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СТАРИНАР

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фото: Петар Милојевић; цртеж: Александар Капуран

Sur la couverture : Localité Velika humska chuka, un collier provenant d'un trésor découvert lors de fouilles en 2022, 15–14. avant JC (Musée national, Niš)
Photo : Petar Milojević ; Dessin : Aleksandar Kapuran

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PRESERVING THE DANUBE LIMES IN SERBIA: A REVIEW OF THE BIODETERIORATION OF TRAJAN'S BRIDGE*

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Abstract. – One of the most significant monuments of the Roman Danube Limes is Trajan's Bridge, built in the period from 103 to 105 AD. The remains of the pillars on the Serbian bank of the Danube were partially restored four decades ago. Today, the pillar that is closest to the river, which has not undergone conservation, is subject to different types of deterioration. During the comparison between the present condition of the pillar and those recorded in photos over the last six decades, we can estimate that its level of material loss has not overly changed. However, the difference in biological growth is visible. The primary aim of this study was to record the degree of infestation and endangerment of the monument and check for possible risks of mortar deterioration where mortar is in direct contact with severely infested bricks. Moreover, a characterisation of the biodeteriogens was performed. Special emphasis was given to fungi as the main agents of deterioration. A precise assessment of the level of risk they pose to this monument was made as a basis for the formulation and implementation of appropriate conservation treatments. Severe macrofouling by epilithic lichenised fungi and mosses was documented. Even areas where pillars lack visible infestation, thriving microbial communities characterized by the presence of various fungal structures, as well as structures of trichal *Cyanobacteria,* and *Chlorophyta* were recorded. Similarities between communities documented on brick, mortar, and stone surfaces, estimated via Sørensen's quotient of similarity, were high, with the highest similarity documented between mortar and stone.

Key words. – biodeterioration, Trajan's Bridge, lichens, Danube Limes in Serbia, historical mortars, historical brick, historical stone, Roman monument

In the last few years, several projects have been initiated in the Republic of Serbia with the aim of researching and documenting the condition of building remains of the former Roman Danube border, originating from the period from the 1st to the 6th century, drawing up conservation and management plans necessary to preserve all their values, to include them in the life of local communities and to make them accessible to visitors. The main project is connected to the creation of the nomination dossier named *Frontiers of the Roman Empire – The Danube Limes (Serbia),* conducted by the Institute of Archaeology and Institute for the Protection of Cultural Monuments of Serbia – Belgrade, with the aim of including the Limes

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Fig. 1. View from the northwest to the remains of Trajan's Bridge on the Serbian bank of the Danube in July 2021 (Archive of the Institute of Archaeology, Belgrade)

Сл. 1. Поглед са северозапада на остатке Трајановог моста на српској обали Дунава у јулу 2021. године (Архива Археолошког института, Београд)

on the UNESCO World Heritage List.[1] The scientific project *Mortar Design for Conservation – Danube Roman Frontier 2000 Years After (MoDeCo2000)*[2] examines the lime mortars of these historic buildings and tries to offer recommendations for the production of conservation mortars while respecting the principles of compatibility and preservation of the environment, using local raw materials and traditional technologies. The latest inclusion is the project *Promising Natural Alternatives for the Cultural Heritage Safeguard: A Force of Natur*e *(PROTECTA),* aimed at investigating causes of the decay of cultural heritage objects and buildings in Serbia, and the development of non-invasive, eco-friendly, and safe biocontrol methods to combat deteriogenic microorganisms responsible for the damage, in order to achieve sustainable restoration and conservation.

Among the most significant monuments of the Roman Danube Limes, which today stretches through several European countries, is Trajan's Bridge, the remains of which can be found today on both sides of the river: in present-day Serbia (the village of Kostol) (Fig. 1), and Romania (the town of Drobeta–Turnu Severin) (44°37'8.3"N 22°40'3.1"E). This once monumental structure consisting of masonry pillars on the banks and in the river bed that, together, supported the wooden construction had a total length of 1,130 m.[3] It was designed by Apollodorus of Damascus and built in the period from 103 to 105 AD in order to enable the crossing of the Danube river by the Roman army under Emperor Trajan during the conquest of *Dacia.* Along with the bridge, two forts were erected for its protection – *Pontes* on the present-day Serbian side of the river and *Drobeta* in today's Romania.[4]

The masonry remains of Trajan's Bridge situated in Serbia were archaeologically investigated in 1979 and 1980,[5] after which two embankment pillars out of four were partially restored [6] (Fig. 2). Initially, three pillars were on dry land, while the environment of the

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[1] UNESCO 2022; Korać et al. 2014, 32–34.

[2] Nikolić, Jovičić 2022, 9–11.

[3] Bjelić 2020, 23; More on wooden construction, see in: Gušić 1996, 259–261; Mehrotra, Glišić 2015, 65–72; Bjelić 2020: 22–32.

[4] Гарашанин, Васић 1980, 7–8.

[5] Ibid, 7–50; Гарашанин et al. 1984, 25–84.

[6] Гушић 1985, 77.

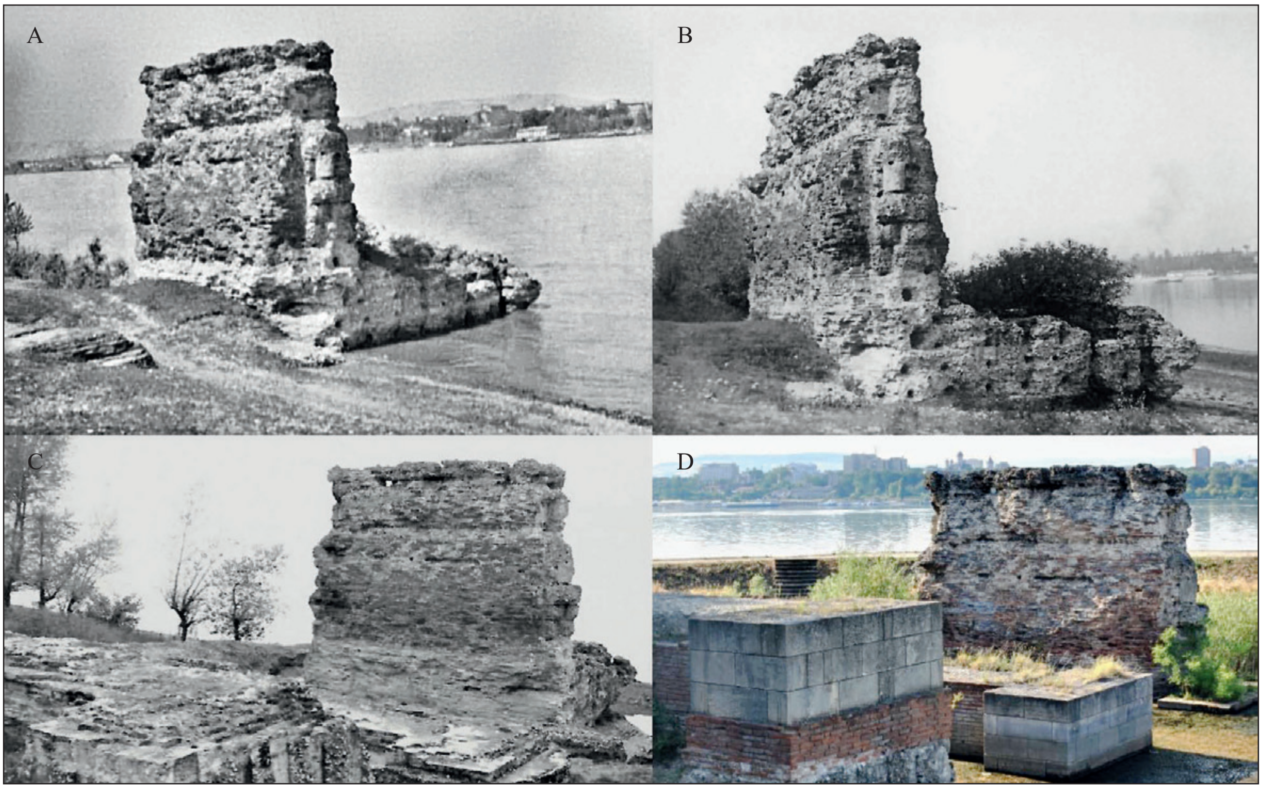

Fig. 2. Remains of Trajan's Bridge before excavations: from 1963 (A), and 1973 (B) (Archive of the Institute of Archaeology); after excavations in 1981 (C) (Archive of the Institute of Archaeology); and from 2021 (D) (Archive of the MoDeCo2000 *project)*

Сл. 2. Остаци Трајановог моста пре ископавања: из 1963. године (А) и 1973. године (В) (Архива Археолошког института, Београд); након ископавања 1981. године (С) (Архива Археолошког института, Београд); и из 2021. године (D) (Архива пројекта MoDeCo2000*)*

one closest to the river depended on the water level (Figs. 1 & 2). Construction of the Iron Gate hydropower plant system caused the rise of the Danube, which led to the building of the defensive embankment around the remains, into which water later penetrated.

Since the monument is in the area of constant water level and humidity fluctuation, and is additionally influenced by air pollution, wind, and precipitation, it was necessary to carry out the initial assessment of its present state of preservation (preparing it for future steps in the conservation plan). This has been done through the implementation of the MoDeCo2000 and PROTECTA projects, which incorporated archaeological and architectural assessments, recorded which building materials were used, implemented laboratory research of mortars, as well as reviewed the state of its conservation (report on the degree of biodeterioration).

The factors that impact building materials are: external environment, the design and choice of materials, construction practices, the properties of the materials themselves, execution of the building works, as well as subsequent maintenance.[7] Due to the influence of the environment (moisture and water, salts, air pollution, variations in temperature, exposure to fire, dynamic loading, and soil subsidence), and the properties of authentic and conservation materials and their use and maintenance in construction, there are many types of deterioration of building materials. Some of these, based on environmental factors, are frost damage, salt crystallisation, formation of different compounds and crusts, dissolution and leaching, erosion, swelling, spalling, cracking, and biological growth on masonry.[8]

The diagnostic process of collecting information and observing the monument for the purpose of its

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[7] Van Hees et al. 2004, 644–645.

[8] Ibid, 645.

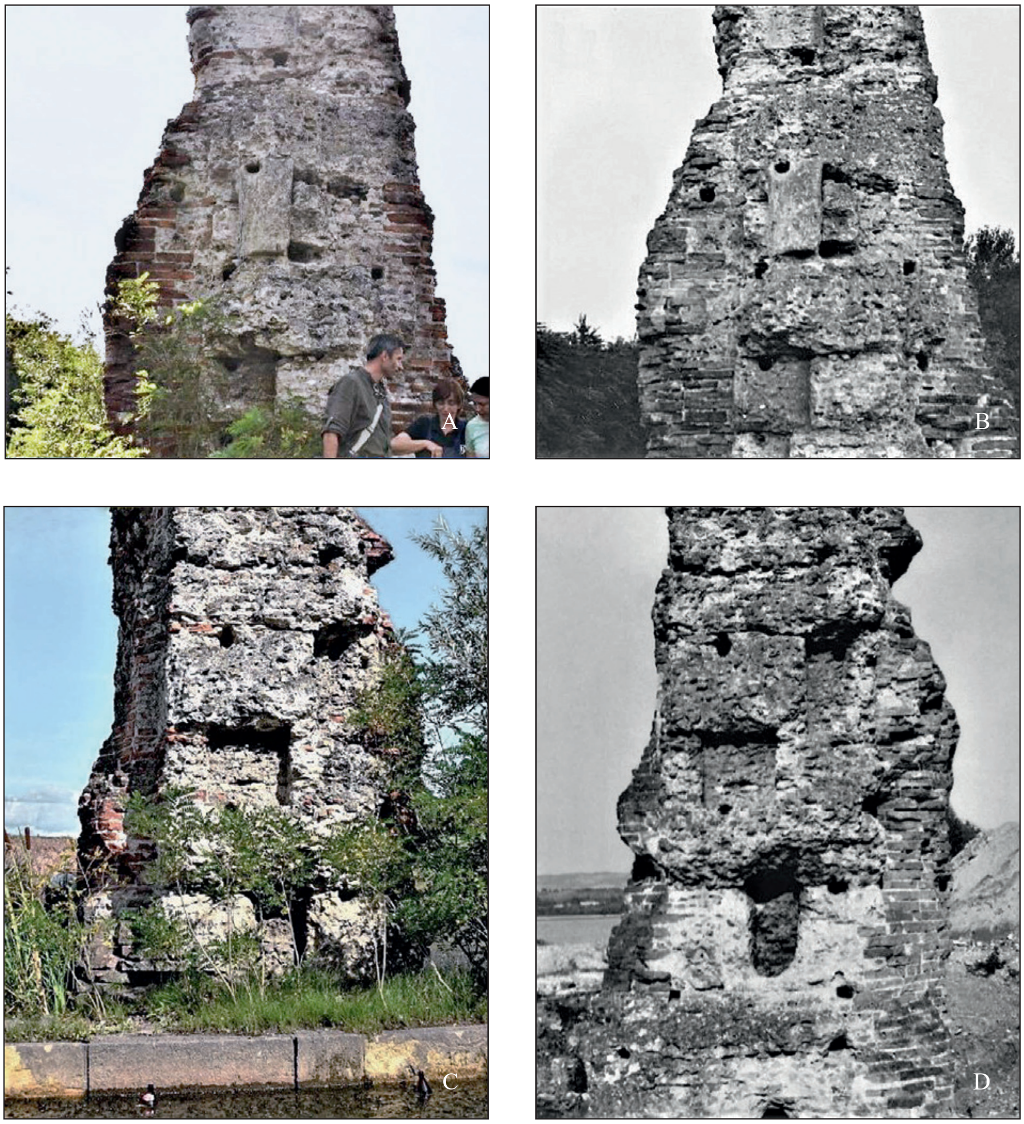

Fig. 3. Eastern side of the pillar – remains in 2020 (A), and in 1979 (B);
western side of the pillar – remains in 2020 (C), and in 1979 (D)
(Archives of the Institute of Archaeology, Belgrade – 1979 and the MoDeCo2000 *project – 2020)*

Сл. 3. Источна страна ступца – остаци у 2020. години (A) и 1979. години (B);
западна страна ступца – остаци у 2020. години (C) и 1979. години (D)
(Архива Археолошког института, Београд за 1979. годину и архива пројекта MoDeCo2000 *за 2020. годину)*

protection and conservation includes the research of the materials used for its construction and later interventions. We can recognise four components in the process of a building conservation assessment: background research (construction, materials, historical development, past interventions and uses); survey of the condition (in general and of components, in order to mark the symptoms of deterioration that can lead us to the conclusions regarding possible causes); detailed analyses (for the investigation and confirmation of deterioration causes); and prognosis with risk assessment (in order to propose the preventive or remedial treatments).[9]A significant step in this process is the determination of the degree of biodeterioration of the investigated monument.

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[9] Henry, Stewart 2011, 165.

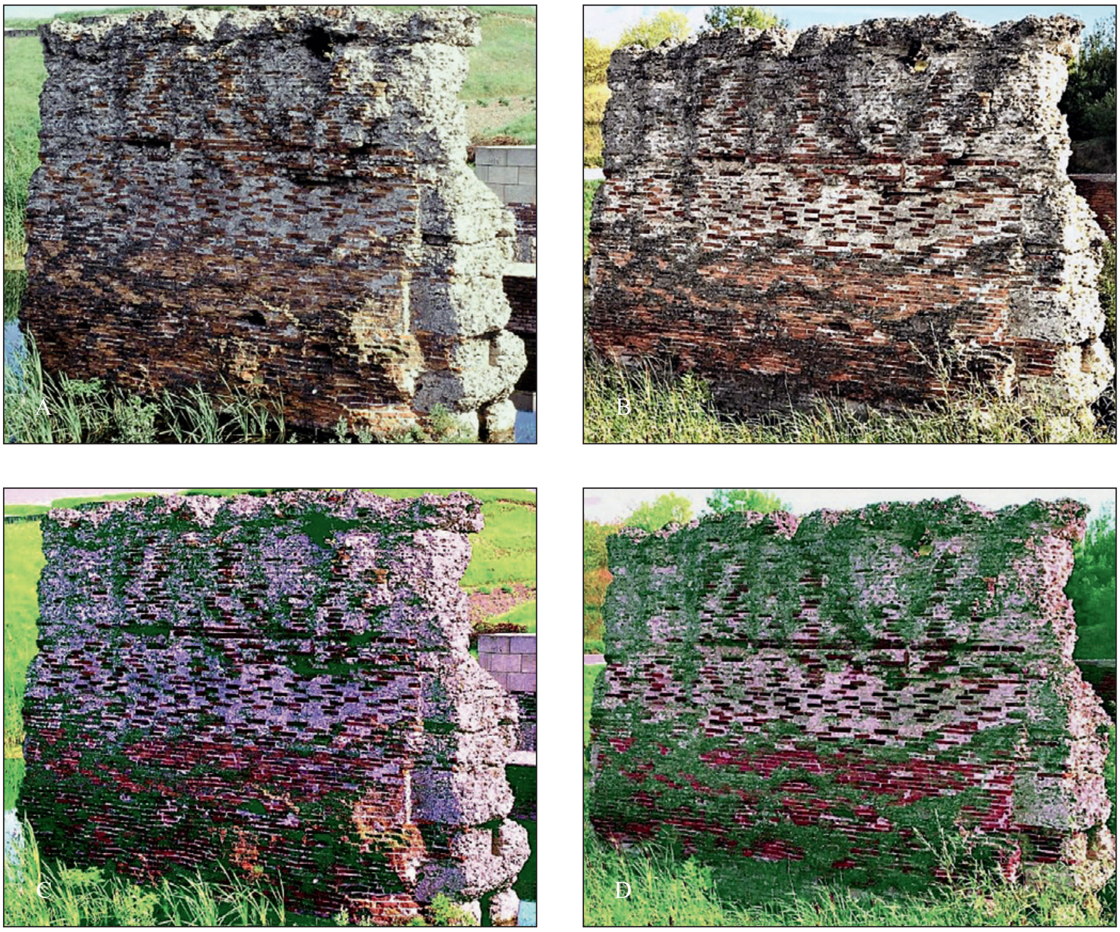

Fig. 4. Biocolonisation on the northern side of the pillar: in the period from the late 1980s to the 1990s (A) (Archive of the Institute of Archaeology), and in 2020 (B) (Archive of the MoDeCo2000 *project); from the late 1980s and 1990s (C) and from 2020 (D); images enhanced using DStretch app (RGB0) (Archive of the* MoDeCo2000 *project)*

Сл. 4. Колонизација на северној страни ступца: у периоду касних осамдесетих и током деведесетих година 20. века (А) (Архива Археолошког института, Београд) и у 2020. години (B) (Архива пројекта MoDeCo2000*); из касних осамдесетих и током деведесетих година XX века (C) и из 2020. године (D); фотографије обрађене уз употребу апликације aDStretch (RGB0) (Архива пројекта* MoDeCo2000*)*

Research aim

The masonry pillar of Trajan's Bridge on the Serbian bank of the Danube, which is closest to the river and which remained unrestored, shows signs of severe deterioration. From comparing its present condition with photographs taken from 1963 up to the rise of the Danube, but also with those taken afterwards, we estimate that the overall change in material loss was minimal (although individual deterioration of the building material itself cannot be recognised in detail due to the lower quality of black and white photos) (Fig. 3). The same conclusion applies to changes in biological colonisation and subsequent deterioration when comparing the present state with images taken in the period from 2004 onwards.[10] However, comparing these images (Fig. 4B, D)[11] with the few colour images created in

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[10] Archive of the Institute of Archaeology (photos from 1963, 1965, 1966, 1967, 1969, 1973, 1979, and 1981); archive of the *MoDeCo2000* project (photos from 2020, 2021 and 2022); archive of the Institute of Archaeology (photos from 2004, 2007, 2008, 2013, 2019 and 2021);

[11] Archive of the *MoDeCo2000* project (2020, 2021, and 2022); archive of the Institute of Archaeology (photos from 2004, 2007, 2008, 2013, 2019 and 2021); the image used (2020) was enhanced using the DStretch application (https://www.dstretch.com/).

the period closer to the that connected to the water penetration (Fig. 4A, C) [12], and by using image software enhancement, we can see a more noticeable difference in the continuous biological colonisation. The growth might have been accelerated after the water penetration, but was evidentially slowed down later again to its natural rate.

Through this study, the characterisation of the biodeteriogens was performed, focusing on lichens (lichenised fungi) as the most dominant colonisers, in order to obtain data on the actual degree of biological endangerment of the monument.

MATERIALS AND METHODS

Study site and sampling points

Trajan's Bridge had twenty masonry pillars with platforms in the river bed that supported the wooden structure, as well as the approach masonry sections on both sides of the river that each consisted of four pillars, the remains of which are still visible (28 pillars in total)[13]. These masonry parts of the bridge were built of brick, stone, and lime mortar. Underwater research established that pillars that carried the platforms with a wooden construction are in the river bed, but with varying degrees of preservation.[14] As for the remains of the approach section on the territory of Serbia, one pillar was preserved at a height of 8.38 m,[15] while the other pillars were preserved only at the foundation level. After the excavation, a partial restoration of two pillars on the bank and the platform simultaneously built with the best-preserved pillar was executed. However, the best-preserved pillar remained unprotected in this process. Since the visual observation of the monument most often does not provide sufficient data for the report on its endangerment, different steps were taken in our research in order to investigate the state of the pillar more thoroughly using laboratory research. The first one was the determination of biodeteriogens.

To identify the main agents of biodeterioration of the pillar closest to the river, *in situ* microscopy and sampling were carried out on several parts of its masonry structure. The samples were taken mainly from the northern side of the pillar, but some samples also came from its eastern and western sides. This was conditioned by the possibility of a physical approach to the infested surfaces and, thus, on the inaccessible southern side only a visual inspection of the structure was performed together with a photographic record.

In situ microscopy

Direct observation of lichen growth on brick, mortar, and stone surfaces was performed using the portable Dino-Lite Edge digital microscope AM-7915MZTL. Image processing and measurements were achieved via DinoCapture 2.0 v1.5.39.A software. Identification of the documented lichenised fungi, based on the morphology of thalli and observed reproductive structures, was performed using a dichotomous key by Smith.[16]

Lichenometry

The maximum diameter of the single largest observed lichen thalli with uniform radial growth (later identified as *Lecanora campestris*) was measured to date the minimum age at which the surface of Trajan's Bridge was exposed to lichenised fungi colonisation, per the LL method described in Bradwell 2009[17], and taking into account the documented radial growth rate of *L. campestris* in a range from 0.12 to 2.3 mm per year.[18]

Bryological analyses

Mosses growing on the surface of bricks, mortar, and stone were carefully removed with a scalpel and transported to the laboratory, under sterile conditions, to be identified using a dichotomous key by Frey *et al*.[19]

Optical microscopy

A non-aggressive adhesive tape method was applied to collect samples for optical microscopy.[20] In laboratory conditions, samples were stained with Lactophenol Cotton Blue-glycerol mixture and analysed with a Zeiss Axio Imager M1 microscope using AxioVision Release 4.6 software. Detected cyanobacteria and algae were identified on the basis of cellular mor-

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[12] Archive of the Institute of Archaeology (the photos are not dated, but they present the monument after the completed restoration works, and it is known they were made before 2004); the image used (late 1980s, or 1990s) was enhanced using the DStretch application (https://www.dstretch.com/).

[13] Гарашанин, Васић 1980, 8; Bjelić 2020, 22.

[14] Karović, Nenadović 2014, 95–97.

[15] Гарашанин, Васић 1980, 16.

[16] Smith 2009, 1–1046.

[17] Bradwell 2009, 61–69.

[18] Joshi et al. 2021, 1–16.

[19] Frey et al. 2006, 1–512.

[20] Urzì, de Leo 2001, 1–11.

phology using identification keys by John *et al.*,[21] Komárek and Anagnostidis,[22] and Ettl and Gärtner.[23]

Isolation and identification of culturable fungi

Samples for the isolation of culturable fungi were collected with sterile swabs, after which the swab tips were immersed in 2 ml microtubes (Sarstedt Inc.) with 1 ml of a 30% glycerol-Mueller Hinton Broth medium, frozen, and transported to the laboratory under sterile conditions. From each microtube, after a 1 min treatment in a vortex mixer (ZX3, VELP Scientifica), 100 µl was pipetted onto plates containing Potato Dextrose Agar (PDA) supplemented with streptomycin (500 mg L^{-1}; streptomycin sulphate salt, Sigma-Aldrich) to suppress bacterial growth. Inoculation was performed in triplicate. Inoculated plates were incubated in an oven (UE 500, Memmert) at 25±2 ºC for 7 days. After the incubation period, morphologically different colonies were reinoculated onto PDA and incubated under the same conditions to obtain axenic cultures. Isolated fungi were identified based on colony morphology and the microscopic characteristics of reproductive structures, observed with the Zeiss Axio Imager M1 optical microscope and AxioVision Release 4.6 software, using identification keys.[24]

Ecological statistics

The similarity between the communities documented on brick, mortar, and stone surfaces was evaluated using Sørensen's quotient of similarity (QS) calculated using the following formula:[25]

$$Qs= 2a / (2a + 2b+ 2c)$$

where *a* – number of taxa common to two analysed substrata; *b* – number of taxa unique to one analysed substratum; *c* – number of taxa unique to the other analysed substratum.

RESULTS

Preservation state of the investigated pillar

The most pronounced deterioration of Trajan's Bridge is represented by different processes of loss of building materials. It is mostly related to bricks, then stone, while its least expression is on mortar. The bricks that formed the face of the preserved pillar, as the most exposed to external factors, are substantially effected by degradation in the upper and in the central parts of the pillar, while the bricks in the lowest zone are in a better condition. Preservation state varies, however, for parts that are always above the water and for those whose submergence depends on the variations in the level of the Danube. Most of the bricks are in a state of spalling and erosion, and the alveolisation of a number of bricks with occasional cracking is also noticeable, as well as the formation of deposits, with pronounced discolouration in the lowest zone. As for the porous stone in the core of the pillar, the most pronounced deterioration is alveolisation with erosion, but there is also scaling and delamination of the stone blocks of the restored platform, which is mostly in water. The mortar is the least affected by these changes, but alveolisation with erosion can be observed as well. The northern side of the pillar has the highest level of deterioration of materials (Fig. 5).

The bricks of the researched pillar are the most endangered by biodeterioration, followed by the stone. However, the mortar is visually mostly spared of infestation. The most pronounced presence of all colonizers, on the best-preserved pillar, is on the exposed bricks of its northern side. They are somewhat less present on the southern side, where its visible structure is also made of exposed bricks. On the eastern and western sides, where stone is mostly observed, colonisation is less common. Looking at the north and south sides of the pillar, a concentration of organisms in the form of vertical strips is noticeable, which seem to "flow" from the top of the monument to its base and are scattered on the protruding parts of the ruin, while the recessed and relatively flat parts of the structure are mostly spared. This can indicate the presence of a precipitation flow that is stopped in protruding spots, creating favourable conditions for the growth of fungi and other organisms. The growth of plants is also detected on the investigated pillar, especially on its top.

In situ assessment of surface infestation

Severe macrofouling in the form of lichen infestation of brick (Fig. 6A–D) and stone (Fig. 6F–H) was documented both visually and in more detail via *in situ* microscopy (Fig. 7). On the basis of the visual observation alone, a gradient of colonisation, in terms of substrate bioreceptivity, could be established, with

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[21] John et al. 2003, 1–896.

[22] Komárek, Anagnostidis 2005, 1–786.

[23] Ettl, Gärtner 2014, 1–773.

[24] Raper, Fennell 1965, 736–737; Samson et al. 2019, 1–481.

[25] Krebs 1999, 1–620.

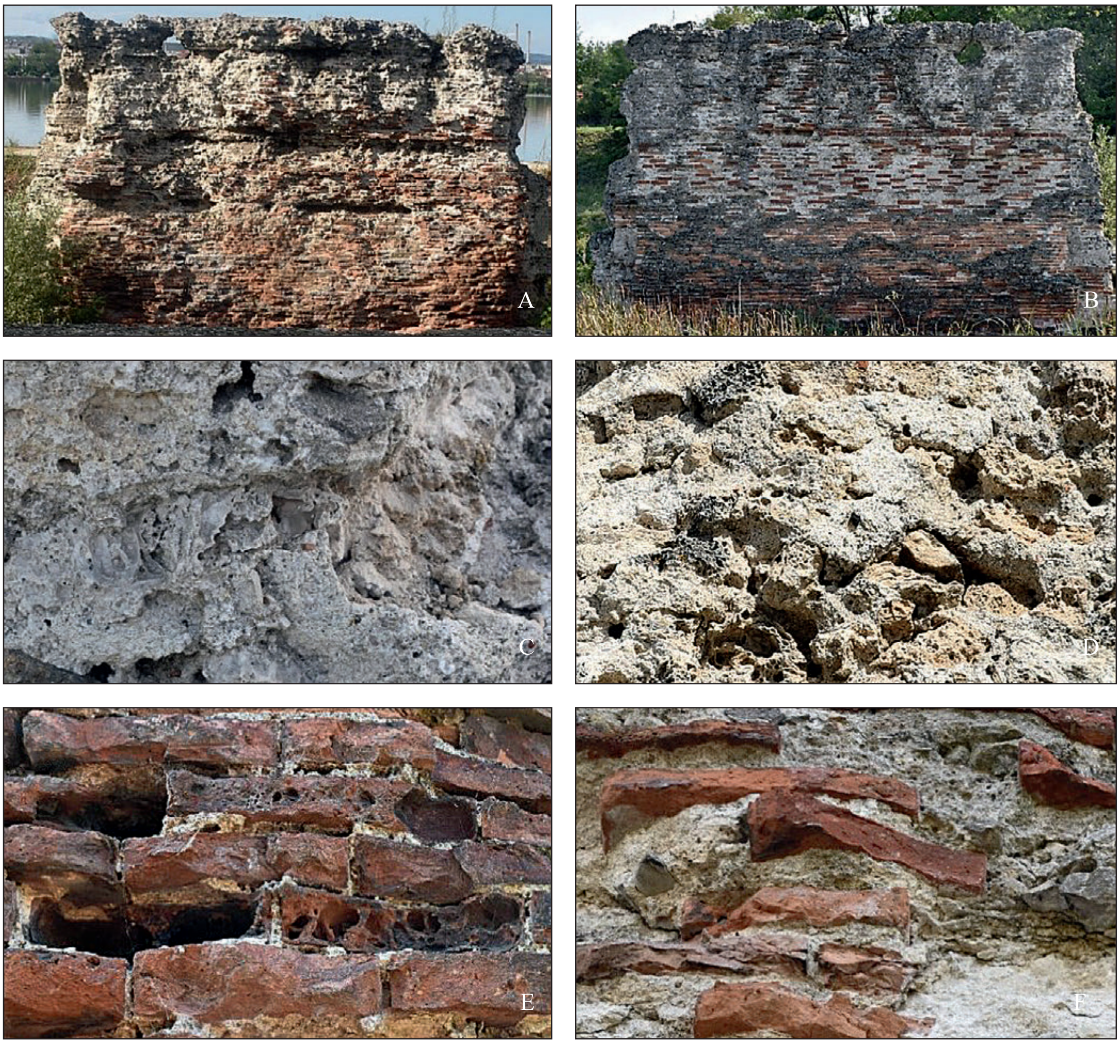

Fig. 5. Deterioration of the pillar – southern side in 2020 (A), and northern side in 2022 (B); stone and mortar deterioration in 2020 (C, D); brick and mortar deterioration in 2021 (E), and in 2022 (F) (Archive of the MoDeCo2000 *project)*

Сл. 5. Детериорација ступца – јужна страна у 2020. години (А) и северна страна у 2020. години (B); детериорација камена и опеке у 2020. години (C, D); детериорација опеке и малтера у 2020. години (Е) и у 2022. години (F) (Архива пројекта MoDeCo2000*)*

bricks being the most susceptible to lichen infestation, followed by stone, while large mortar surfaces, for the most part, remained clean. Predominant colonisers were epilithic crustose and foliose lichens identified as *Caloplaca decipiens*, *Lecanora campestris*, *L. dispersa*, *Parmelia saxatilis*, *Porpidia crustulata*, as well as lichens of the genera *Verrucaria* and *Xanthoria.* Based on the diameter of the largest lichen thalli of *L. campestris*, and taking into consideration the minimal growth rate of this lichen and the fact that, to the best of our knowledge, the studied part of the bridge was never cleaned of infestation and restored, it was calculated that the lichen colonisation of Trajan's Bridge could have started as early as 800 years ago.

In contrast to the bricks and stone, the mortar was, for the most part, free from infestation (as was previously mentioned), with the only exception being thin connecting layers between bricks (Fig. 6A). In these instances, colonisation presumably occurred due to the proximity of the infested bricks and the inoculum transfer between closely positioned and connected bricks. Furthermore, the lower part of the pillar, often submerged in water, was easily distinguishable from the rest of the pillar by the grey discolouration and was,

Fig. 6. Lichen and moss infestation of brick (A–D), mortar (E), and stone (F–H) of the pillar in September, 2022; lower, usually submerged part is discoloured and infestation free (I–J) (Archive of the PROTECTA *project)*

Сл. 6. Инфестација опеке стубца лишајевима и маховинама (A–D), малтера (E) и камена (F–H) у септембру 2022. године; доњи, углавном потопљени део је изгубио боју и нема инфестације (I–J) (Архива пројекта PROTECTA*)*

for the most part, free from infestation (Fig. 6I–J), with the occasional dried lichen and moss thalli occurring on bricks.

In addition to lichenised fungi, fully developed moss thalli in the form of scattered tufts are sporadically present on bricks, as well as stone and the thin layer of mortar in some instances (Fig. 6E). These were determined to be *Brachythecium glareosum*, *Grimmia pulvinata*, *Pohlia* sp. and *Tortula muralis*, with *G. pulvinata* and *T. muralis* being the most prevalent mosses documented at the studied location (Table 1).

Microbial proliferation on brick, mortar and stone surfaces

Optical microscopy of the adhesive tape samples, taken from the surface of bricks, mortar and stone, has revealed the dominant presence of lichen soredia in mass (Fig. 8A), rhizines, as well as in clusters and/or chains of melanised mycobiont, alone (Fig. 8B) or in association with green algae in different phases of lichen primordia formation. Melanised clusters of cells, very reminiscent of microcolonial fungi (MCF), were also found permeating stone fragments (Fig. 8C). In

Documented taxa	Sample type	Mortar	Stone	Brick	Submerged area
Fungi					
Filamentous fungi					
- Ascomycota -					
Alternaria alternata	SS	+	+		+
Alternaria infectoria	SS	+	+	+	+
Alternaria sp.	SS	+	+		
Aspergillus flavus	SS	+			+
Aspergillus niger	SS		+	+	
Aspergillus nidulans	SS			+	
Aspergillus ochraceus	SS	+			
Cladosporium cladosporioides	SS	+	+	+	+
Cladosporium sphaerospermum	SS	+	+	+	+
Epicoccum nigrum	SS	+	+		+
Penicillium sp.	SS				+
- Zygomycota -					
Rhizopus stolonifer	SS		+		
- Unidentified -					
Mycelia sterilia – Moniliaceae	SS	+	+	+	+
Mycelia sterilia – Dematiaceae	SS	+	+	+	+
Yeasts	SS	+		+	+
Lichenized fungi					
- Ascomycota -					
Caloplaca decipiens	IM/AT		+	+	
Lecanora campestris	IM/AT			+	
Lecanora dispersa	IM/AT		+	+	
Parmelia saxatilis	IM/AT		+	+	
Porpidia crustulata	IM/AT			+	
Verrucaria sp.	IM/AT			+	+
Xanthoria sp.	IM/AT		+		
Plantae					
Bryophyta					
Brachythecium glareosum	VS			+	+
Grimmia pulvinata	VS	+	+	+	
Pohlia sp.	VS	+		+	
Tortula muralis	VS	+		+	
Chlorophyta					
Apatococcus sp.	AT	+	+		
cf *Haematococcus pluvialis*	AT				+
Desmococcus olivaceus	AT	+	+	+	+
Trebouxia sp.	AT		+		

AT – adhesive tape; IM – *in situ* microscopy; VS – voucher specimen; SS – sterile swab

Table 1. List of taxa documented on various substrata of the pillar;
microbial proliferation on brick, mortar and stone surfaces (Archive of the PROTECTA *project)*

Табела 1. Листа таксона забележених на различитим супстратима стубца;
пролиферација микроорганизама на површинама опеке, малтера и камена (Архива пројекта PROTECTA*)*

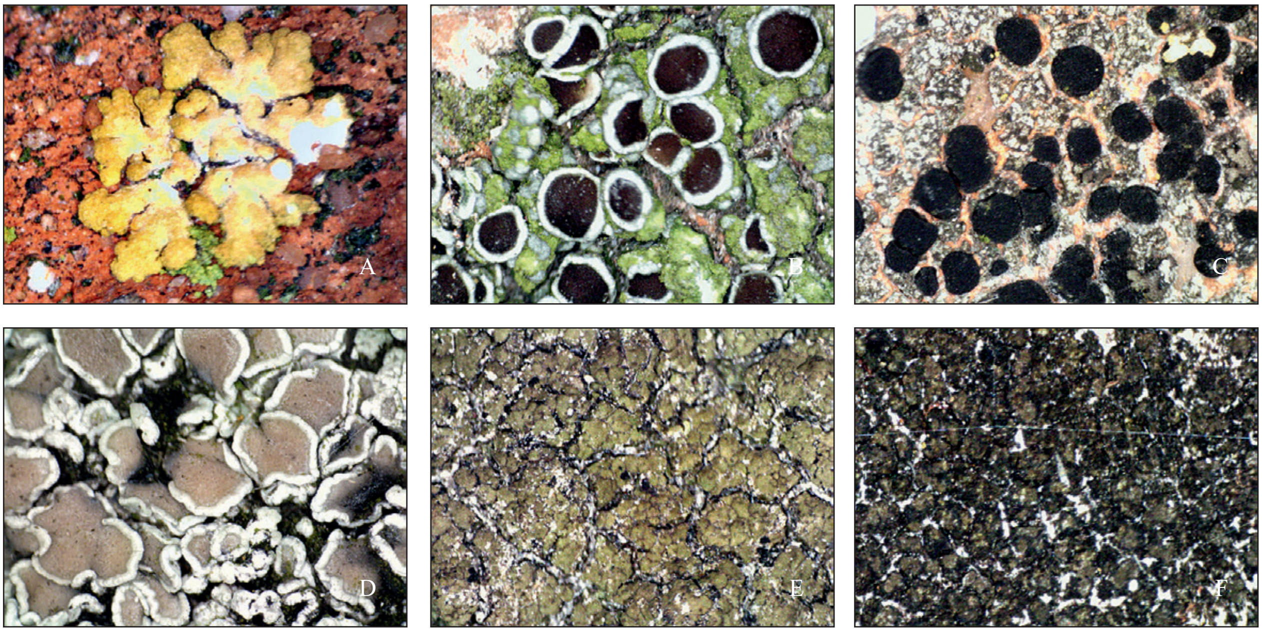

Fig. 7. Details of epilithic foliose and crustose lichen thalli, with clearly visible apothecia, observed via in situ *portable microscope (Archive of the* PROTECTA *project)*

Сл. 7. Детаљи епилитских фолиозних крустозних талуса лишајева, са јасно уочљивим апотецијама, посматрани in situ *преносним микроскопом (Архива пројекта* PROTECTA*)*

addition, various fungal structures, such as fragmented hyphae (Fig. 8G) with visible chlamydospores, sporangia, darkly pigmented ascospores presumably of *Pleosporales*, chains of *Alternaria* dictiospores, *Epicoccum* and *Helminthosporium* conidia, as well as a mass of *Cladosporium* conidia, alone or bound to trichomes and spider webs (Fig. 8D–F), were very abundant within all samples. Furthermore, green algae from the genera *Apatococcus* and *Trebouxia*, as well as *Desmococcus olivaceus*, were observed as phototrophic biofilm components on stone and mortar, all of whom are known to be lichen photobionts. These findings prove that the mortar, although clean and lacking visible infestation, possesses a thriving microbial community. The biofilm from the occasionally submerged area of the bridge, as expected, was dominated by phototrophic microorganisms, i.e., green algae, among them *Haematococcus pluvialis* and *D. olivaceus,* and trichal Cyanobacteria (Fig. 8H–K), although a mass of soredia and various associations of mycobiont and photobiont could also be observed.

Culturable mycobiome

A total of 12 filamentous fungi from six genera were determined as part of the culturable mycobiome of different substrata of Trajan's Bridge (Table 1). The greatest diversity was documented on mortar and stone, with eight fungal species each, followed by the previously submerged area of the bridge (7) and finally the exposed bricks (5). The majority of isolated fungi were *Ascomycota*, with *Rhizopus stolonifera,* the only isolated representative of the phylum *Zygomycota*. With four documented species, the genus *Aspergillus* was the most diverse, followed by *Alternaria* (3) and *Cladosporium* (2). Furthermore, the culturable mycobiome also consisted of several yeasts, as well as fungi that did not form reproductive structures in the cultures, i.e., *Mycelia sterilia* of both the *Moniliaceae* and *Dematiaceae* families.

On the basis of the results obtained by the isolation of the culturable mycobiome, together with microscopic analysis and identification of lichen and moss specimens, Sørensen's quotient of similarity was calculated to determine the similarity between the communities documented on brick, mortar, and stone surfaces, as well as those present on the surface of dry and occasionally submerged parts of the bridge. Due to species overlap, the obtained QS values for mortar-stone-brick substrates were especially high (in the range from 0.57 to 0.65) with the highest similarity documented

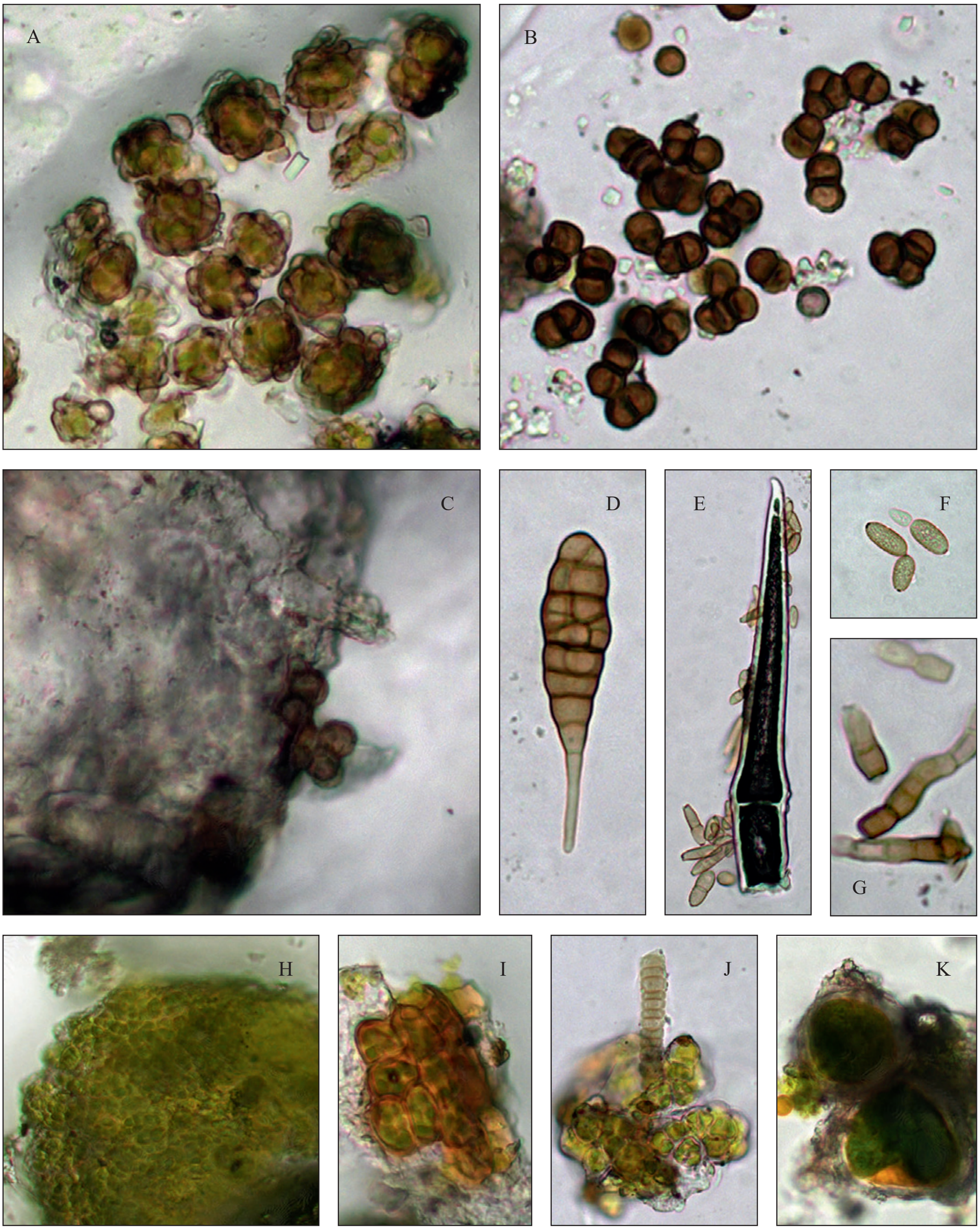

Fig. 8. Optical micrographs of adhesive tape samples from various substrata of the pillar:
A. soredia in mass; B. melanised clusters of mycobiont; C. melanised clusters permeating stone substrata;
D. Alternaria-like dictiospore; E–F. Cladosporium-like conida in mass; G. melanised hyphal fragments;
H–K. various stages of green algae and trichal cyanobacteria (Archive of the PROTECTA project)

Сл. 8. Оптичке микрографије узорака адхезивне траке са различитих супстрата стубца:
A. соредије у маси; B. меланизовани кластери микобионта; C. меланизовани кластери који прожимају камени супстрат; D. диктиоспоре Alternaria типа; E–F. Cladosporium*–конидије Cladosporium типа у маси; G. меланизовани хифални фрагменти; H–K. различите фазе животног циклуса зелених алги и трихалних цијанобактерија (Архива пројекта* PROTECTA*)*

between mortar and stone incorporated within. Furthermore, a high QS value of 0.57 was also obtained when comparing communities documented on dry and on occasionally submerged parts of the bridge.

DISCUSSION

It is a well-known fact that "maintenance-free materials do not exist".[26] The detection of the possible damage that can occur as a result of external or internal factors and proposing the actions to avoid the damage is a necessary activity prior to conservation. In the case of the remains of Trajan's Bridge in Serbia, it is very important to consider the change in the environment and its influence on building materials, since some materials are stable in one environment, but can be very vulnerable in different conditions.[27] This is especially important for the remaining parts of the bridge pillars not initially constructed in water, but now partially submerged in the varying water level.

In a travelogue from the end of the 19th century, Felix Kanitz (1829-1904) wrote that the pillar of Trajan's Bridge in its upper part was damaged due to weather conditions. He also pointed out that the local villagers tore off the stone blocks lining the shorter sides of the pillar, and that the core had hardened so much that they could not do anything to it.[28] There is no mention of the condition of the brick lining on the longer sides. However, there is a possibility that some bricks were damaged or have even completely disappeared over time, due to the separation of entire units by villagers, but more probably due to the tearing of their fragments (as a result of the impossibility of complete unit separation from the strong concrete core to which the brick lining was tightly bound).

Biological colonisation on the Trajan's Bridge did not spare a single part of the preserved pillar, and it affected all the types of construction materials present. The predominant presence of lichens and mosses indicates that the bricks, stone, and mortar possess a very high bioreceptivity and are in the later stages of biological succession, characterised by the advanced state of the deterioration process. This is not unexpected, since colonisation of monuments is greater on substrates with higher porosity or rough surfaces that retain more moisture.[29] Although known to be involved in the process of bioprotection against a multitude of weathering factors, lichen infestation, as pronounced as the one observed on the studied site, has great ecological implications due to its marked paedogenetic activity, which causes reduced substrate cohesion. Lichens deteriorate substrate biogeochemically, via oxalate formation, carbonic and various organic acids that form cation–ligand complexes with minerals altering the substrate composition (biocorrosion), and biomechanically, due to hyphal penetration, contraction of the thalli and adhesion of rhizines, which are assumed to act cooperatively and penetrate a few millimetres into the porous material over the course of several decades.[30] Furthermore, due to their slow but constant growth, they cover considerable areas with undesirable chromatic alterations.[31] All of the lichens documented in the study are from the surrounding rocks and are known colonisers and deteriogens of cultural heritage monuments worldwide, with the only exception being *L. dispersa,* which has been previously reported to cover significant areas but does not induce evident modifications.[32] The chemical and mechanical activity of mosses is, likewise, not without consequences as damage, usually manifested as discolouration, green-grey patches, and extracted minerals, is known to occur over an extended period of time. Their biodeterioration capacity is largely due to the accumulation of Ca^{2+} within the thalli, such is the case with *G. pulvinata*[33]*,* for whom this process is very well documented. Furthermore, the death of mosses can cause indirect damage to monuments by enriching and increasing the humus content that supports the growth of successive species of the higher plant.[34] Since plant growth has been recorded on the pillar of Trajan's Bridge, especially on its top, it is necessary to carefully register the species and make decisions regarding its elimination, since the removal of higher plants should be done as soon as they grow.[35] Their appearance starts in the mortar joints, and the roots can exert mechanical stresses on this bonding material, which get higher as the plants grow, eventually causing the failure of the structure.[36]

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[26] Balksten, Strandberg-de Bruijn 2021, 367.

[27] Henry, Stewart 2011, 122.

[28] Kostić 2011, 217–218.

[29] Henry, Stewart 2011, 152.

[30] Ibid; Salvadori, Municchia 2016, 39–54.

[31] Ljaljević-Grbić et al. 2017, 304–310.

[32] Piervittor et al. 1998, 263–277; Guiamet et al. 2012, 339–344.

[33] Dakal, Cameotra 2012, 36.

[34] Ibid.

[35] Elena Charola 2016, 18.

[36] Steiger et al. 2011, 230.

The culturable mycobiome, dominated by *Ascomycota* and *Zygomycota* to a lesser extent, represents a typical fungal community that originated from the surrounding soil and vegetation, and was deposited via the air onto the surface of the monument. Although frequently isolated from this type of cultural heritage object, for the most part they possess no role in the process of biodeterioration, since their particular ecology does not allow them to establish critical mass easily or at all on these extreme types of substrata. In that sense, they only act as allochtoon transients that do not participate in the complex process of microbial deterioration. On the other hand, an actively growing microbial community, documented in the samples analysed via microscopy, mainly consisted of various lichen structures and black yeast-like MCF permeating the stone and leading to the development of the *biopitting* phenomenon, i.e., the formation of pits in sizes ranging up to 2 cm in diameter and depth.[37] The dominance of extremophile organisms, such as these, is expected in the studied type of monument since they are the most adapted to the stressful factors (UV, temperature fluctuation, periods of water freezing in fissures, etc.) that characterise it.

It is not precisely known for how long Trajan's bridge was in use. Dion Cassius (c.155 – c.235) wrote that it was demolished by the Romans as early as the time of Hadrian so that it would not be used by barbarians to cross the Danube, while Procopius (500–565) wrote that the bridge gave way under the rush of the river. Most likely, after the abandonment of the province of Dacia by the Roman state in 271, the bridge definitely lost its purpose. Since it provided an opportunity for barbarians to make quick incursions, it soon began to represent a threat to the border region. This is probably why the upper structure was removed, and the last recorded use of the bridge was during the time of Constantine the Great. At a low water level in 1858, 16 pillars in the river bed were visible. Two of them were destroyed in 1909 for the purpose of unhindered navigation.[38]

The study conducted on the current state of preservation of the remains of Trajan's Bridge can be also important for scientific research in disciplines outside of biology and conservation, showing the significance of multidisciplinary research of historical monuments. The calculation that lichen colonisation of Trajan's Bridge could have started as early as 800 years ago can be interesting for landscape research. This estimate was based on the lowest known growth rate of measured lichen thalli of *L. campestris*. However, bearing in mind that the radial growth rate of many foliose and crustose lichens increases early in life and later approaches a constant growth rate for an indefinite period of time or enters senescence when growth rate decreases, and is dependent of ever-changing environmental factors, the precise time period in which the studied pillar was subjected to the deteriogenic activity of lichens is difficult to establish. It is nonetheless important since we do not have records of any pillar cleaning treatments and the time period necessary for the establishment of such large thalli is considerable.

Based on the visual inspection of the damage to Trajan's Bridge and on the determination of biodeteriogens given in this study, in combination with the results of the research on building materials, along with the additional assessment of the influence of the factors of the external environment, the most adequate method of its preservation should be proposed. It is also important to mention that all present building materials need to be treated together, as mutually interdependent, since bricks and stone bonded with mortar form a unity in the structure, and "a single system".[39] The biological growth of the masonry is dependent of external conditions, but also of the bioreceptivity of the colonised materials, with the presence of water being the most important factor. The elimination of biological growth is an easy task but it is known that it will regrow again as long as favourable conditions exist. Moreover, resistance can be developed to the applied chemicals (biocides) and, in the end, can cause the growth of more aggressive forms of infestations.[40]

In this process, possible architectural/restoration works that will protect the investigated monument from external factors must be considered as well. Modern architectural interventions need to provide protection, but can also add value to the monument itself, thus encouraging its conservation.[41] However, determining the priorities in this process is extremely important, and our activities must physically protect the monument, but not endanger its influence and significance,[42] offering a carefully balanced relationship between

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[37] Sterflinger, Piñar 2013, 9637–9646.

[38] Mirković 1986, 112–113; Гарашанин, Васић 1980, 8, 11.

[39] Odgers, Henry 2012, 47.

[40] Elena Charola 2016, 18.

[41] Hebbelinck et al. 2001, 31.

[42] Nikolić 2018, 34.

what we inherited and our contribution to its preservation for the future.

CONCLUSIONS

A visual inspection of the remains of the most preserved pillar of the Trajan's Bridge on the Danube bank in Serbia showed that bricks and stone present in its structure have a severe level of deterioration, among which the most pronounced are the loss of brick material and the one caused by biodeteriogens. Brick and stones, according to the visual observations on site, have a high level of bioreceptivity. However, laboratory research showed that the mortar itself is very receptive as well, and that the whole structure is in the later stage of biological succession and the advanced state of the deterioration. The exposed location and the environment favourable for the biocolonisation together with the high level of bioreceptivity of the used building materials make this monument very prone to infestation.

During future studies on the monument, it will be necessary to conduct deep research into all deterioration types registered in this review and plan the necessary interventions in accordance with the results of the research. Furthermore, only by including the analyses of all external and internal factors that can influence the state of the monument in further research can we adequately recognise the causes of deterioration and minimize its actions, which can sometimes lead to damage to the whole structure. One of the conservation plan segments must be connected to the threat from biological agents, with proposals for the future maintenance of the monument and maximum reduction of further biological colonisation.

Given that this study has shown a high degree of biodeterioration of Trajan's Bridge's remaining pillar, that other types of deterioration are present as well, and that the monument has been generally decaying, it is necessary to prepare plans for its protection in the very near future, and try to slow those processes down, which are mostly natural and thus inevitable, but whose direction of development and dynamics we can certainly influence with adequate maintenance.

In order to develop future conservation plans for the preservation of precious monuments of the Danube Limes in Serbia that aspire to be part of the UNESCO World Heritage List, it is very important to do the research on their building materials and construction, as well as the state of their preservation. As such, the study of the biological colonisation of the remains of Trajan's Bridge represents a valuable contribution to this development.

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ОЧУВАЊЕ ДУНАВСКОГ ЛИМЕСА У СРБИЈИ: ПРИКАЗ БИОДЕТЕРИОРАЦИЈЕ ТРАЈАНОВОГ МОСТА

Кључне речи. – биодетериорација, Трајанов мост, лишајеви, дунавски лимес у Србији, историјски малтери, историјска опека, историјски камен, римски споменик

Један од најзначајнијих споменика римског дунавског лимеса, који се данас протеже кроз неколико европских земаља, јесте Трајанов мост, подигнут у периоду од 103. до 105. године. Његови остаци се налазе у данашњој Србији и Румунији. Од некада величанственог моста са зиданим ступцима који су носили дрвену конструкцију, данас су остали само ступци на обалама, са делимично познатим стањем очуваности оних под водом. Остаци стубаца на српској страни Дунава откопани су и делимично рестаурирани пре четири деценије. Данас је стубац најближи реци, који није био подвргнут конзервацији, подложан различитим врстама детериорације, пре свега због високог нивоа спољних утицаја. Према визуелном опажању, најизраженији видови детериорације споменика су везани за губитак материјала опеке и инфестацију опеке и камена, док је малтер, чини се, најмање угрожен. Приликом поређења садашњег стања ступца са оним видљивим на фотографијама током последњих шест деценија, можемо проценити да се степен његове детериорације није драстично променио. Међутим, разлика у биолошком расту је видљива када се садашње стање упореди са оним забележеним близу тренутка када је вода продрла у простор одбрамбеног насипа изграђеног због подизања нивоа Дунава у склопу активности хидроенергетског система Ђердапа. С обзиром на наведено, примарни циљ овог истраживања је био да се утврди степен инфестације и угрожености споменика и утврди потенцијални ризик детериорације малтера у контакту са инфестираном опеком. У ту сврху су окарактерисани забележени биодетериогени. Посебан акценат је стављен на гљиве као познате главне узрочнике процеса детериорације како би се прецизно утврдио степен ризика који присутна фунгална заједница представља за овај споменик а све у циљу формирања и имплементације адекватног протокола за конзервацију. Констатована је интензивна колонизација опеке и камена епилитским лишајевима родова *Caloplaca*, *Lecanora*, *Parmelia*, *Porpidia*, *Verrucaria* и *Xanthoria*, као и маховинама родова *Brachythecium*, *Grimmia*, *Pohlia* и *Tortula*. На деловима споменика који нису имали видљиву инфестацију или симптоме биодетериорације такође је констатовано присуство бројних фунгалних структура, као што су соредије лишајева, кластери микроколонијалних гљива и споре за полно и бесполно размножавање меланизованих гљива, али и структуре трихалних *Cyanobacteria* и *Chlorophyta* rodova *Apatococcus*, *Desmococcus*, *Haematococcus* и *Trebouxia*. Укупно 12 филаментозних гљива из 6 родова су изоловане са површине споменика. Доминирају представници раздела *Ascomycota* (*Alternaria*, *Aspergillus*, *Cladosporium*, *Epicoccum* и *Penicillium*), док је забележена само једна гљива из раздела *Zygomycota*. Сличности између заједница забележених на опеци, малтеру и камену, процењене према Соренсеновом индексу сличности, високе су и у опсегу од 0,57 до 0,65, са највећом сличношћу забележеном између малтера и камена.

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