Chapter

The Impact Study of the Exchanges between the Microorganism Communities on the Surfaces from Constanta Roman Mosaic and Anthropic Interactions

Verginica Schröder, Daniela Turcanu-Carutiu, Adina Honcea, Sorin Grigore and Loreley-Dana Jianu

Abstract

The constituent elements of the Roman Mosaic from Constanta are damaged under the activities of microorganisms present both on surfaces and in the airborne microbes. The predominance of microorganisms on the different surfaces of the edifice has led to multiple damage such as discoloration, pigmentation, wall degradation and exposed ceramic objects. Through this study we aimed to invest the diversity of microorganisms on the various substrates and levels as well as microclimate conditions. From the samples collected there were isolated and identified microorganisms, many of them with pathogenicity risks for staff and visitors. Thus, for the improvement of the surrounding conditions of the Roman Mosaic exhibition room, the need for management is aimed at reducing the microbial contaminations, based on understanding the changing conditions in the microclimate and decreasing the damage biofilm. Our study can be seen in a broader procedural in the current COVID-19 pandemic conditions.

Keywords: Roman Mosaic, microbial contaminations, antropic, COVID-19 pandemic conditions

1. Introduction

The Roman mosaic building is an ancient construction located in the old town of the Peninsular area of Constanta, Tomis in antiquity, port on the Black Sea called in antiquity Pontus Euxinus. It was discovered after the works made for the construction of some blocks of flats in the autumn of 1959. The archeological campaigns dedicated to the Romanian edifice extended over five years, until 1965. To these were added those from 1965 to 1968, which aimed to conduct further research on construction [1–3].

The archeological complex arranged along the cliff, on the four terraces, has remained almost unchanged since its opening, but it is too little used and appreciated today in relation to its real cultural value.

The archeological importance of the Mosaic meant that in the period 1970–1976 a steel, concrete and glass structure was built above the monument, in the area of the original mosaic, for protection.

The imposing construction, erected in the 3rd or 4th century e.n. (possibly under the emperor Constantine the Great, 306–337), it took place in antiquity on three terraces, cut in the slope of the cliff and leveled to ensure the stability of the construction. It was located right on the quays of the old Tomitan port, from which opened its first suite of rooms. It included eleven vaulted chambers used as warehouses for cargo brought by merchant ships calling at the port.

The configuration of the third level is totally different from the other two. Here we are dealing with a huge hall (101 m long, 21.45 m wide), non-compartmentalized and sumptuous, with over 2,000 m^2 of polychrome mosaic. Its decoration is luxurious, and the improvised tribune on its north-eastern side shows that it had a destination that served the commercial purposes of the port, the place where merchants and transporters met, where business was negotiated, and the prices of products were fixed. In ancient times it was probably covered with a huge vault supported by pillars. Today, part of the long wall and one of the side walls are still preserved from this room.

The first, preserved on a length of 65 m with a maximum height of 5.40 m (it is preserved only at the southern end and gradually descends to the north, to the level of the foundation), has a rhythmic succession of pilasters embedded in it. It seems that there were fifteen such pilasters delimiting fourteen fields. The walls were clad in marble, and the pillars had marble slabs at the top that mimicked the shape and decoration of capitals. Their decorative motifs were predominantly vegetal.

The wall was pierced by an entrance from the current Ovidiu Square, later closed and used as a niche, in which was probably exposed the bust of an emperor (among the sculptural fragments discovered here was the bust of Constantine the Great). Another peculiarity of the room is formed by the few steps that made up the small platform attached to the same wall and also covered with marble. It was probably used as a tribune for the auctions of goods that took place here.

The mosaic carpet stretched across the room.

The floor is therefore kept on a length of 49.80 m and a maximum width of 16.60 m, covering an area of about 800 m^2. The tiles that compose it have variable dimensions, depending on the size and details of the motifs represented. It measures 2–3 cm in the border and 1–2 cm in the central part. The materials used are marble and natural stone of different colors: white, red, bluish-green, black, cream. They are arranged in straight rows, especially in the border and in winding lines for more complex motifs. They are placed on a bed of lime mortar mixed with a lot of crushed brick [2, 3].

These technical characteristics make it possible to classify the work in two of the categories of the typology proposed by several researchers. Thus, from the technical point of view, this mosaic combines the two styles: opus tesselatum (especially the border) and opus vermiculatum (especially the center and the circular, undulating motifs). The carpet has two component parts: the border and the central painting.

The frame has a rectangular shape and a width of 6.20 m. It is composed of several continuous, different strips, which frame and highlight the central representations. These are, in order of their inward succession:

- a rectangular frame delimiting a white band on which stretches a wave of ivy, with the leaves arranged symmetrically, sometimes with the tip up, sometimes with it down, at equal distances. The spell is red, and the leaves have white, red, and green tiles on the inside;

- the next band contains the motif of the simple rope, symmetrical with another identical rope, together with which it frames a much wider motif, formed by symmetrical intersections of circles; the circles are reddish in color, and their petal-shaped intersection is white; each quadrilateral delimited by intersections in the middle of a circle has in the center a rectangular group of white tiles. The motif is the same used in one of the lodges at Histria;

- after the other rope follows the well-known motif of the Etruscan wave, white, oriented to the left. It is symmetrically arranged with another reddish color and oriented in the opposite direction. Together they frame a band containing the triple braid, which is also called David's knot.

The frame that accompanied the western side of the mosaic, preserved in a proportion of 10%, includes the same motifs, in the same sequence, with one exception: the insertion of "oblique lines that form paleograms".

The central painting consists of an alternation of rectangles and circles inscribed in squares. It seems that there were three such circular fields, but the first two were preserved almost intact [4].

The first circle, the one from the south-west, with a diameter of 7 m, is inscribed in a square with polychrome sides, along which a row of disc halves and one of isosceles triangles (the so-called motif of wolf teeth or saw teeth). Between one of the corners of the square and its corresponding circular arch is a stylized brick, kantharos, with white grooves arranged vertically on its lower half and with S-shaped handles. From it they extend in the two opposite parts, supporting, as it were. This part of the circular border, two ivy twigs. Next to one of the ivy leaves is a white dove, the only zoomorphic representation in the composition [1–4].

The circumference of the circle is adorned by a row of wolf teeth arranged in a mirror with those on the sides of the square frame and by the Etruscan brick wave, oriented to the left. Inside the circle, the simple rope meanders delimiting several geometric medallions, each in turn comprising different motifs: stylized vessels, swastikas, vegetal representations, weapons - double crossed axes and other geometric shapes.

The other preserved circle, to the northwest, is filled with circular scales. Their size decreases at the same time as approaching the center. Apparently, this was the center circle of the carpet. Between the two circles was preserved a rectangle divided by rows of simple rope into several regularly arranged squares. The other dividing rectangle, located in the south, includes a set of various geometric shapes (squares, rhombuses and triangles), which include different motifs: woven rope in four, zig-zags, chessboard, pelta, petals, gamma cross, etc.

In the northwest, the color and stylistic unity of the mosaic is interrupted. The patterns are continued more carelessly, and the colors differ. There are several repairs performed in this part. It is noteworthy, however, that the craftsmen, who reconstituted this degraded part then, chose the most expensive and most difficult method of repairing a mosaic.

The carpet in the Roman mosaic building is loaded with geometric motifs well known in the Roman world and used in most works of art in this category. The simple rope, the Etruscan wave, the wolf's teeth are usually used in the art of mosaic as framing motifs, as decoration for frames or frames surrounding other more important decorative elements. They have a delimiting role and highlight a much more important representation, like the frame of a painting. The whole ensemble is executed with care, having a unitary composition, formed by various images, combined in a rich and carefully chosen color framework.

Integrated in a monumental architectural ensemble, partially preserved, the Roman Building with Mosaic preserves, perhaps best, the image of the ancient Tomis in a time of economic flourishing (4th - 6th century AD).

Despite the cultural openness and projects that easily fit into the (international) architecture of the moment, the obsessive economic principle has left a deep mark on the architecture of the '60s and' 70s, by transforming projects, in many situations, with the implementation and by deterioration over time, due to the use of poor-quality materials. For this reason, perhaps even more so for reasons related to incompatibilities between the old and the new structure, the exposure of the monument and the creation of an artificial environment for it, the protective construction of the building and the monument itself today show visible damage.

The implications of microorganisms in biodegradation are well known [5, 6] but data on exchanges between microorganisms and eco-physiological principles which relate to the rules of association of microbial communities are limited [7, 8]. Biodegradation is a complex phenomenon so therefore environmental factors, and especially factors that induce a selective growth climate for microorganism, are very important [9–12].

A recent complex study found that the development of fungal communities is conditioned by microclimate factors inside the space, while bacteria vary significantly with a number of behavioral anthropogenic factors (ventilation, type of material used on the floor, number of occupants in the space) [13].

In a few works about the museum indoor spaces were the information on the exchanges and interactions between the communities of microorganisms in the closed environment [14] or the different materials substratum [15].

A recent study [16] shows that the relationship between microorganisms on surfaces and those from the aerosol load requires supplementary studies because there is little information on the matter.

The studies on the interactions between mentioned microorganisms might have an important and promising perspective taking into account human health [17]. It is known that microorganisms in the environment in which humans work have an important role on general health and on the adaptation of the immune response [18].

The present work is a first study in that the surfaces of a building that is several centuries old with evidently degradation have been analyzed. Located inside the covered enclosure, these archeological components are obviously influenced by microorganisms agglomerated on the surface, but also by other source such as the brick side wall, the presence of people who take care of and protect the space, the penetration of air through the vents, etc.

The study aimed to assess the microbial communities from this complex museal space, the degree of the interaction between associations from Roman Mosaic surfaces and airborne microbiota, the persistence in space of pathogens with infection high risk, when the flow of tourists was very limited, in the current COVID-19 pandemic conditions.

2. Materials and methods

2.1 Sampling and macroscopic evaluations

In order to make the diagnosis regarding the biological activity, the surfaces of the pieces from the Roman mosaic were analyzed (**Figure 1**). The biological samples were collected using a fine scalpel, in sterile Eppendorf tubes or in sterile containers with a volume of 10 mL. The samples were kept at low temperatures of 2–4^0 C until processing.

Areas from the mosaic, as well as from adjacent parts, such as the eastern wall, built of brick and with an obvious biological contamination and degradation, were identified as relevant points for analysis (**Figure 2**).

The autotrophic biofilm was analyzed at this level, arranged in successive layers, portions with intense degradation processes, in the upper area, as well as mineral deposits.

The macroscopic and microscopic observations of the degraded areas were performed.

To identify the biodegradation, sampling for laboratory analysis was performed from the affected portions, (**Figure 3**) but the techniques were minimally invasive, avoiding the production of new colonization points or deterioration.

All, biological analysis was performed in the laboratory by making microscopic observations.

2.2 The microbial contaminations methods assessment

Contact sampling methods have been developed to assess surface contamination collecting by surface 1 cm^2 with sterile moistened swabs touching the surfaces

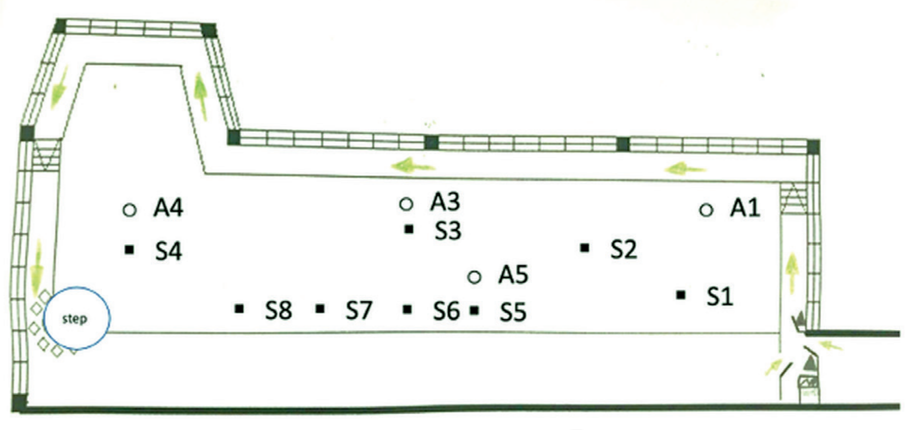

Figure 1.
The Roman mosaic museum samples map and codifications (A1-A4 air contamination, S1-S8 surfaces biocontamination assessment, the green arrow is the route of tourists).

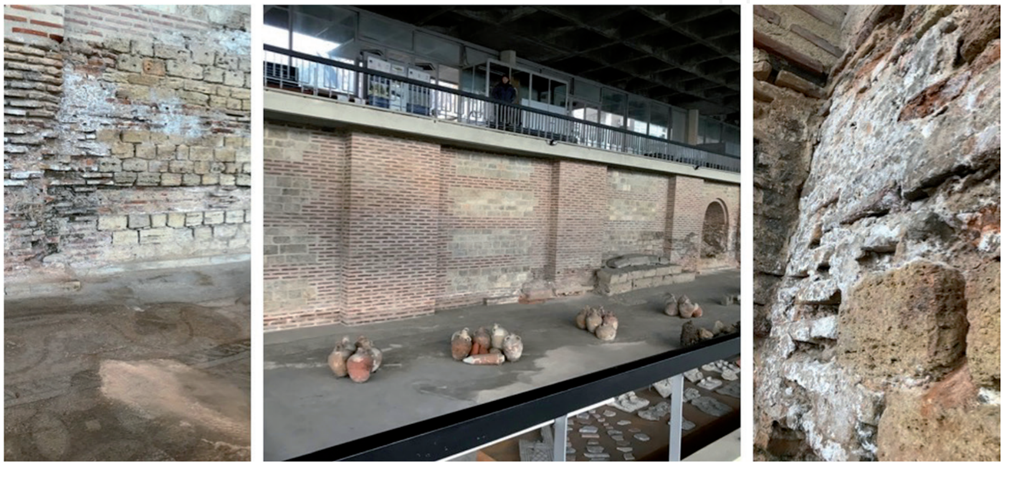

Figure 2.
The details of wall built of brick analyses.

Figure 3.
Appearance of the biological attack on tesserae.

and rapid inoculation on the culture medium. The swab should be pre-moistened with a sterile rinse medium [19]. Also, they were introduced in 1 mL of saline solution after which the inoculation was performed [20].

The growth media used were Columbia blood (isolates pretentious hemolytic bacteria), Tryptic Soy Both (non-selective medium), Sabourad (selective fungi), nutritive agar (non-selective), Mannitol egg Yolk Polymyxin agar, MYP Agar (selective for *Bacillus cereus*).

The identification of microbian species was performed on morphologically criteria (colony characteristics, microscopic on culture smear), biochemical criteria (catalase, oxidase), Biomerieux VITEK microbial identification system.

2.3 Settle plates

Petri dishes with a diameter of 9 cm were used, placed in four points, fifteen minutes exposure, at approximately 1 m from the floor and respectively from the wall or the passage stairs.

Thus, the determination of the presence of microorganisms in the air is made by the method of sedimentation on nutrient/culture media which assumes that after cultivation there is a count of all the colonies developed on the culture medium and thus establishing the total number of microorganisms on the plate and colony forming unit calculated (CFU/cm^2). The Index of Microbial Air Contamination (IMA) was calculated according to [21, 22].

2.4 Microscopical analysis

Small sample fragments were placed in about 5 μL of distilled water, and the cell suspensions were placed on the slide and analyzed under an optical microscope.

The colonization level and the presence of biological particles were monitored under a microscope, the degree of biodegradation was quantified, and the links between the microbiota and the substrate were identified by staining techniques. Rapid staining techniques, methylene blue or acetic acid were applied for staining. Fluorochrome acridine orange (AO) was used to identify morphological details of the microorganisms, with an excitation filter of 488 nm and an emission filter of 515 nm.

DOI: http://dx.doi.org/10.5772/intechopen.99057

3. Results

3.1 Macroscopical and microscopical analysis

Relevant areas for analysis were identified as affected areas of the mosaic (**Figure 4a** and **b**) and adjacent parts, such as the eastern wall, built of brick and with obvious biological contamination and degradation (**Figure 4c** and **d**). The autotrophic biofilm was noticed at this level, arranged in successive layers, parts with intense processes of fungal contamination, in the upper area, as well as mineral deposits.

Microalgae are commonly found on calcareous substrates and can induce degradation by release of acids, accumulation of ions and alteration by pigmentation, fragmentation of the substrate and the formation of crusty, dark or patinated-looking surfaces [23].

Microscopic analysis shows lichen-like structures with an upper cortex formed exclusively by tightly woven and welded hyphae, as well as a gonidial layer with algal symbiosis indicating chlorophyll activity (red coloration) (**Figure 5a**). The middle layer (medullary) consists exclusively of mycelial hyphae, loosely braided (green coloration), (**Figure 5a**).

The biological activity by substrate attachment is very evident (**Figure 5**), as well as the formation of mobile structures, detached from the substrate or colony,

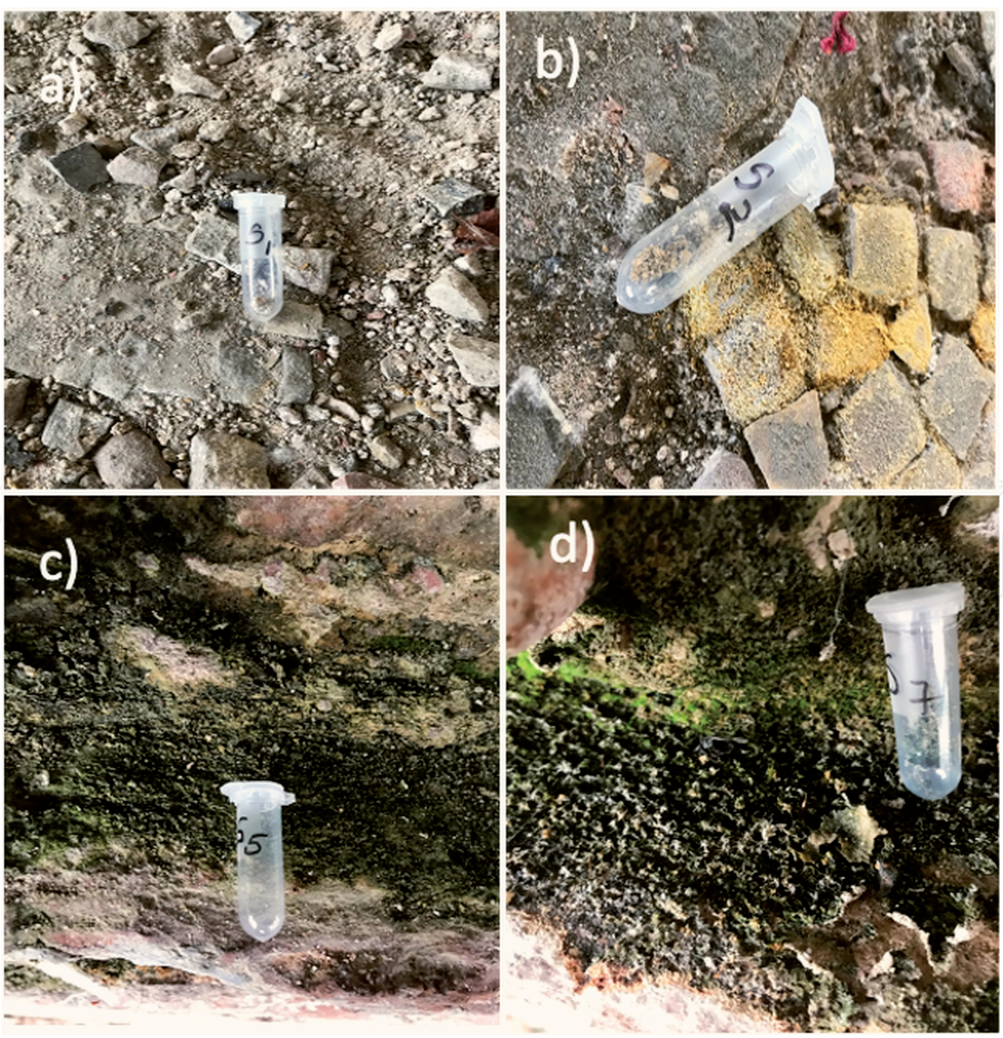

Figure 4.
The affected areas of the mosaic by chromatic alteration and destructions (a, b), the biodegradation of wall surfaces (c, d).

of the goniocyst type, which include photobiont cells surrounded by hyphae arranged in a single layer (**Figure 5c**).

In the S 8 sample (**Figure 6**) intense biological activities were identified, including various colonies of microalgae (**Figure 6d**) as well as fungal activity (**Figure 6a–c**).

The massive microorganisms colonization, highlighted in the samples, explains the various depigmentation, or changes in shades, or the biogenic pigmentation that was identified on the analyzed surface. Bacteria induce changes by discoloration as well as by dissolving various materials generating biodegradation of the substrate through specific metabolism [24].

The crushing of the substrate is noticeable in many samples and can be generated, for the most part, by the impact produced by the bacterial metabolism combined with the chemoheterotrophic metabolism of the fungi [25].

Fungi (hyphae or resistance morphotypes) induce degradation on the substrate by dissolution or the formation of pigmented or discolored areas [26].

The analyzed samples were noted free of hyphae and completely attached to the substrate by obvious interactions. The samples show emission in green by staining with AO which suggests active metabolism.

3.2 Microbiota isolated on nutritive medium

The isolated microorganisms from the museum space, respectively mosaic surfaces, included several forms of which 15 are bacteria (*Bacillus ssp. Aeromonas salmonicida,*

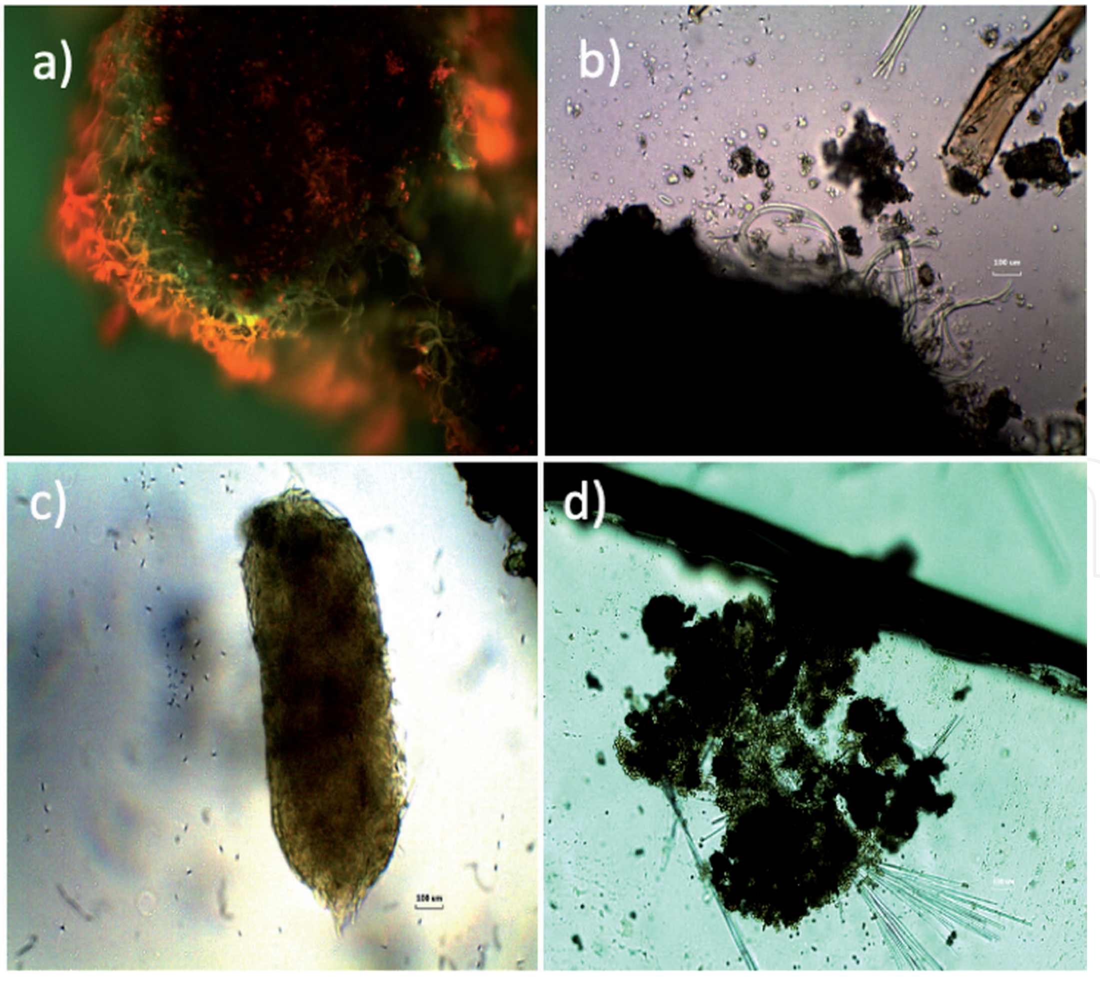

Figure 5.
The microscopical examination of biological samples a) autotrophic forms, in epifluorescence with acridine orange staining, b) biological associations in light microscopy images, c) goniocyst, d) fungal contamination, optical microscopy, bar = 100 µm (x400 magnification).

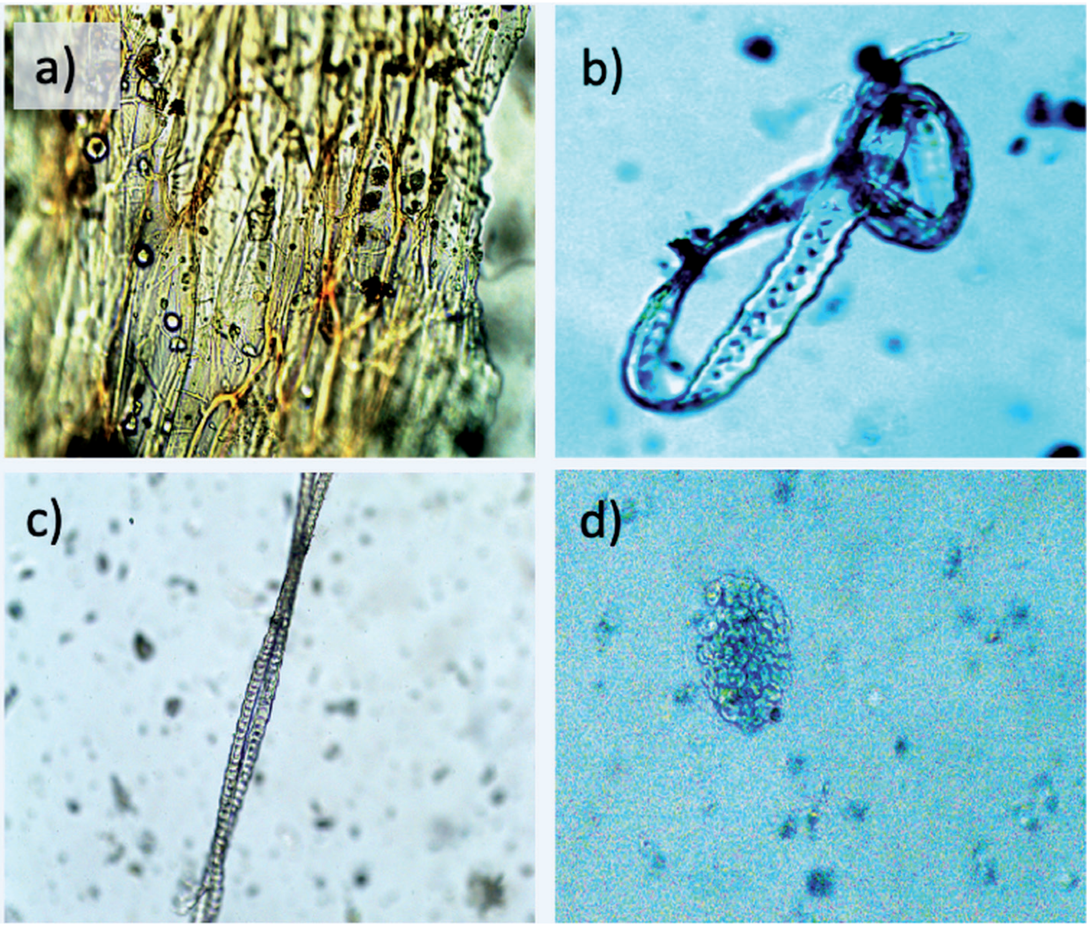

Figure 6.
The microscopic details of fungal morphotypes (a, b, c) and microalgae (d).

Kocuria rosea, Clostridium histolyticum, Corynebacterium urealyticum, Acinetobacter lwoffii, Corynebacterium pseudodiphtericum, Bacillus licheniformis, Bacillus clausii, Staphylococcus epidermis, Micrococcus luteus, Corynebacterium ssp., Pseudomonas ssp.) and 11 fungi (*Aspergillus flavus, Aspergillus niger, Rizopus sp., Penicillium ssp, Candida sp., Alternaria* sp.) and five unidentified species.

Quantitative evaluation of microorganisms highlighted the microorganism load of surfaces near the eastern wall (S 5, S 7) of the analyzed space (**Table 1**) where the number of bacterial colonies was 1921.8 CFU/cm^2 and 2328.1 CFU/cm^2, respectively. The fungal colonies being also remarkable in the S 5 sample, the data indicating 3146.8 CFU/cm^2 (**Table 1**). The low CFU/cm^2 density values were recorded in the central area of the space (S 1, S 3).

The air is not a favorable environment for the development of microorganisms, but in the air, there are microorganisms that come from the substratum, humans contact and another different sources and these organisms could be transmitted through air currents.

The air microbiota is influenced by a series of factors: the degree of air ventilation, temperature, relative humidity, human presence and agglomeration, etc.

From the total samples analyzed by the isolated colonies, one can notice the density of fungi with over 4000 CFU/m^3 in the area destined for the passage of tourists (**Table 2**), passage with stairs (A 1) as well as near the brick wall (A 5). These data confirm the possibility of favorable factors with risk of passage to the mosaic, in the portions with cement and brick substrate where humidity and porosity are the main factors that maintain the development of fungi.

A large part of the microorganisms with pathogenicity for humans can be transmitted by air inside the exhibition hall, but at the same time they can represent

The microbiota type	CFU/cm^2				
	S 2	S 3	S 5	S 7	S 8
Bacteria	131.2	81.2	1921.8	2328.1	31.2
Fungi	50	128.1	3146.8	84.3	81.2
Total colonies	**181.2**	**209.3**	**5068.7**	**2412.5**	**112.5**

Table 1.
The microbial density (CFU/cm^2) on mosaic surfaces.

The microbiota type	IMA (CFU/m^3)			
	A 1	A 3	A 4	A 5
Bacteria	112.5 x 10^2	112.5 x 10^2	253.1 x 10^2	356.2 x 10^2
Fungi	4790.6 x 10^2	159.3 x 10^2	178.1 x 10^2	4809.3 x 10^2
Total colonies	**4903.1 x 10^2**	**271.8 x 10^2**	**431.2 x 10^2**	**5165.6 x 10^2**

Table 2.
The airborn microbial density (CFU/cm^3), the index of microbial air contamination (IMA).

degradation factors for the exhibited museum objects, mosaic and other vestiges of the museum complex (**Figure 7**).

The samples highlight the microbiological load in the air column, the isolation from repeated samples suggesting an amplified activity, especially of the species isolated on Columbia blood environment that show hemolytic activity.

Many species are found in the native, resident microbiota of the colon. Among the isolated species, *Bacillus cereus*, *Corynebacterium urealyticum*, they attracted attention on surface of mosaic samples.

In terms of habitat and pathological implications, resistance by the formation of spores at these bacteria can maked occasionally be isolated from environmental sites, which are only sources of transmission. The ease of airborne transmission of endospores is very obvious.

The most important colonies, present in all the analyzed points, are from the group of gram-positive bacilli, the genus *Bacillus* being highlighted in all the samples (*B. pumilus, B. cereus*) and *B. clausii* bacteria only in airborne (**Figure 8**).

The genus *Bacillus* includes aerobic or facultative anaerobic forms with a diversity of physiological characteristics: mesophilic, thermophilic, psychrophilic, alkalophilic, acidophilic, halotolerant or halophilic species. These characteristics also explain the presence of this genus on analyzed surfaces, with the highest frequency and diversity (**Figure 8**), being very tolerant.

Bacillus cereus grows in poor soils and frequently contaminates food. The implications in the current infectious pathology of the identified bacilli, which aerobically endospore, are the followings: wound infections, lung infections, urinary tract infections, food poisoning, rectal fistulas.

Another genus identified is *Clostridium*. Bacteria gathered from this genus are sporulated anaerobic bacteria, widespread in the soil and present in the colon of humans and animals. The pathogenic potential of anaerobes is manifested only in conditions that favor their access to the internal medium and allow them to grow. Regarding their sensitivity to antimicrobial agents, it is important to mention that *Clostridium* sp. and gram-positive non-sporulated anaerobes have retained their natural sensitivity to antibiotics, but infections with anaerobic bacteria are often mixed, including different morphotypes.

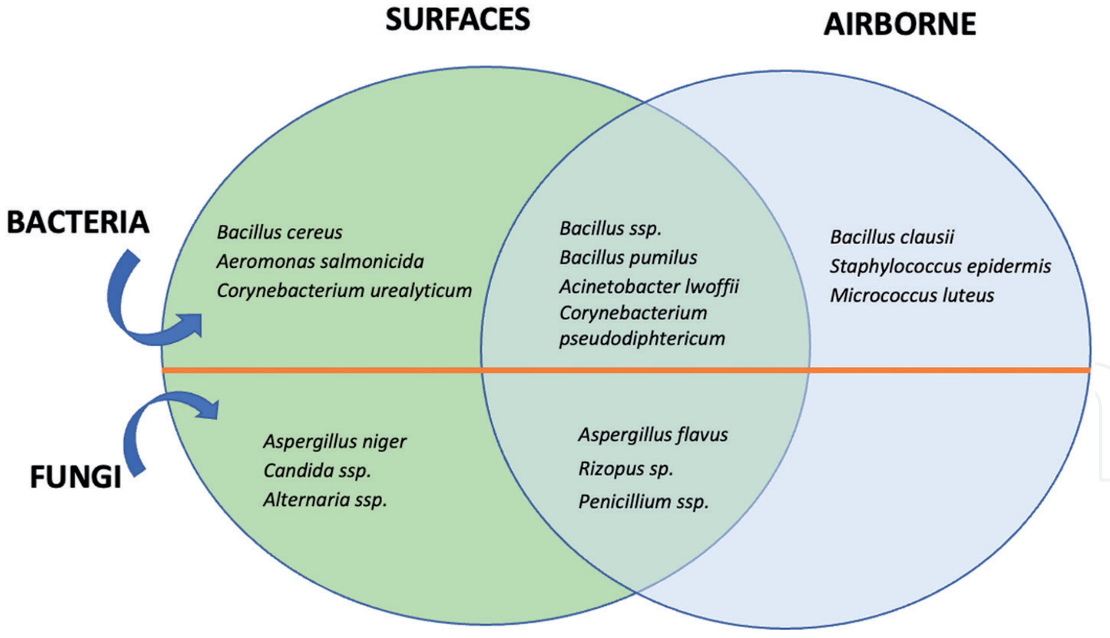

Figure 7.
The common and specific microbiota for surfaces and airborne samples diagram.

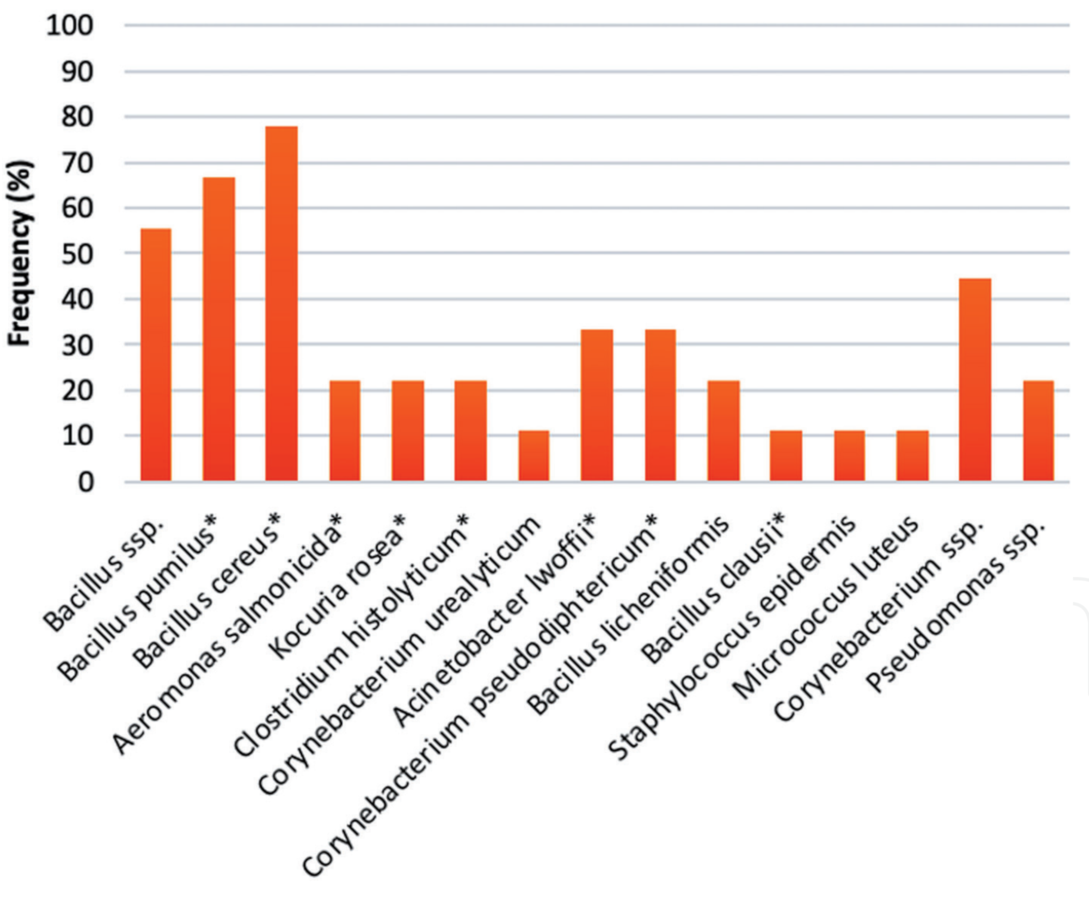

Figure 8.
*Bacterial frequency (%) isolated all samples; * VITEK microbial identification system used.*

The study of Perez et al. 2021, highlighted the fact that in the biodegradation process there are successive sequences of biochemical reactions that favor the growth of different microbial communities. The first stages of this reaction complex are hydrolysis and bacterial acidogenesis in which class *Clostridia* are involved followed by class *Bacilli*, which explains the large number of colonies in

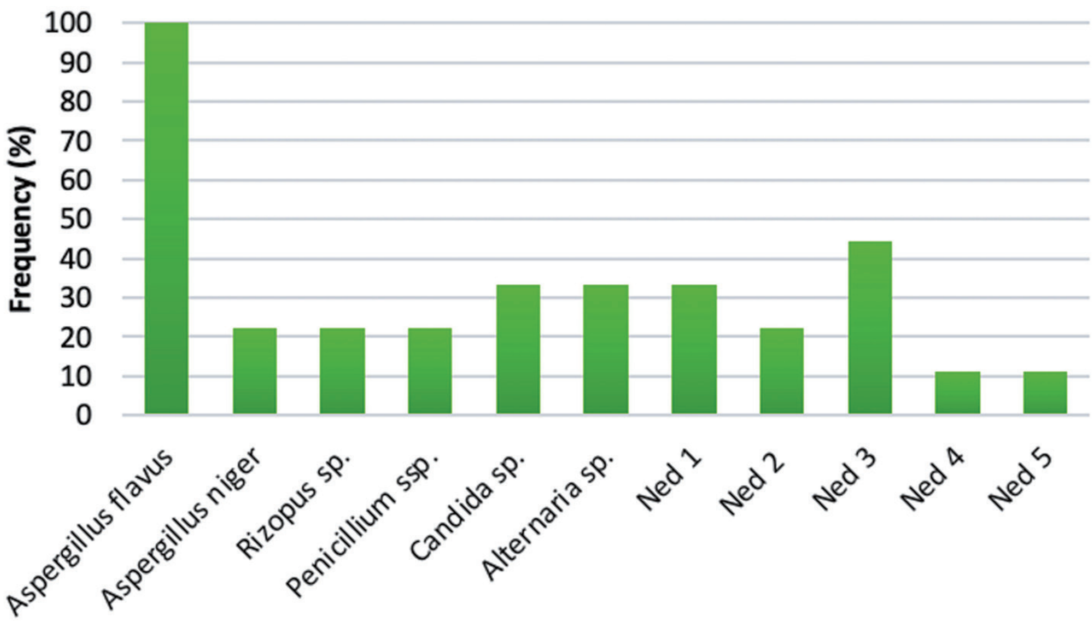

Figure 9.
Fungal frequency (%) isolated from all samples (ned 1-ned 5 unidentified species).

these categories isolated in samples taken and analyzed from the surfaces of Roman mosaic pieces named *tesserae* [27].

Another bacterial category that attracts attention, isolated from the analyzed surfaces (S1) is *Staphylococcus epidermidis* and *Micrococcus luteus*. The species is ubiquitous in skin biotopes [28]. The species *Staphylococcus epidermidis senso stricto* is a species of major medical interest, being characterized by high pathogenic potential. The presence in the samples was repeated and confirmed in the laboratory by morphological analysis of the colonies, microscopy and biochemistry.

Kocuria ssp. appears in the analyzed samples and be noticeable it stands out by the fact that the genus includes commensal forms in humans, but also in animal species. *Kocuria* spp. is also present in the environment but may have pathogenic potential in people with compromised immune systems [29].

Another species identified is *Corynebacterium urealyticum* which has the human skin as its habitat. Coryneform bacilli are distinguished by the fact that they can occasionally move from one ecological niche to another and can accidentally cause diseases (urinary tract infections, skin infections, endocarditis [30].

From the category of fungi from Ascomycota, the most dominant presence of the forms of *Aspergillus* as well as of the type of lower fungi *Rhizopus* sp. can be noticed (**Figure 9**). Also, several genera grew on nutritive media *Penicillium* ssp., *Candida* sp., *Alternaria* sp., and other five colonies were unidentified.

The genus *Aspergillus* comprises over 461 species, mostly found in saprobiosis in the environment and are rarely parasitic [31].

Aspergillus flavus is known for its ability to generate mycotoxins (aflatoxins) when grown on food substrate. As a pathogenicity, in immunodeficient subjects, it produces pulmonary aspergillosis and chronic sinusitis.

Aspergillus niger is a cosmopolite micromycete, frequently isolated from soil, but also from many organic substrates. It develops a series of mycotoxins such as aflatoxins, aspergillin, aspergin. It is frequently involved in the appearance of aspergillomas and in immunocompromised subjects causes skin, lung and systemic infections.

The genus *Rhizopus* develops rapidly in culture media but also in natural environments an invasive mycelial apparatus, with a fluffy appearance at maturity colored in black, due to sporangia. Characteristic of the genus are the rhizoids with which it attaches to the substrate. *Rhizopus* species are generally non-pathogenic but can cause zygomycosis in debilitated patients manifested by processes of tissue necrosis [31].

By analyzing the microbiota in the air of the exhibition hall one can predict the degree of danger of a future infection of the mosaic carpet, the exhibits and which are the groups and species of microorganisms that represent the main threat to an infection of heritage objects. Last but not least, through this analysis it is possible to establish the risk of infecting the people present inside the museum complex, both employed staff and visiting public.

4. Conclusions

The results showed that the Roman Mosaic are significatively affected by multiple biological systems with biodeterioration high potential (algae, lichens, bacteria and fungi).

The species thus isolated and identified were analyzed in correlation with the implications in biodegradation, they affect the components of archeological interest, as well as in terms of pathogenic risk, taking into account the sources of anthropogenic contamination of mosaic components.

The presence, in this exhibition space, of several pathogenic genera (bacteria and fungi), confirmed as having an important pathogenic potential, draws attention to the management of cleaning and preparation of the space before and after the passage of visitors.

Acknowledgements

This work was supported by a grant of the Romanian Ministry of Research and Innovation, CCCDI – UEFISCDI, project number PN-III-P1-1.2-PCCDI-2017-0476/51-PCCDI/2018, within PNCDI III, ACRONIM: ARHEOCONS.

Conflict of interest

The authors declare no conflict of interest.

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