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Microbial Damage to Manuscripts Preserved in Historical Monuments

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Abstract. This study investigates the microflora of rare manuscripts preserved in the historical monuments of Samarkand city, specifically in the Sherdor Madrasa and the Ulugbek Observatory. The research identified the primary bacterial species responsible for the deterioration of manuscripts based on the density and enzymatic activity of heterotrophic bacteria present in the samples. Microbiological analysis of the damaged manuscripts revealed the presence of bacteria belonging to the genus *Bacillus*, and fungi of the genera *Fusarium*, *Alternaria*, *Verticillium*, *Aspergillus*, and *Penicillium*. All nine isolates exhibiting enzymatic activity were examined at the molecular level. The identified species included *Bacillus sp.*, *Bacillus aryabhattai*, *Bacillus mojavensis*, *Bacillus simplex*, *Bacillus halotolerans*, *Bacillus tequilensis*, and *Bacillus subtilis*, all belonging to the family *Bacillaceae*.

Keywords: microbiome, micromycete, bacteria, manuscripts, paper.

1 Introduction. Historical manuscripts are essential works of art that reflect a nation's history and culture; therefore, their preservation is of great importance. Today, the conservation and restoration of historical manuscripts— considered invaluable elements of cultural heritage—are being increasingly enhanced through advances in technology and scientific knowledge, thereby expanding opportunities to safeguard these works for future generations.

One of the major challenges in the preservation of manuscripts is their biological degradation. The most critical step in addressing this issue is the identification and examination of microorganisms that cause or accelerate deterioration. Determining the viability and metabolic activity of microorganisms that damage paper-based materials, as well as identifying their species, is key to selecting appropriate conservation and restoration strategies. In recent years, the role of microorganisms in the deterioration of historical monuments and the manuscripts preserved within them has become an important subject of study.

Previous research has shown that deterioration observed in ancient manuscripts is not caused by a single group of microorganisms, but rather by the complex interactions of multiple microbial communities [1]. Materials of cultural significance not only possess historical, aesthetic, scientific, or ethnographic value, but also provide a rich supply of nutrients and energy sources for microorganisms due to their broad environmental exposure [2]. Microorganisms—including bacteria, archaea, and micromycetes—are capable of colonizing all types of cultural heritage artifacts, including manuscripts, often causing irreversible structural and aesthetic damage [3]. The term *"biodegradation,"* first introduced by Hueck in 1965, was defined as *"undesirable changes in material properties caused by the vital activity of living organisms"* [4,5].

The microorganisms that develop on manuscripts are influenced by atmospheric and environmental factors [6]. Researchers have emphasized that the presence of insects inside cultural heritage structures contributes to microbial development, as mold grows rapidly on the chitin and protein residues left by these animals [7]. Other studies have examined the effects of light, temperature, and air pressure on microorganisms inhabiting historical artifacts and stone structures, showing that these parameters influence microbial community composition [8]. To better understand biological degradation in the preservation of cultural heritage, studies conducted in three historical sites in Italy analyzed indoor air by measuring temperature, humidity, and airflow. These factors were found to correlate with the growth of microorganisms, leading researchers to conclude that controlling environmental conditions can help prevent mold growth that damages artifacts [9]. Another study examining Latin American artifacts investigated the relationship between microorganisms and environmental factors that cause deterioration. Researchers analyzed the structural materials' composition, physicochemical properties, atmospheric pollutant concentrations, and associated microbial communities, demonstrating that these three major factors act synergistically in the degradation of historical objects [10].

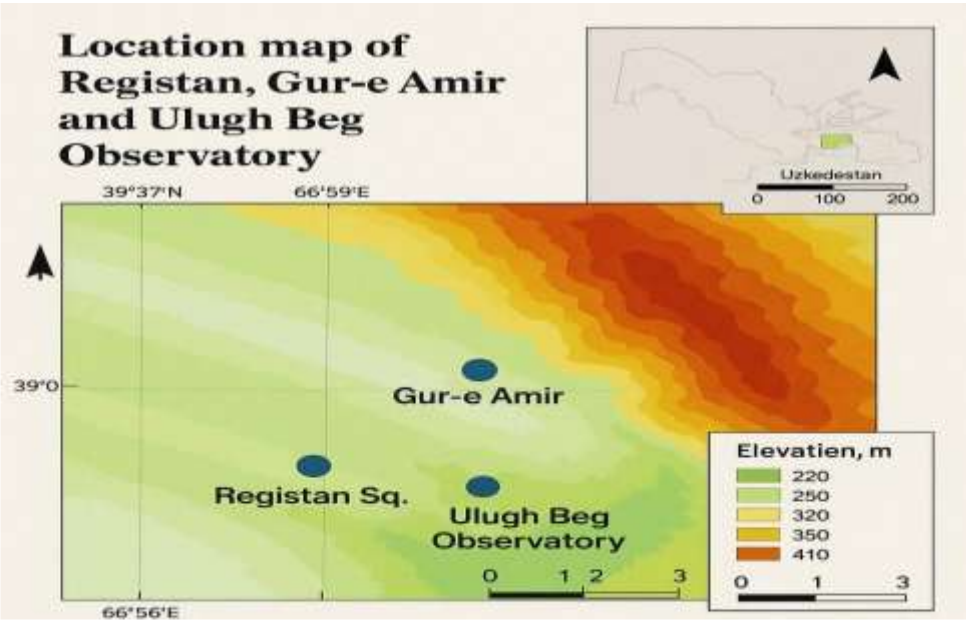
Research on historical manuscripts is carried out using microbiological and molecular methods [11,12]. Fibers used in paper production, animal- and plantderived adhesives, inks, pigments, binders, and other supplementary components found in manuscripts serve as excellent carbon sources for microbial proliferation. The hygroscopic nature of paper further facilitates microbial growth. The biological degradation of paper is directly proportional to its organic matter content and moisture level [14]. Moreover, detecting the production of enzymes by microorganisms involved in the microbiological deterioration of paper is essential for identifying the species that play a primary role in degradation [15]. Most microorganisms are capable of producing hydrolytic enzymes necessary for the breakdown of cellulose or collagen. Enzymatic activity (such as cellulase, amylase, and protease) plays a crucial role in the deterioration of organic materials, especially in libraries and archives [16].

Because microbial metabolic activity is significantly higher during the early stages of growth, artifacts are more prone to physical and chemical damage during this period. After this phase, structural changes occur in historical and archaeological materials, while microbial metabolic activity slows considerably [17]. The biodegradation of paper in historical manuscripts and archival documents is a major concern for libraries and archives worldwide. Therefore, understanding the primary causes and mechanisms of microbiological degradation in historical objects is highly important for libraries and museums [18].

The deterioration observed in rare manuscripts preserved in historical monuments—including handwritten and printed documents and books of historical value from ancient to modern times—is closely associated with environmental conditions that encourage microbial growth, particularly warm and humid storage environments [19].

2. Materials and Methods

In our study, the microbiome composition of three historically significant architectural sites located in Samarkand—considered a cultural and scientific center—was investigated. These sites include the mausoleum of Amir Temur, the Sherdor madrasah, and the Ulughbek observatory, all constructed between the 14th and 19th centuries.



The relationship between air and surface samples was examined. These investigations were conducted in order to identify the microflora that may contribute to the deterioration of historical monuments.

Figure 2. Damage to manuscripts caused by microorganisms.

Historical manuscripts should be stored in well-ventilated preservation rooms and must not come into direct contact with external walls or the floor, as such contact leads to increased humidity and condensation. There are various methods used for the detection of microorganisms, which are generally classified into phenotypic and molecular approaches. Phenotypic methods are primarily based on culturing microorganisms on nutrient media, whereas molecular techniques do not require the cultivation of microbial cells. These independent approaches are further divided into three major groups: microscopic, molecular, and immunological methods. Each group includes a variety of techniques applied for characterizing existing microorganisms. Since each method has specific limitations, the simultaneous use of multiple techniques is often necessary to obtain the most accurate results. Among microscopic methods, scanning electron microscopy (SEM) is considered an effective tool for identifying microorganisms present on paper materials.

For many years, techniques based on prolonged cultivation of microbial cells on nutrient media have been employed to identify microorganisms responsible for the degradation of cultural heritage objects. These approaches are effective for revealing the physiological and biochemical potential of isolated organisms, yet they provide little information about the diversity of complex microbial communities. Traditional culture-based methods enable the isolation of only living and cultivable organisms under standard laboratory conditions, which accounts for merely 0.1–1% of naturally occurring microorganisms. Because only a small fraction of bacterial communities can be cultivated, only a limited portion can be fully characterized and named. Advances in molecular techniques have introduced alternative approaches for assessing microbial diversity in cultural heritage materials without the need for culturing or isolation steps. These developments allow for the investigation of the microbiological diversity associated with heritage objects by directly analyzing microbial DNA.

Sampling from manuscripts. In studies involving cultural heritage objects and materials, the sampling area—regardless of the size of the artifact—must remain intact and unaffected. If invasive sampling is required depending on the condition of the object, the procedure must be carried out within established guidelines. In particular, the paper composition of rare artifacts and manuscripts makes it impossible to establish a standard sampling procedure. Sterile gloves must be worn during sampling, and all collected specimens should be analyzed under aseptic conditions in a laboratory environment. To avoid damaging the object, adhesive tape used for sampling should have low tack and be transparent for microscopic observation. If necessary, various stains may be applied upon transferring the sample to a slide to improve visualization of microbial structures. These sampling methods are suitable for culture-based inoculation or for preparing aqueous suspensions and do not harm the artifact in any way.

When microbial growth on the paper surface is extensive, tape sampling becomes difficult. In such cases, extremely fine tweezers may be used to collect microscopic samples from highly degraded corners or edges of the material. However, invasive sampling must not compromise the value of the artifact.

In our study, the microbiome of historic monuments located in the culturally significant city of Samarkand was investigated. The diversity of microbiomes present on different structural materials of historical buildings was characterized. Samples were collected from the historical monuments of Samarkand during two seasons—autumn and spring. Samples were taken from various materials found in the monuments, including marble stone, wooden doors, bricks, archaeological remains, deteriorated walls, and damaged manuscripts.

Samples were obtained using the classical microbiological swabbing technique (Figure 3). For this purpose, sterile cotton swabs were prepared and immersed in 0.9% physiological saline solution. The collected samples were inoculated onto meat-peptone agar (MPA), potato-dextrose agar (PDA) (1000 ml water; infusion prepared from 200 g peeled potatoes; 20 g dextrose; 20 g agar powder; pH 5.6 ± 0.2), and Czapek agar media, and incubated in thermostats at temperatures ranging from 20°C to 38°C. Pure isolates were obtained by repeated streaking on nutrient media. Morphological characteristics were examined, and isolates were identified using standard taxonomic keys.

Figure 3. Sampling from damaged sections of the walls and from manuscripts of historical monuments in Samarkand

Samples collected from the existing manuscripts of the Amir Temur mausoleum, the Sherdor madrasah, the Shohi Zinda mausoleums, and the Ulughbek observatory were incubated on the above-mentioned culture media for 6–7 days (Figure 3) to study the development of the associated microflora. For obtaining pure cultures from these samples, the standard sub-culturing technique commonly used in microbiology was applied on PDA (potato-dextrose agar) medium.

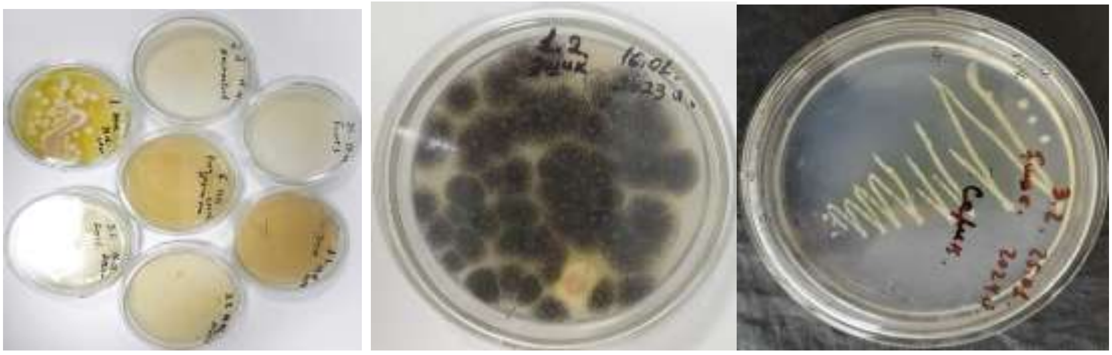


Figure 4. Microbial colonies developed from samples collected from damaged wall sections and manuscripts of historical monuments in Samarkand

In the next stage of the study, pure cultures were isolated from the microbial colonies that developed on the samples (Figure 4). To identify the species of the pure isolates, the morphological characteristics of the microorganisms were examined (Figure 9). The microscopic structures and morphological features of the microorganisms were studied using XSP-136B and OLYMPUS BX41 light microscopes at 400× magnification.

3 Results and discussions. Studies on microbial contamination of manuscripts have generally focused on problems caused by microfungi rather than bacteria. One of the reasons for this is the difficulty of isolating bacteria commonly found in libraries and archives—such as *Cytophaga*, *Cellfalcicula*, *Cellulomonas*, *Serratia*, and *Nocardia*—under laboratory conditions. The most frequently identified bacterial species in manuscripts include *Bacillus atrophaeus*, *Bacillus cereus*, *Bacillus licheniformis*, *Bacillus subtilis*, *Lysinibacillus fusiformis*, *Microbacterium aerolatum*, *Psychrobacillus psychrodurans*, *Staphylococcus epidermidis*, *Staphylococcus pasteuri*, *Staphylococcus saprophyticus*, *Staphylococcus succinus*, and *Streptomyces ambofaciens*. Alongside species of the genus *Bacillus*, *Streptomyces ambofaciens* exhibited pronounced cellulolytic activity. In addition, bacterial species belonging to the aerobic order Actinomycetales and the anaerobic order Clostridiales, both capable of degrading cellulose, were also detected on the artifacts.

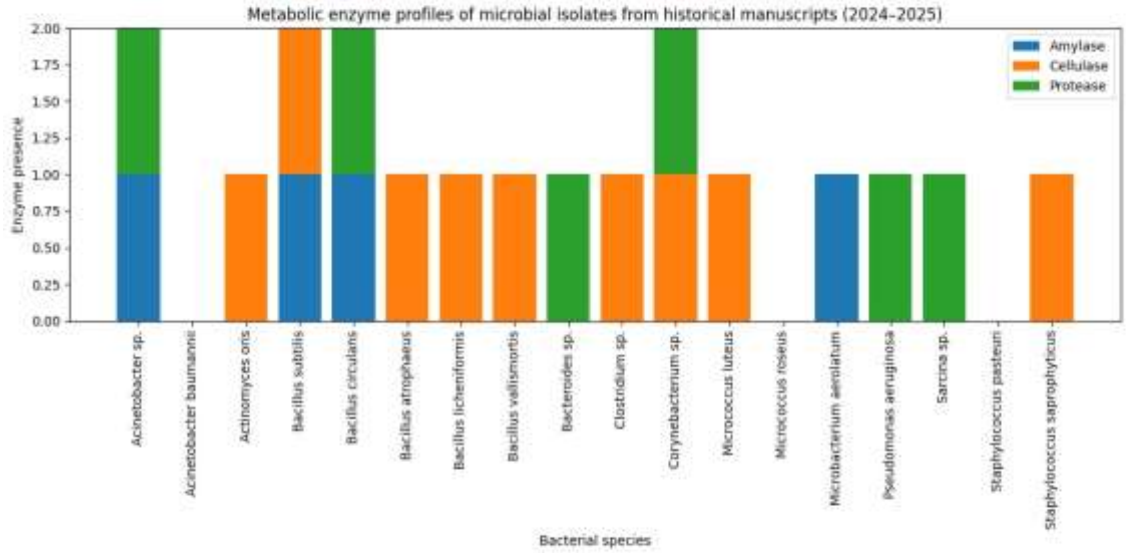


Figure 1. Microbial isolates from historical manuscripts and their metabolic enzymes (2024–2025)

The bacterial isolate obtained from the ancient manuscript (book) was morphologically identified as belonging to the rod-shaped bacterial group. It exhibited spore-forming characteristics and was either aerobic or facultatively anaerobic—features typical of bacteria within the genus *Bacillus*. Therefore, this isolate was identified as *Bacillus* sp. A second bacterial isolate displayed the same morphological characteristics. In addition, spherical (coccus-shaped) bacterial isolates were also detected.

Moreover, samples collected from the deteriorated walls of historical monuments and from damaged manuscripts preserved in Samarkand yielded microfungal isolates belonging to the genera *Aspergillus*, *Penicillium*, *Fusarium*, and *Alternaria*. The morphological characteristics of all microorganisms were examined using light microscopy.

4 Conclusions. Microorganisms are easily dispersed through air currents and are commonly present in the aeromicroflora of storage areas within historical monuments. When the relative humidity in the environment reaches 65% or higher, the temperature is 20°C or above, the moisture content of paper is 8–10%, and the bound water activity (aw) exceeds 0.65, microorganisms can utilize these factors to grow (Figure 1). The relative humidity required for fungal growth is lower than that for bacterial growth. Therefore, the environmental conditions in historical monuments are generally more favorable for the proliferation of fungi than for bacterial growth.

Research in this field over many years has explored various methods for controlling microorganisms in museums and historical sites. The use of insecticides and fumigants is limited due to health risks, life-threatening hazards, high cost, potential chemical reactions with artifacts producing toxic by-products, and environmental harm. As an economically viable and environmentally safer alternative, temperature-based treatments, along with chemical and ecological approaches, are commonly applied for the preservation of cultural heritage objects.

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