recovered from the back of the left dragon.

Table 3. Vegetal and animal colonizers.

Samples *(scalpel, forceps)*	**Sampling areas**	**Macroorganisms**
sample 15	basin edge	Bryophyte, Mosses
sample 7	dragon on left side (back)	*Stellaria media* (*Caryophyllaceae*)
sample 4	dragon on right side (wing)	Larvae (*Diptera*, *Coleoptera*)
sample 5	dragon on right side (wing)	Adult insects (*Psocoptera*, *Hemiptera*)

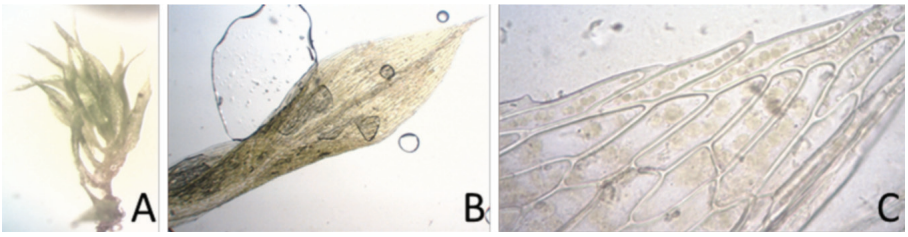

Figure 6. Gametophyte sampled on basin edge. Stereomicroscope (A), optical microscope 10X (B), and 40X (C) images.

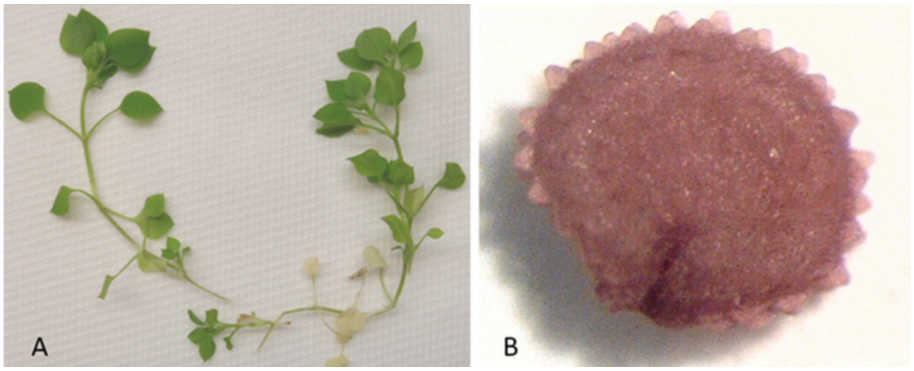

Figure 7. (A) Stellaria media (Caryophyllaceae); (B) Stereomicroscope 40X its seed.

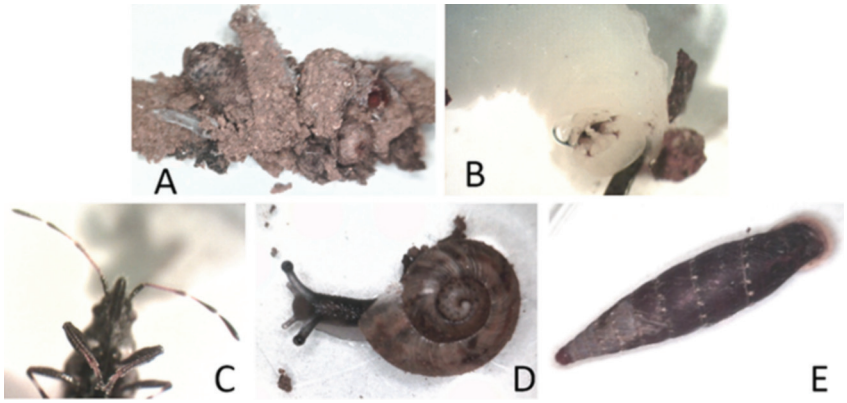

Figure 8. (A) Dipteran pupae; (B) Coleoptera larva; (C) semiaquatic bugs Velia sp. (Heteroptera: Gerromorpha); (D, E) terrestrial pulmonate (probably Aegopinella sp. and Leucostigma sp.).

A suitable environment and the presence of energy sources with organic and inorganic nutrients contribute to biological colonization on exposed stone, favouring also the settlement of different taxa of invertebrates (Figure 8A-E). Several pupae of mycophagous dipteran specimens were collected from the stone (Figure 8A). It was impossible to determine the species, as mycophagous flies can only be distinguished by the morphological characteristics of adult males, and larval samples must be reared to adulthood to obtain an adult male specimen. However, their presence is supported by the growth of fungi, algae and lichens. An Arthropodes community was also represented by some coleopteran larvae (Figure 8B) and the semiaquatic bugs (Heteroptera: Gerromorpha) belong to the genus *Velia* (Figure 8C). The latter actively forages and sucks up prey, usually soft-bodied arthropods, collected from the surface of the dragon on the right side. In addition to the indicated species in Figure 8 several individuals of Psocopetera were also collected in the same areas.

The Molluscan community was represented by a terrestrial pulmonate gastropod: the small land glass snail (probably *Aegopinella nitidula* of the family Oxychilidae, Figure 8D) and the door snail (*Leucostigma* sp. of the family Clausiliidae, Figure 8E); these were observed on the stone supporting algal and lichenic growth.

These pulmonates are herbivorous, living on fresh and/or dead plant material. They can also graze on algal films as they have specially modified spadelike radular teeth. They also commonly feed on fungi.

All these animals contribute to the diffusion and propagation of new algae and fungi colonies by means of their spores, soredia, algal cells and fecal pellets or by becoming entrapped among their body parts (such as hairs). These taxa were recovered in cracks or in hidden areas of the dragons and their activity, growth and swelling can induce physical stress and mechanical breaks. Direct destructive action can be both physical, caused mainly by grazing, and chemical, caused by the dropping of excrements. Indirect damage results from organic substances accumulated on the stone surfaces, which can serve as nutritive substrata for heterotrophic microorganisms (bacteria and fungi).

The destroyers damage the constituents of the substrate while utilizing organic substances for their metabolic requirements. Insects, fungi and most bacteria are destroy-

ers. The consumers can be considered the least dangerous group in this field because they feed on living matter from other micro and macro organisms. On the other hand, they have a crucial role in controlling the growth of 2 other biodeteriogens, lichen-eating snails and grazing animals.

Despite the potential importance of invertebrates inhabiting our heritage and historical monuments, only scant literature is available [15] on the various aspects of biodeterioration of stones due to invertebrates. Further study into the taxonomy, recruitment, species richness, community, composition, ecological significance, biology, physiology and reproduction of species that are potential biodeteriogens for historical monuments is still needed.

5. Conclusion

The biological sampling on the decorative elements and basin surfaces was performed on the Fountain of the two Dragons in Palermo. The samples were collected from areas affected by chromatic alterations, deposits, exfoliations, incrustations, biological or pigmented patinas. Afterwards, characterization of plant residues and insects was performed by microscopic investigations in order to reveal the main macro-biological systems inhabiting the fountain. Cultivation-based techniques were then employed in association with molecular analysis to give a complete overview of microbial taxa able to colonize the surfaces of the artifacts.

Pigmented bacteria belonging to *Arthrobacter* spp. and *Paracoccus* spp. were revealed in collected samples that were characterized. In general, these bacteria develop on stone artworks causing pigmentation and discoloration [16, 17].

Fungi have a crucial role in the discoloration and mechanical degradation of stone in cultural heritage; hyphae penetrate deeply into materials and can release extracellular enzymes (enzymatic degradation and pigments production). Moreover, fungal spores are wind-dispersed and often extremely abundant in outdoor air. The main fungal colonies revealed in the samples belong to *Alternaria* and *Phoma* genera. The mentioned fungal genera are typically soil fungi that can induce mechanical exfoliation of stone material by hyphae penetration or production of different pigments and organic acids [18].

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Biographical notes

Enza Di Carlo graduated in "Biological Science" at the University of Palermo and specialized in "Microbiology and Virology" at the School of Medicine at the same university. She is a Research Fellow at the Laboratory of Biology and Biotechnology for the Cultural Heritage (LABBBC) of the Department of Biological, Chemical and Pharmaceutical Sciences and Technologies (STEBICEF) at the University of Palermo, where she carries out microbiological monitoring of environments for the preservation and use of Cultural Heritage.

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Gaetano Corselli D'Ondes, Architect; for more than thirty years he has worked in the field of architectural restoration, in particular stone materials, frescoes, stuccoes, mosaic coatings and iron ores. He served at the Superintendency BB.CC.AA. of Palermo between 1993 and 2003 by participating, among other works, in the restoration of Maredolce Castle and took care of the underground architecture of Palermo. Currently, he provides consulting services in the field of restoration at Mediterranean Engineering on behalf of which he also directed the work of the Martorana church in Palermo.

Silvana Lo Giudice, Architect in Palermo, she is specialized in "Conservation of Historical Architecture". From 1993 she has been a member of the *Salvare Palermo* Association, Cultural and Scientific Disclosure Commission and Advisor of the Board of Directors of the New *Salvare Palermo* Foundation. From 2010 she has been a member of the editorial office of *Per;* she is a member of the Restoration Committee in the role of coordinator of restoration intervention in Palermo and permanent art exhibitions. She is author of several publications (*Le radici della Fotogrammetria a Palermo*, 1992; *L'impiego dei fotogrammi del volo Italia (1989) e dell'ortofotocarta, da essi derivata, per l'aggiornamento delle tavolette 1:25.000 relative al territorio comunale di Palermo*, 1993; *I risultati della tesi di laurea nel volume Il territorio: questo sconosciuto*, 1997; *Il CD multimediale "al di là del mare", Comune di Gioiosa Marea*, 2001; *Disegni in luce, Comune di Palermo*, 2002; in *Palermo Normanna*. *Proposte di itinerari didattici*, *Assessorato regionale BB.CC.AA. e P.I. – Soprintendenza BB.CC.AA. di Palermo,* 2003; *La chiesa Holy Cross a Palermo*, 2008); since 2008 she has been *Dirigente Tecnico Architetto del ruolo tecnico dei Beni Culturali*.

Summary

Stone works of art located outdoors are exposed to natural deterioration, due to several physical, chemical and biological factors. Biological macro- and micro-systems (e.g. weeds, insects, algae, mosses, fungi and bacteria) may induce the biodeterioration of stone materials. In February 2014, the state of conservation of the Fountain of the two Dragons in Palermo was examined, during which time biological colonization in some areas of the fountain and statues, was also detected. The monument represents an excellent substrate for the development and growth of microrganisms and organisms that need a continuous supply of water. Direct solar irradiance promotes the development of photoautotrophic organisms, bryophyte flora, in addition to several microbial genera, responsible for pigmentation, discoloring, efflorescence. After autotrophic colonization, some organisms find an ideal habitat also during wintertime, including two species of gastropods and two species of Hemiptera, omnivorous, a species of Collembola, mostly fungivorous. This interdisciplinary study has enabled the detection and identification of different biological communities, providing information for a subsequent project of preventive conservation of the fountain and its surroundings.

Riassunto

I manufatti lapidei esposti all'aperto sono sottoposti a processi naturali di deterioramento, dovuti a fattori di natura fisica, chimica e biologica. I macro e micro sistemi biologici (per esempio semi, insetti, alghe, muschi, funghi e batteri) possono causare il deterioramento dei materiali lapidei. Nel Febbraio del 2014 è stato effettuato uno studio sullo stato di conservazione della Fontana dei due Draghi, Palermo, durante il quale sono state individuate diverse aree interessate da colonizzazione biologica, sia all'interno della fontana e sia sulle statue decorative. Il monumento rappresenta un substrato di crescita eccellente per lo sviluppo di microorganismi e di organismi che necessitano di un continuo supporto di acqua. Inoltre, l'irradiamento solare diretto promuove lo sviluppo di organismi autotrofi, briofite oltre a diversi generi microbici responsabili dei fenomeni di pigmentazione, alterazioni cromatiche, efflorescenze. Dopo la colonizzazione autotrofica, molti organismi trovano l'habitat ideale anche durante il periodo invernale, incluse due specie di gastropodi e due specie di Hemiptera, onnivori, una specie di Collembola, principalmente fungivoro. Questo studio interdisciplinare ha permesso di identificare differenti comunità biologiche, fornendo informazioni utili per un futuro progetto di conservazione preventiva della fontana e il suo ambiente circostante.