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Algal and Cyanobacterial Biofilms on Calcareous Historic Buildings

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Abstract. Major microorganisms in biofilms on external surfaces of historic buildings are algae, cyanobacteria, bacteria, and fungi. Their growth causes discoloration and degradation. We compared the phototrophs on cement-based renderings and limestone substrates at 14 historic locations (47 sites sampled) in Europe and Latin America. Most biofilms contained both cyanobacteria and algae. Single-celled and colonial cyanobacteria frequently constituted the major phototroph biomass on limestone monuments (32 sites sampled). Greater numbers of phototrophs, and especially of algae and of filamentous morphotypes, were found on cement-based renderings (15 sites), probably owing to the porosity and small pore size of the latter substrates, allowing greater entry and retention of water. All phototrophic groups were more frequent on Latin American than on European buildings (20 and 27 sites, respectively), with cyanobacteria and filamentous phototrophs showing the greatest differences. The results confirm the influence of both climate and substrate on phototroph colonization of historic buildings.

Both historic and modern buildings are subject to the deteriorative and degradative action of the environment and living organisms, normally referred to as “weathering”. These processes can be accelerated, or even initiated, by microorganisms, whose activities are potential threats to the maintenance of modern buildings, as well as historic and cultural property. Since the organisms are present on the surface of the materials, in biofilms, their activities are localized and concentrated at these points [4].

The major groups of microorganisms detected in the superficial biofilms are algae, cyanobacteria, heterotrophic bacteria, and fungi; protozoa are also frequently present. Fungi and cyanobacteria are particularly adapted to survive repeated drying and rehydration occurring on exposed building surfaces [11]. Many cyanobacteria and the most prevalent fungi on external surfaces of buildings are darkly pigmented. Their growth leads to discoloration (aesthetic deterioration) of the surface. However, they can also actively degrade the structural materials by production of acid metabolites [10], siderophores or other chelating materials [12], and osmolytes, which can degrade siliceous minerals, as well as by penetrating into the substrate by unknown mechanisms [11].

The phototrophs, algae and cyanobacteria, have been considered to be the primary colonizers [8], conditioning the inert surfaces for the growth of heterotrophic organisms, such as fungi. There have been a number of regional studies on the phototrophs present on historic buildings [1, 11, 12, 16], but differences in methodology impede the comparison of results from different climates and on different substrates. We used a standard adhesive tape technique [5] to identify the phototrophs present on calcareous substrates composing the external surfaces of 14 historic buildings in Europe and Latin America.

Materials and Methods

Forty-seven samples of biofilms were taken from 14 historic buildings in Europe and Latin America by using the adhesive tape method [5]. Substrates sampled were cement renderings of churches in Porto Alegre, Brazil, and external walls of constructions in Dorset, UK, and Valladolid, Mexico; mortar renderings in Anhatomirim, Florianopolis, Brazil, and in the necropolis of Carmona, Spain; and limestone monuments in Blaye, France; Uxmal, Kabah and Tulum, Mexico; and London, UK. All the surfaces showed visible discoloration, generally

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Fig. 1. Statue on the Codz Poop building at the Mayan site of Kabah, Mexico, showing typical black biofilms.

grey/black in appearance, such as that shown in Fig. 1. Table 1 lists the buildings/sites sampled, with their historical details.

For microbiological analysis, tape samples were placed directly on solid Modified Knop's Medium (MKM) [5] and were incubated at 28°C in an illuminated BOD incubator. Plates were examined after 4 h to visualize rehydrated microorganisms in situ to establish the major biomass and then at various intervals up to 5 weeks. Some organisms were isolated in liquid MKM by repeated subculture. Algae and cyanobacteria were identified by their morphology, according to Holt et al. [9], Prescott [13] and Smith [15].

Results and Discussion

The major groups of microorganisms detected in the superficial biofilms were cyanobacteria, algae, and fungi, although bacteria and protozoa were also noted. Table 2 shows the various groups of algae and cyanobacteria that were detected on the surfaces. Most of the biofilms contained both cyanobacteria and algae. The aqueous dilution method of isolation, where used, yielded almost 100% filamentous organisms, whereas the tape method of culture detected similar numbers of filamentous organisms but many more non-filamentous phototrophs. On the limestone monuments, cyanobacteria of Bergey's sub-groups 1 and 2 [9] frequently constituted the major phototroph biomass. These are the single-celled and colonial types, which have previously been found to be prevalent on limestone buildings [12, 6]. In the present survey, there were 0.84 filamentous organisms detected per site on limestone substrates, compared with 2.73 on cement and mortar. The equivalent figures for single-celled and colonial types were 3.56 and 4.13, respectively. Higher total numbers of phototrophs, and especially of algae, were found on the cement and mortar sites, and these were the only samples that yielded diatoms as part of the biofilm. Algal numbers per site were 1.38 for limestone and 2.73 for the cement group, with the equivalent cyanobacterial numbers being 3.03 and 4.13. All the above figures were evaluated using the χ^2 test and were found to be significantly different from each other at the 1% level. Significant differences were also found between European and Latin American sites. All groups of phototrophs were more frequent on the surfaces of Latin American buildings (Table 3). It has previously been noted, in Europe, that the season of the year in which samples are taken does not affect the major species of algae and cyanobacteria detected [11]. Our samples were taken only once from each site, and the month in which this occurred is stated in Table 2. We noted no obvious differences between the organisms detected at similar sites in different seasons of the year, where such samples were available.

The data show that both substrate and environment are important in determining phototroph colonization. The higher temperatures and humidity at the Latin American sites will obviously promote microbial growth, leading to greater overall colonization. The much greater preponderance of cyanobacteria over algae, and of filamentous over coccoid morphotypes, at Latin American sites may be a result of high insolation. Ultraviolet irradiation is inhibitory to most microorganisms, but many cyanobacteria produce protective pigments [2, 3, 14], noted in this survey by the common occurrence of dark-colored cells such as the brown-sheathed *Scytonema* species shown in Fig. 2. In addition, filamentous microorganisms may gain protection from high UV levels at the surface by an increased ability to penetrate below the surface of the substrate.

A difference in major microorganisms colonizing buildings of various materials in the same environment has also been reported by Tomaselli et al. [16]. All the substrates sampled in our study were calcareous in nature. The most important difference between limestone (a natural rock) and the other, artificial substrates is probably the porosity, which is generally lower in limestone. Cement is mainly calcium silicate hydrate, which is very

Table 1. Buildings and sites sampled

Substrate	Building(s)	Built	Situation	Location
Limestone	La Citadelle	10^{th},13^{th} &17^{th} centuries	Town (on Gironde estuary)	Blaye, France
	War monument	1930	City center	London, England
	Mayan buildings	6^{th}–10^{th} centuries	Rural	Uxmal & Kabah, Mexico
	Mayan buildings	9^{th}–16^{th} centuries	By Caribbean sea	Tulum, Mexico
Cement and mortar	Fort of Santa Cruz	18^{th} century	Small island off the mainland	Island of Anhatomirim, Brazil
	Domestic wall	Ca. 1900	Village	Morcombelake, England
	Roman Necropolis	1^{st} and 2^{nd} centuries. Excavated late 19th, early 20th centuries	Town outskirts	Carmona, Spain
	Hotel	18^{th}/20^{th} centuries	Town center	Valladolid, Mexico
	Five churches	19^{th} century	City center	Porto Alegre, Brazil

Table 2. Major algae and cyanobacteria detected in biofilms on calcareous historic buildings in Europe and Latin America

	Limestone				Cement/mortar				
Site: Month: No. sites	Blaye/ Aug 19	Euston/ Dec 4	Uxmal and Kabah/Feb 6	Tulum/ July 3	Anhato/ April 4	Dorset/ July 1	Carmona/ Feb 3	Valladolid/ Feb 2	POA Aug 5
Synechocystis/coccus[a]	+	+	+	+	+	+	+	+	+
Gloeocapsa/thece[a]	+		+	+			+	+	+
Pleurocapsales	+	+	+	+	+	+	+	+	+
Oscillatoriales[c]		+			+		+	+	+
Nostocales[c]	+				+	+	+	+	+
Stigonematales[c]	+			+					
Coccoid chlorophytes	+	+	+	+	+	+	+	+	+
Protococcus[b]	+		+			+	+	+	+
Stichococcus[c]	+		+			+		+	+
Klebshormidium[c]						+			+
Trentepohliales[c]	+	+	+	+		+	+	+	+
Diatoms						+			+

[a] *Chroococcales*
[b] *Protococcus* is a coccoid chlorophyte, separated here because of its frequent occurrence.
[c] = filamentous morphotypes.

Table 3. Types of phototrophs detected per sampled site in Europe and Latin America

Type of phototroph	European buildings (27 sites sampled)	Latin American buildings (20 sites sampled)
Filamentous	0.56	2.65
Single-celled/colonial	3.15	4.45
Algae	1.56	2.15
Cyanobacteria	1.44	5.00

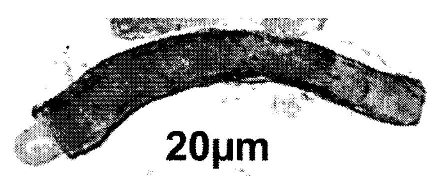

Fig. 2. A *Scytonema* species with a thick, highly pigmented brown sheath. Similar forms, typically seen as short filaments in situ on walls, were detected in several samples from Latin America.

porous and retains a lot of water in its fine pores. The porosity of the substrate is related to the penetration and retention of water, which, in turn, affects microbial colonization. Algae are more frequent on humid than on dry sites [7] and therefore are more favored on these wetter, cement-based substrates. The increased prevalence of filamentous phototrophs on these materials may be explained by their greater ability to grow in wetter environments, as shown by the isolation rates of filamentous versus non-filamentous phototrophs in the aqueous dilution method, and indicating an increased dependency of filamentous organisms on free water. This difference is most marked for the cyanobacteria.

There are no previous publications on the differences in phototrophic microflora on natural and artificial calcareous substrates. These results suggest that the greater availability of free water at the surface of cement modifies it in such a way that it is more readily colonized. The ongoing development of low-porosity, cement-based composites should lead to the production of less susceptible building materials in the future.

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