Black Sea Journal of Engineering and Science
doi: 10.34248/bsengineering.1882575

Open Access Journal
e-ISSN: 2619 – 8991

Research Article
Volume 9 - Issue 2: 962-970 / March 2026

METAGENOMIC IDENTIFICATION OF BIODETERIORATIVE MICROORGANISMS ON THE STONE SURFACE OF THE ŞEYH EDEBALI TOMB

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Abstract: Biodegradation is a process that occurs in historical structures due to the release of enzymes, organic acids, and other chemical compounds by microorganisms, and it poses a significant threat to the integrity of cultural heritage. In this study, metagenomic analyses were used to determine the microbial diversity present on the walls of the Şeyh Edebali Tomb and to reveal the biodegradation processes occurring on the stone surface. The microbial taxa on the stone surface were identified, and the dominant microbial groups associated with biodegradation were determined. Our results showed that there is a high microbial diversity on the tomb's stone surface, which allowed us to uncover the metabolic impacts of these groups in the biodegradation processes. Particularly, the microbial communities abundant on the stone surface have been shown to possess biofilm formation potential, the ability to adapt to different environmental conditions, and biodegradation properties. The families *Pseudomonadaceae* (59.53%), *Xanthomonadaceae* (22.36%), *Comamonadaceae* (8.75%), and *Sphingomonadaceae* (5.41%) were identified as the most dominant families. The genera *Pseudomonas* (84.74%), *Sphingomonas* (7.28%), and *Stutzerimonas* (1.27%) were the most abundant. It is predicted that the species within these taxonomic groups contribute to biodegradation processes through different metabolic activities. Identifying the microbial diversity on the stone surface will guide the preservation of historical stone architecture and the transfer of these architectural works to future generations.

Keywords: Bilecik Şeyh Edebali tomb, Biodeterioration, Metagenomic sequencing analysis, Microbial taxonomy

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Received: February 05, 2026
Accepted: March 12, 2026
Published: March 15, 2026

Cite as: Özdemir, F & Tüzer, M. E. (2026). Identification of microorganisms causing biodeterioration on the stone surface of the Şeyh Edebali Tomb by metagenomic sequencing analysis. *Black Sea Journal of Engineering and Science*, *9*(2), 962-970.

1. Introduction

The city of Bilecik, which has a small surface area, is known for its rich history, with many civilizations having previously inhabited the area. While the city's remains date back to the Early Bronze Age, its primary historical significance comes from being the land where the Ottoman Empire was founded. One of the important historical structures from the founding period of the Ottoman Empire is the Şeyh Edebali Tomb, dedicated to Şeyh Edebali, the spiritual leader of Osman Bey, the founder of the Ottoman Empire. The Şeyh Edebali Tomb is located at the top of a hill, slightly higher than the Orhan Gazi Mosque, in proximity to it. The tomb was built during the reign of Orhan Gazi and was renovated during the period of Sultan Abdulhamid II. This structure, of great historical importance, is believed to have used various natural stone materials in its architecture. Over time, the exterior of this well-preserved structure has undergone erosion due to environmental effects and biological impacts from living organisms.

The surfaces of stone materials undergo wear and degradation as a result of the effects of microorganisms (Scheerer et al., 2009). Biological weathering is the process of deterioration and erosion caused by the metabolic activities of microorganisms (enzymes, organic acids, or other chemical compounds) on building materials (Gaylarde et al., 2003; Dakal and Cameotra, 2012; Nuhoğlu et al., 2017; Ding et al., 2020). Organic acids can dissolve the mineral components of stone materials and impair their structural integrity (Gu and Katayama, 2021). Furthermore, the metabolic products of microorganisms contribute to the progression of biological weathering by exerting a harmful effect on building materials (Özdemir et al., 2020). Microgeochemical events regulate nitrogen, iron, and sulfur metabolism on the stone surface, affecting the biodestruction process on the stone (Liu et al., 2022; Li et al., 2025). The formation of bacterial biofilms also accelerates the wear processes on the stone surface (Yadav and Purchase, 2025). The presence of biological weathering in historical structures alters their external appearance, blurs the details, and seriously impacts the durability and long-term preservation of the structures. Recently, DNA sequencing-based methods have increasingly been used to identify the microbial composition in historical buildings and to obtain information about the active members of biological degradation processes. This is due to the lack of a method in isolation techniques that reflects the entire microflora and the inability to isolate and identify groups that cannot be cultured. Metagenomic approaches, as a method that enables direct analysis of the genetic material of environmental microorganisms and determination of microbial diversity in the analyzed environment, have become a molecular technique used to identify the microbial composition in historical sites (Li et al., 2025). This method is used not only for historical structures but also for determining the microbial composition of soil (Kocak et al., 2023), water (Pat et al., 2024), and various surfaces (Bao et al., 2023). Through metagenomic analyses of the surfaces of historical illicit marble statues, the storage locations and the geographical transfer process of these artifacts have been identified (Piñar et al., 2019).

Li et al. (2025)conducted metagenomic analyses on the doors and walls of the Ji family's residential buildings, finding that microbial diversity was higher in the damaged areas. A study of the stone surfaces of the Baekje Royal Tombs also used metagenomic analysis, noting that microbial diversity varied in samples taken over different years (Lee and Chung, 2022). In the case of the Dingtao M2 tomb (Huangchangticou tomb), changes in the surfaces affected by microbial degradation were identified through metagenomic sequencing analysis (Wang et al., 2024). Additionally, data on different enzyme activities that could be active in the degradation processes were also obtained. Metagenomic analyses have been used to determine microbial metabolism and biodegradation processes, and to assist in the restoration and preservation of cultural heritage (Wu et al., 2023).

In this study, the aim is to obtain a comprehensive understanding of the total microbiota present on the tombstone, its diversity, and the effects of microbial communities on biological weathering processes through metagenomic analyses. The goal is to identify different microorganism species present on the tombstone surface and their relative abundance through metagenomic sequencing analyses. By identifying the bacteria responsible for wear, the study aims to determine the biodegradation processes of the tombstones.

2. Materials and Methods

2.1. Sample collection

Sterile swab samples were collected from the stone surface outside the Şeyh Edebali Tomb and brought in sterile containers to the Bilecik Şeyh Edebali University Molecular Biology and Genetics Research Laboratory. These samples were used for complete DNA isolation and metagenomic analyses.

2.2. Genomic DNA isolation

Genomic DNA isolation was performed using the "Quick-DNA™ Fecal/Soil Microbe Miniprep Kit, Cat. No.: D6010" from Zymo Research. The quantity and purity of the isolated DNA were determined fluorometrically with Qubit. DNA was isolated from samples taken from the same sampling point and the DNA samples were combined and then sequencing was performed. The V3-V4 regions of the 16S rRNA gene to be used in species identification were amplified using the SimpliAmp Thermal Cycler with 341F-805R primer sequences. PCR reaction mixture ratios were prepared to a total of 25 µL for each sample (Table 1). PCR reaction conditions are given in Table 2.

Library creation and sequencing were performed as a service procurement from Ficus Biotechnology (Ankara-Türkiye). The steps involved in these steps are outlined below.

Table 1. PCR reaction components and quantities for amplification of the V3-V4 vector 16S rRNA gene region used in metagenome analyses

PCR components	Amount (µL)
10x buffer	2.5
$MgCl_2$ (25 mM)	0.65
dNTP (10 mM)	0.5
Forward primer	0.5
Reverse primer	0.5
Taq polymerase enzyme	0.4
dH_20	18.35
DNA	1

Table 2. PCR reaction conditions for amplification of the V3-V4 vector 16S rRNA gene region to be used in metagenome analyses

Condition	Temperature (°C)	Time (sec/min)	Cycles
Pre-Denaturation	95	10	1
Denaturation	95	45	35
Annealing	50-55	45	35
Extension	72	1	35
Final extension	72	3	1

2.3. Library preparation and sequencing process

Library preparation for 16S rRNA V3-V4 amplicon products was performed using Illumina's "Nextera XT DNA Library Prep Kit, Cat. No.: FC-131-1096" and indexing was performed using "TG Nextera XT Index Kit v2 Set A (96 Indices, 384 Samples), Cat. No.: TG-131-2001." PCR purification was performed using Beckman Coulter's "AMPure XP beads." Sequencing was performed using Illumina's Miseq platform as paired-end (PE) reads of 2x150 bases. A minimum of ≥ 30,000 reads per sample was obtained.

2.4. Metagenomic Data Analysis

FastQ files obtained from Illumina Miseq100 were acquired as demultiplexed raw reads for each sample. Illumina Miseq100 reads were analyzed with FastaQC software (Andrews et al., 2010) to assess sequence quality, and primer sequence trimming was performed in Trimmomatic v0.32 (Bolger et al., 2014). Demultiplexed and raw reads were filtered out of low-quality data using CLC Genomics Workbench (Qiagen, USA). The Kraken application assigns taxonomic labels to short DNA sequences with high precision and speed (Wood and Salzberg, 2014). For reporting, OTU classes were converted separately to txt files. Statistical analysis of the Shannon and Simpson indices was used to compare samples. To statistically assess the diversity detected at the species level, Shannon and Simpson indices were calculated for all bacterial species (Table 3). These values reflect the species diversity within a population (Feng et al., 2017).

3. Results and Discussion

A sterile swab was used to collect samples from the stone surface of the Şeyh Edebali Tomb. The sampling was done from a high point, specifically out of reach of people. This minimized human influence on the microbiota forming on the stone surface and ensured an area that reflected its natural structure. A visual representation of the sampled area and its surface features is provided in Figure 1.

Figure 1. Sample stone surface of the Şeyh Edebali Tomb

In this study, metagenomic analyses of combined samples taken from the stone surface of the Şeyh Edebali Tomb in Bilecik were performed using the 16S rRNA V3-V4 gene region. Following the sequencing process, the Shannon and Simpson indices of the sample were calculated. These values were used to statistically assess diversity at the species level. The Shannon Diversity Index was used to evaluate the within-sample (alpha) diversity of the metagenome. This index provides insight into both community richness and evenness. In this sample, the Shannon index was calculated as 4.41, indicating a highly diverse microbiota on the stone surface of the tomb. The Simpson index, determined to be 0.977 in the metagenomic analysis, also reflects high species richness and an even distribution. Since these values represent species diversity and distribution homogeneity within a population, it can be concluded that the bacterial density on the surface of the tomb is potentially high and diverse. In metagenomic analyses conducted on the surfaces of various historical monuments, high values in these indices have also been reported to indicate a dense and diverse microbiota in those areas (Xing et al., 2023; Li et al., 2025). In the metagenomic analyses, *Pseudomonadaceae* (59.53%) and *Xanthomonadaceae* (22.36%) were identified as the dominant families on the tomb's stone surface, while *Comamonadaceae* (8.75%) and *Sphingomonadaceae* (5.41%) were also found in relatively high abundance. The abundance of the families *Enterobacteriaceae*, *Rubrobacteraceae*, *Alteromonadaceae*, *Vibrionaceae*, *Burkholderiaceae*, and *Propionibacteriaceae* was found to be below 1% (Figure 2, Supplementary Tables).

Table 3. Shannon and Simpson indices of samples taken from the tomb: Diversity at the species level

	Diversity at the species level	
Sample	Shannon Index (H) / (H / LN (N))*	Simpsons Index (D-1)*
Stone surface	4.41 / 0.8511	0.977

In the study conducted at the Alcobaça Monastery, *Bacillaceae-1* was reported as the most dominant family (30.51%), with *Sphingomonadaceae* (2.20%) and *Rubrobacteraceae* (1.23%) also present in the environment (Silva et al., 2024). Similarly, in the study by Xing et al. (2023), the *Proteobacteria* group was found to be the most abundant across all samples. In the study conducted at the Qinling Tomb, the phyla *Cyanobacteria*, *Proteobacteria*, and *Actinobacteria* were found to be highly dominant, and these taxonomic groups were reported to play significant roles in the microbial communities forming on the stone surface. The same study also indicated that ecological factors, particularly temperature, influence the bacterial communities on the tomb's stone surface (Xing et al., 2023). Similarly, in our study, *Proteobacteria* and *Actinobacteria* were also found to be dominant groups.

As a result of the metagenomic analyses, the bacterial population at the genus level was identified, in descending order of abundance, as consisting of the genera *Pseudomonas*, *Sphingomonas*, *Stutzerimonas*, *Stenotrophomonas*, *Rubrobacter*, *Klebsiella*, *Escherichia*, *Vibrio*, *Hydrocarboniclastica*, and *Lysobacter* (Figure 3). Among the stone surface samples, the genus *Pseudomonas* was found to be the most dominant, with a relative abundance of 84.74%. The next most abundant genus was *Sphingomonas*, with a proportion of 7.28%. *Stutzerimonas* also appeared as a significantly represented genus, with a relative abundance of 1.27%. The remaining genera were each found to be present at less than 1%.

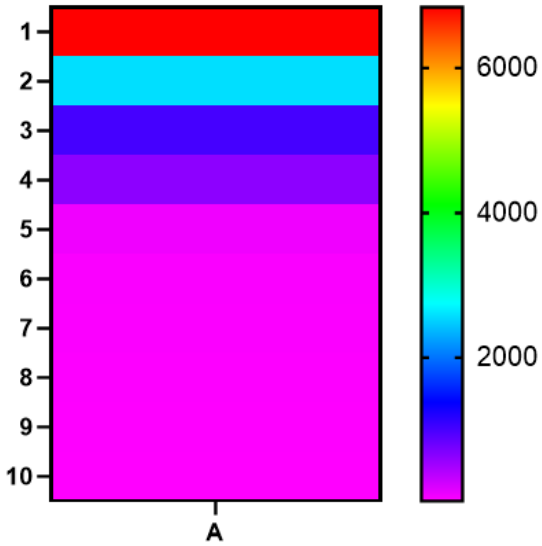

Figure 2. Percentage distribution graph of the 10 highest bacterial families of the stone surface 1. *Pseudomonadaceae*, 2. *Xanthomonadaceae*, 3. *Comamonadaceae*, 4. *Sphingomonadaceae*, 5. *Enterobacteriaceae*, 6. *Rubrobacteraceae*, 7. *Alteromonadaceae*, 8. *Vibrionaceae*, 9. *Burkholderiaceae*, 10. *Propionibacteriaceae.*

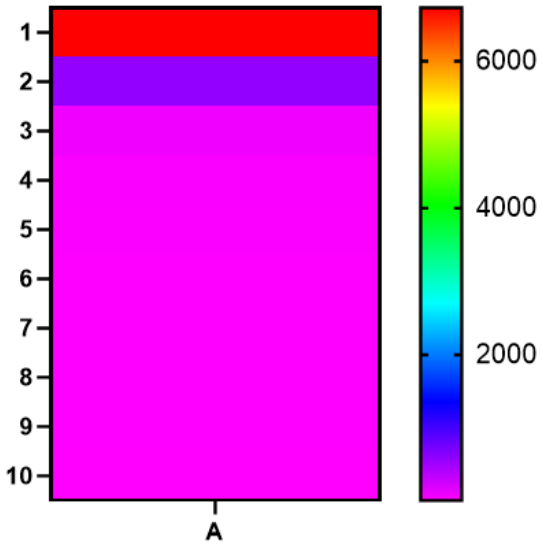

Figure 3. Percentage distribution graph of the 10 highest bacterial genera of the stone surface. 1. *Pseudomonas*, 2. *Sphingomonas*, 3. *Stutzerimonas*, 4. *Stenotrophomonas*, 5. *Rubrobacter*, 6. *Klebsiella*, 7. *Escherichia*, 8. *Vibrio*, 9. *Hydrocarboniclastica*, 10. *Lysobacter*.

Metagenomic sequencing analyses revealed that the genus *Pseudomonas* was the most dominant genus on the stone surface (84.74%). Within this genus, prominent species such as *Pseudomonas aeruginosa*, *Pseudomonas putida*, and *Pseudomonas fluorescens* are known to play a role in the biodegradation of various organic and inorganic compounds. In particular, *Pseudomonas aeruginosa* is known to be effective in breaking down industrial pollutants such as petroleum hydrocarbons. In an isolation study investigating the biodegradability of microorganisms isolated from petroleum-contaminated water and soil samples, it was found that 3 out of 46 isolates had high degradation capabilities. Based on tests conducted at different concentrations, isolate number 13, which showed the best growth, was selected and demonstrated a 73% biodegradation rate at a pyrene concentration of 80 ppm. According to 16S rRNA sequence analysis, this isolate was identified as *Pseudomonas hydrolytica* (Ulusoy and İşçen, 2023). The ability of *Pseudomonas* species to degrade aromatic compounds is attributed to their extensive enzymatic systems. These species convert complex organic compounds into simpler and less toxic substances, particularly through the action of oxygenase enzymes. *Pseudomonas* is also notable for its rapid adaptability to environmental conditions, making it an ideal microorganism for the effective degradation of various pollutants (Nogales et al., 2019). Additionally, the biofilm-forming abilities of these microorganisms provide a protective environment for microbial communities. This facilitates the accumulation and concentration of corrosive substances in the environment, thereby increasing the rate of biodeterioration on the stone surface (Li et al., 2025). In the study conducted by Sakr et al. (2018), *Pseudomonas aeruginosa* was identified as one of the dominant groups on the yellow granite stone surface of King Psusennes I's tomb. It was reported that due to the acidolytic activities and biofilm formation of this microorganism, the pink color of the granite was altered to the gray color of clay minerals.

Based on the findings obtained from this study and supported by other studies in the literature, it can be concluded that the genus *Pseudomonas* possesses significant potential for biodegradation due to its various metabolic activities. The strains of this genus are found in high abundance on the tomb’s stone surface, likely due to their ability to survive under a wide range of environmental conditions. Additionally, the potential biodegradation capabilities of other genera, even when associated with symbiotic interactions, could also contribute to the deterioration of the tomb’s outer surface. Genera such as *Sphingomonas* and *Stenotrophomonas* are known to have biodegradation potential. Several species belonging to these genera have been reported in the literature to possess the ability to break down various industrial pollutants (Zhang et al., 2022). In their study, Li et al. (2025) identified varying abundances of the bacterial genera *Pseudomonas*, *Serratia*, *Sphingobium*, and *Sphingomonas* on historic buildings and reported that these genera play an active role in the deterioration processes of such structures (Li et al., 2025).

The genus *Sphingomonas* was identified as the second most abundant bacterial group as a result of metagenomic analyses. Members of the *Sphingomonas* genus possess metabolic capacities that provide advantages for survival and growth under nutrient-limited conditions and stress factors such as UV radiation, temperature, and dryness. In particular, certain *Sphingomonas* species are capable of degrading resistant aromatic compounds such as petroleum derivatives and pesticides (Gambino et al., 2021). These bacteria possess enzymatic systems that play a critical role in the degradation of aromatic compounds (Fenibo et al., 2019). *Sphingomonas* species have been reported to possess a high capacity for degrading polycyclic aromatic hydrocarbons (PAHs). These microorganisms are capable of metabolically converting PAHs into less toxic compounds. Studies have shown that the enzymatic systems of *Sphingomonas*, particularly oxygenases such as dioxygenases and monooxygenases, can effectively break down PAH molecules. Moreover, the ability of these bacteria to adapt to various environmental conditions and remain active across a wide pH range further enhances their biodegradation potential (Zhang et al., 2022). In addition, their ability to form biofilms allows them to colonize environmental surfaces effectively and sustain long-term biodegradation (Bala et al., 2022). In the study conducted at the Qinling Tomb, it was reported that exogenous pollutants, including PAHs and dioxins, were involved in biofilm formation and biodeterioration processes, and that this condition also influenced microbial diversity (Xing et al., 2023).

Members of the genus *Stenotrophomonas,* one of the most dominant genera on the tomb's stone surface, possess various capabilities such as the degradation of numerous xenobiotic compounds, involvement in nitrogen and sulfur cycles, and the ability to form biofilms (Ryan et al., 2009). These characteristics make them one of the predominant inhabitants of the stone surface.

The first member of the genus *Rubrobacter* was isolated from a radioactive hot spring, and subsequent species have typically been identified from high-temperature natural environments such as thermal springs, volcanic areas, and geothermal sources. Although thermophilic, halotolerant, and radiotolerant species have been described, their primary habitat is soil. In recent years, new species have also been identified from biologically deteriorated monuments. In one such study, three different *Rubrobacter* species were identified from samples taken specifically from biofilm-covered areas, and *Rubrobacter bracarensis* was reported as a novel species (Jurado et al., 2012). Similarly, in the degraded areas of the Bayon Temple in Angkor Thom, PCR–denaturing gradient gel electrophoresis (DGGE) analysis of biofilm samples revealed that the salmon-pink biofilm zone was predominantly composed of the genus *Rubrobacter* (Kusumi et al., 2013). *Rubrobacter* isolates were obtained from samples collected from biologically deteriorated areas of three different monuments, and these isolates were found to be involved in certain biodeterioration processes (Laiz et al., 2009). These findings indicate that *Rubrobacter* species, thanks to their biotolerance capabilities, can be found not only in thermal environments but also in nutrient-poor habitats such as stone surfaces. In our study, we also identified different *Rubrobacter* species present on the stone surface.

It is believed that symbiotic relationships among bacteria on stone surfaces contribute to the high density of bacterial populations. This is because stone surfaces are generally poor environments in terms of organic matter and the necessary conditions for microbial life. For example, *Pseudomonas* species such as *Pseudomonas fluorescens* and *P. aeruginosa* produce pyoverdine, a key compound in iron metabolism, which not only enables the uptake of iron—an essential element for microorganisms—from the environment but also contributes to biofilm formation (Bayram, 2022). In addition, members of the genus *Klebsiella* have the ability to perform biological nitrogen fixation (Bennett et al., 2023). Therefore, they may help meet the nitrogen requirements of other microorganisms in the environment. Moreover, the microbial groups identified in this environment appear to possess various metabolic pathways and enzymes. For instance, *Stenotrophomonas* species can obtain energy by utilizing inorganic compounds such as nitrate, nitrite, and sulfate—just one example of this metabolic diversity. The products resulting from these metabolic pathways have a high potential to serve as substrates for other microorganisms.

At the species level, metagenomic analyses revealed that *Stutzerimonas stutzeri* (9.31%), *Candidatus Pseudomonas adelgestsugas* (4.52%), *Pseudomonas veronii* (3.87%), *Pseudomonas aeruginosa* (3.78%), *Pseudomonas putida* (3.78%), *Pseudomonas amygdali* (2.49%), *Pseudomonas fluorescens* (2.3%), *Rubrobacter marinus* (2.3%), *Escherichia coli* (2.12%), and *Pseudomonas* sp. $NC0_2$ (2.12%) were the most abundant species detected (Figures 4 and 5).

The most dominant species on the tomb's stone surface was identified as *Stutzerimonas stutzeri* through metagenomic analyses. In a study investigating the effects of *Stutzerimonas stutzeri* on tomato growth and N_2O emissions, it was found that tomato growth increased while N_2O emissions decreased. The study reported that *Stutzerimonas stutzeri* strains reduced N_2O emissions by altering the structure of the soil microbial community and the abundance of genes related to the nitrogen cycle (Gao et al., 2024).

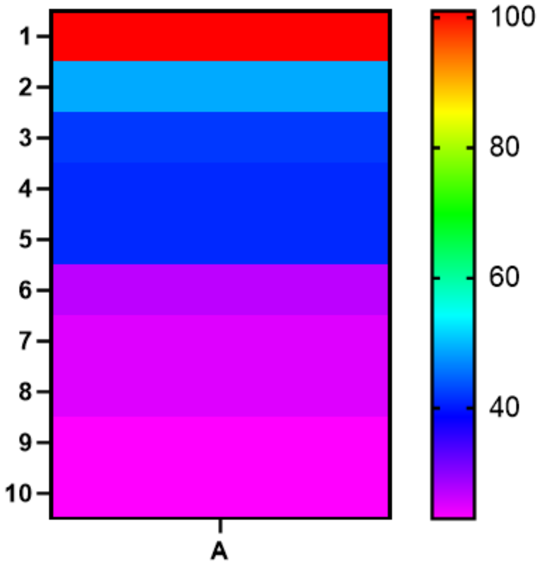

Figure 4. Percentage distribution graph of the 10 highest bacterial species of the stone surface. 1. *Stutzerimonas stutzeri*, 2. *Candidatus Pseudomonas adelgestsugas*, 3. *Pseudomonas veronii*, 4. *Pseudomonas putida*, 5. *Pseudomonas aeruginosa*, 6. *Pseudomonas amygdali*, 7. *Rubrobacter marinus*, 8. *Pseudomonas fluorescens*, 9. *Pseudomonas umsongensis*, 10. *Escherichia coli*.

Ca. Pseudomonas adelgestsugas was identified as the second most abundant species on the tomb's stone surface. According to the literature, it has a symbiotic relationship specifically with insects (Weglarz, 2019). Its high abundance on the stone surface suggests that it may have been transported by insects and subsequently colonized the surface.

As a result of metagenomic analyses, the presence of bacterial taxa with diverse characteristics on the stone surface was also identified. Notably, the coexistence of *Pseudomonas amygdali* and *Rubrobacter marinus* in the same environment is quite intriguing. This is because *Pseudomonas amygdali* is a plant pathogen, while *Rubrobacter marinus* is a species isolated from deep-sea sediment (Chen et al., 2020; Todai et al., 2022).

Based on the results obtained, it was found that although species belonging to the genus *Pseudomonas* were predominant, *Stutzerimonas stutzeri* had the highest relative abundance at 9.31% when evaluated at the species level. These findings indicate the presence of a wide range of taxonomic groups on the stone surface.

Biofilm formation not only provides a habitat for bacterial communities but also plays a significant role in stone surface deterioration by enabling the accumulation and retention of substances involved in biodeterioration (Liu et al., 2022; Yadav and Purchase, 2025). Microbial metabolic activities contribute to the production of various acids, which are actively involved in the deterioration of stone surfaces (Yadav and Purchase, 2025). Bacteria that participate in the carbon, nitrogen, and sulfur cycles are among the most critical agents in biochemical processes that produce acidic or corrosive byproducts. These byproducts can erode stone surfaces and dissolve minerals (Gu and Katayama, 2021). Members of the *Comamonadaceae* family include Fe^{3+} reducing, hydrogen oxidizing, fermenting, and anaerobic denitrifying bacteria. They also include aerobic organotrophic, photoautotrophic, and photoheterotrophic bacteria (Willems, 2014). A potassium ion uptake system identified in the *Xanthomonadaceae* family is thought to play an important role in environmental adaptation (Zhu et al., 2022). The family *Sphingomonadaceae* consists of chemoorganotrophic and facultatively photoheterotrophic bacteria. Some species can degrade xenobiotic and recalcitrant (poly)aromatic compounds. (Glaeser and Kämpfer, 2014). *Stenotrophomonas* species can obtain energy by utilizing inorganic compounds such as nitrate, nitrite, and sulfate. Their potential to form biofilms and high adaptability to their environment have enabled them to thrive in various habitats (Ryan et al., 2009; García et al., 2022). When examining the dominant families and genera present on the stone surface, it is evident that they possess adaptations to extreme environments, the ability to form biofilms, the ability to degrade various aromatic compounds, and metabolic activity in iron, nitrate, and carbon cycles. These characteristics have been highlighted in various studies as being highly significant in the formation of stone microbiota and in potential biodeterioration processes occurring on the stone surface (Liu et al., 2022; Li et al., 2025).

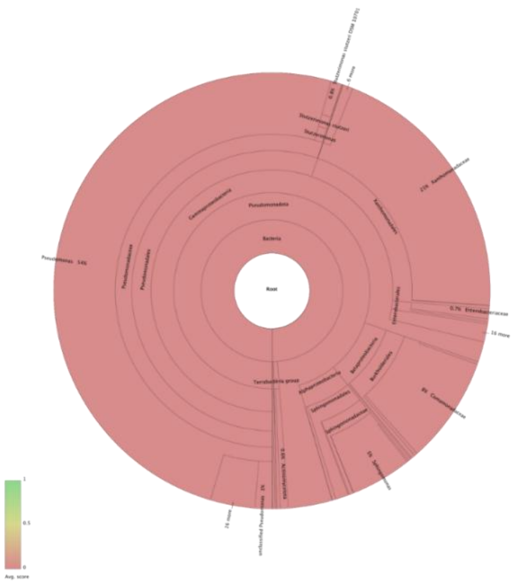

Figure 5. Circular corona graph of species distribution.

Mapping the microbial communities present on stone surfaces is crucial for understanding their roles in various biogeochemical reactions and their involvement in nutrient cycles such as nitrogen, carbon, and iron. The use of advanced omics technologies—such as metagenomics, proteomics, and metabolomics—along with modern analytical methods, will provide deeper insights into the structure of microbial communities and the metabolic processes of these microorganisms. This knowledge is also of great importance for preventing the biodeterioration of historical monuments and structures made from different types of stone. In this way, cultural heritage can be preserved and passed on to future generations for centuries without deterioration.

4. Conclusion

The high microbial diversity observed on the surface of the Şeyh Edebali Tomb also indicates intense metabolic activity occurring on the stone. This suggests that the rate of stone surface deterioration may continue to increase over time. This study aimed to investigate the relationships between bacteria and the biodegradation processes they may induce on stone surfaces, focusing on their abilities to form biofilms—which are critical for bacterial colonization—adapt to extreme environments, carry out metabolic activities, produce organic acids, possess various degradation capabilities, and participate in the carbon, nitrogen, and sulfur cycles. However, in this study, sampling and analysis were limited to only one section of the tomb. To gain a more comprehensive understanding of the microbial diversity of the Şeyh Edebali Tomb from different perspectives, further studies employing advanced techniques such as metagenomic and proteomic analyses are needed. New analytical studies to be conducted on the tomb would provide valuable data, allowing for more detailed insights into the structure and restoration needs of the tomb's outer layers. Moreover, isolation studies could lead to the identification of new species, offering opportunities for the discovery of novel microorganisms. This study represents the first investigation aimed at identifying microorganisms on the outer surface of the Şeyh Edebali Tomb in Bilecik and assessing their potential role in biodeterioration. It also lays the groundwork for and supports future research in this area.

Author Contributions

The percentages of the authors' contributions are presented below. All authors reviewed and approved the final version of the manuscript.

	F.Ö.	M.E.T
C	70	30
D	70	30
S	80	20
DCP	70	30
DAI	90	10
L	70	30
W	100	0
CR	100	0
SR	100	0
PM	60	40
FA	40	60

C= concept, D= design, S= supervision, DCP= data collection and/or processing, DAI= data analysis and/or interpretation, L= literature search, W= writing, CR= critical review, SR= submission and revision, PM= project management, FA= funding acquisition.

Conflict of Interest

No conflict of interest or common interest has been declared by the authors.

Ethical Consideration

Ethics committee approval was not required for this study because there was no study on animals or humans.

Acknowledgements

This study is supported by the Scientific and Technological Research Council of Türkiye (TUBITAK) with project number 2209-A. The authors would like to thank Öznur BİLGİN for her assistance. Permission was obtained from the Ministry of Culture and Tourism, General Directorate of Foundations, Bursa Regional Directorate of Foundations.

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