Product evaluation

Aquashield Active

against photosynthetic microorganisms

JUSTIFICATION.

At the request of the Lurederra Technology Centre, the antimicrobial property against photosynthetic microorganisms of one of the products manufactured and marketed by the company Tecnología Navarra de Nanoproductos S.L. (TECNAN) has been tested.

INTRODUCTION.

Stone materials are susceptible to attack by organisms and microorganisms (bacteria, cyanobacteria, algae, fungi and lichens) that cause their deterioration.

In the case of external stone material, green algae and especially cyanobacteria are the first organisms to colonise it, forming the first greenish patinas that can be observed, since they only need light, water and a few inorganic compounds and also prefer an alkaline substrate. The most important factors determining their appearance are light intensity, humidity, temperature and pH. Their growth is favoured by high humidity, as well as the adherence of dust, organic residues and other substances, so that a muddy layer or patina is formed, which can be exploited by other organisms (lichens, mosses and vascular plants).

Biofilms of cyanobacteria and green algae cause not only aesthetic damage, but also physical and chemical damage. Photosynthetic organisms contribute to the deterioration of stone materials through their respiratory processes, retaining water and releasing acids (carbonic, lactic, oxalic, succinic, acetic or pyruvic) or chelating compounds.

The colonisation of the stone material by microorganisms depends on several factors such as the bio-receptivity of the substrate, environmental parameters (light, temperature, relative humidity, wind, atmospheric pollution, etc.), and specific microclimatic parameters (orientation, exposure to shade, constant wetting by capillarity, etc.).

It seems clear that water availability is one of the most important factors since all organisms need water for their metabolism. Therefore, any measure that reduces the humidity of the stone materials will contribute to prevent or hinder the development of the aforementioned microorganisms. And a very widespread measure is the application of hydrophobic products such as the one tested in this study.

MATERIAL AND METHODS.

Tested product.

Aquashield Active has been tested, a hydrophobic/water-repellent product, which is also an inhibitor of microorganisms, as indicated in its data sheet.

Stone material tested.

The product has been tested on the so-called Puerto de Santa María stone (Cádiz), a bioclastic calcarenite. For this purpose, two 30x20x2 cm test samples were used, which were previously sterilised in autoclave.

Product application.

Each of the stone specimens was divided into two parts, one for the product and one without product, which served as a control. The product was applied on the surface of the specimens according to the manufacturer's instructions, and left to dry for 24 hours.

Test samples after application of the product. In both cases, on the left the treated area with Aquashield Active and on the right the untreated one.

Inoculation of photosynthetic microorganisms.

For inoculation, a BG11 liquid medium culture was used from a natural phototrophic biofilm present on the surface of biodeteriorated stone, specifically from the building of the former Hospital de las Cinco Llagas in Seville, now the seat of the Andalusian Parliament, whose original stone is precisely from the Puerto. This culture contains cyanobacteria *Chroococcus sp., Gloeocapsa sp., Leptolyngbya sp.* and *Phormidium sp.,* and green algae *Chlorella sp., Chlorocococcum sp. and Desmodesmus sp*.

Inoculation was carried out by spraying the culture over the surface.

Incubation.

Given the unavailability of a climatic chamber, incubation was carried out for 6 months in a humid area on a terrace of our facilities where moss naturally develops, with natural light cycles and real meteorology. The test specimens were moistened almost daily by spraying the microbiological culture solution over their surface, so that each time they were moistened, they were re-inoculated at the same time, in order to accelerate the development of photosynthetic microorganisms. Subsequently, the test samples were placed in trays that allowed constant humidity by capillary action, remaining in the same trays for another 6 months in the laboratory, also with natural light cycles and at a constant temperature of 24 ºC approximately.

Location of the specimens in a humid area on a terrace of the IAPH headquarters during the first 6 months (left) and in trays in the laboratory for the remainder of the test (right).

Quantification of photosynthetic growth.

The area of the stone surfaces covered by photosynthetic colonisation was considered as a measure of photosynthetic growth. In order to quantify the development of photosynthetic microorganisms on the surface of the test specimens after incubation time, the areas covered by biofilms were measured. This was carried out in two ways, on the one hand manually using transparent graph paper, and on the other hand by means of digital image analysis, measuring the colonised area with a well-known image processing programme on photographs of the test specimens.

Colourimetry.

Colourimetry makes it possible to determine the colour variations that occur in different materials. It is usually used to evaluate the possible changes in colour in the materials after some treatment (biocide, water repellent, consolidant, etc.), but in this case we have used colourimetry not to calculate the global variation in colour, but simply to calculate the variation towards green, assuming that a change towards green is a consequence of the chlorophyll of the photosynthetic microorganisms that have developed.

A Minolta Chroma meter CR-210 colourimeter was used, which characterises colour in the form of three-dimensional coordinates in the CIE-Lab colour space. This measurement system provides the colour parameters L* a* b*. L* indicates the brightness and a* and b* are the colour coordinates, a* corresponds to the red/green coordinates (+a indicates red, -a indicates green) and b* corresponds to the yellow/blue coordinates (+b indicates yellow, -b indicates blue).

Colourimetric measurements were taken at 10 different points on each tested area of the specimens, before the test started, and at the same points after the end of the test. The more negative the difference of the parameter a* after the test ($\Delta a^* = a^*_2 - a^*_1$), the more chromatic change towards green.

RESULTS.

Development of photosynthetic microorganisms.

Firstly, the water-repellent capacity of the product tested is worth highlighting. When spraying to moisten, the pearl effect is evident on the surface where the product has been applied, while on the untreated surfaces the water droplets are quickly absorbed.

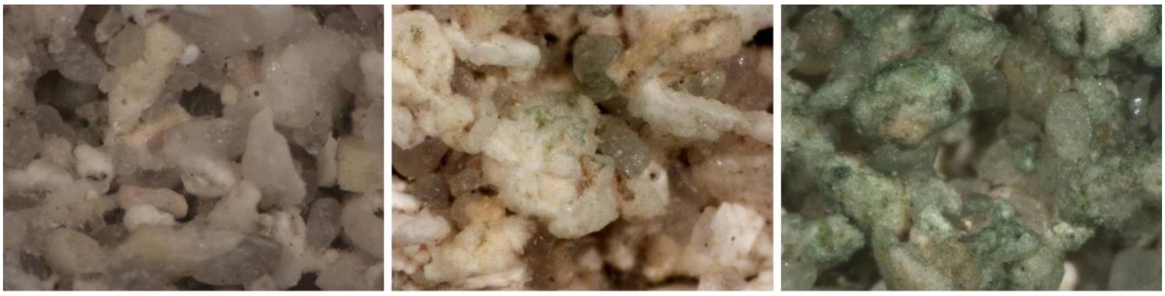
Perhaps this is why the formation of biofilms of photosynthetic microorganisms was very slow at first; it was not until 3 months later that their growth began to be noticed visually in the control or untreated areas. After 6 months, the growth became visually evident, covering practically the entire surface in the case of the untreated areas. The Puerto Stone is a very porous material and its surface shows irregularities, which provide anchorage points and micro-niches for the settlement of microbial cells, thus favouring their growth.

Hydrophobic capacity of the Puerto stone. Given its high porosity, no pearl effect occurs,
but it can be seen how the untreated area is darker due to the absorption of water compared to the treated area.

On the control or untreated surfaces, the development of photosynthetic microorganisms is evident. This development began at 3 months with cyanobacteria, acquiring a blue-green colour, followed by green algae. At 6 months the development of photosynthetic microorganisms was already more than evident to the naked eye, and was complete by the end of the trial. In some places, blackish-brown spots are visible on the green biofilms; this is fungal growth. Cyanobacteria and green algae contribute through photosynthesis to a significant increase in the organic carbon content of the stone, favouring the growth of heterotrophic microorganisms (bacteria and fungi) that live on the extracellular organic matter synthesised by the living algae, or feed on the dying algae. We therefore see the ecological succession that occurs naturally, and which in theory would continue with the development of lichens and bryophytes.

On the other hand, in the areas treated with Aquashield Active, the surface area colonised by photosynthetic microorganisms is smaller, and more cyanobacteria than green algae can be seen. After 12 months of testing, we see incipient colonisation in these treated areas. However, the vitality of the microorganisms is the same as in the untreated areas, so the product is not biocidal, but it does delay microbial growth.

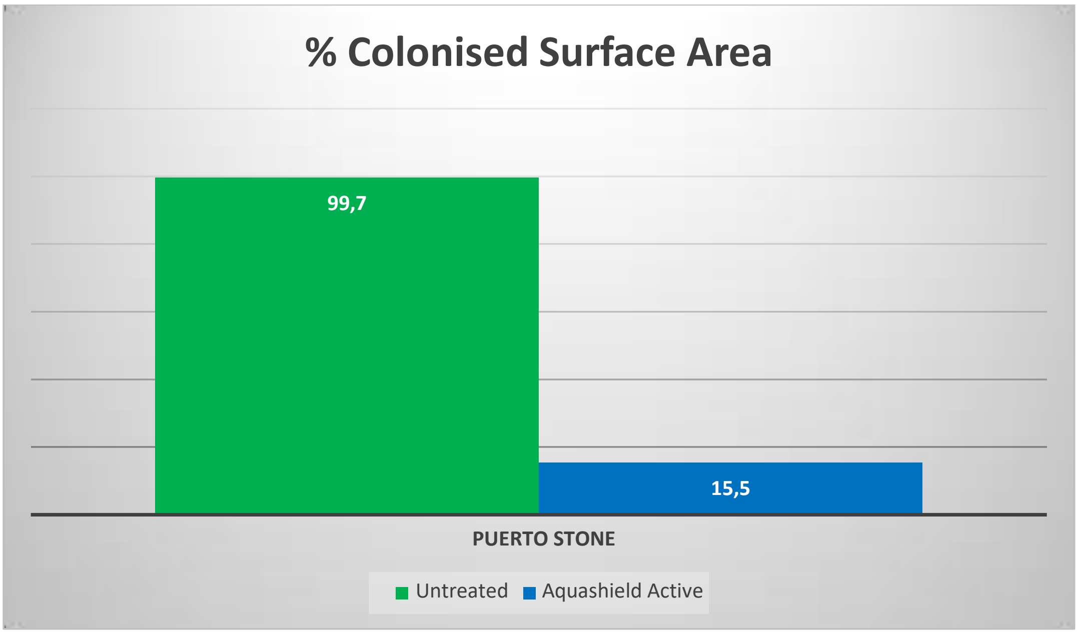
Evolution of the Puerto stone specimens during the test. From top to bottom, at 3, 6, 9 and 12 months.
In all cases, on the left the area treated with Aquashield Active and on the right the untreated area.



Stereomicroscope images (7x) of uncolonised Puerto stone (left), incipient colonisation in area treated with Aquashield Active (centre) and full colonisation in untreated area (right). Regardless of whether they are treated or untreated, the photosynthetic microorganisms present have the same vitality.

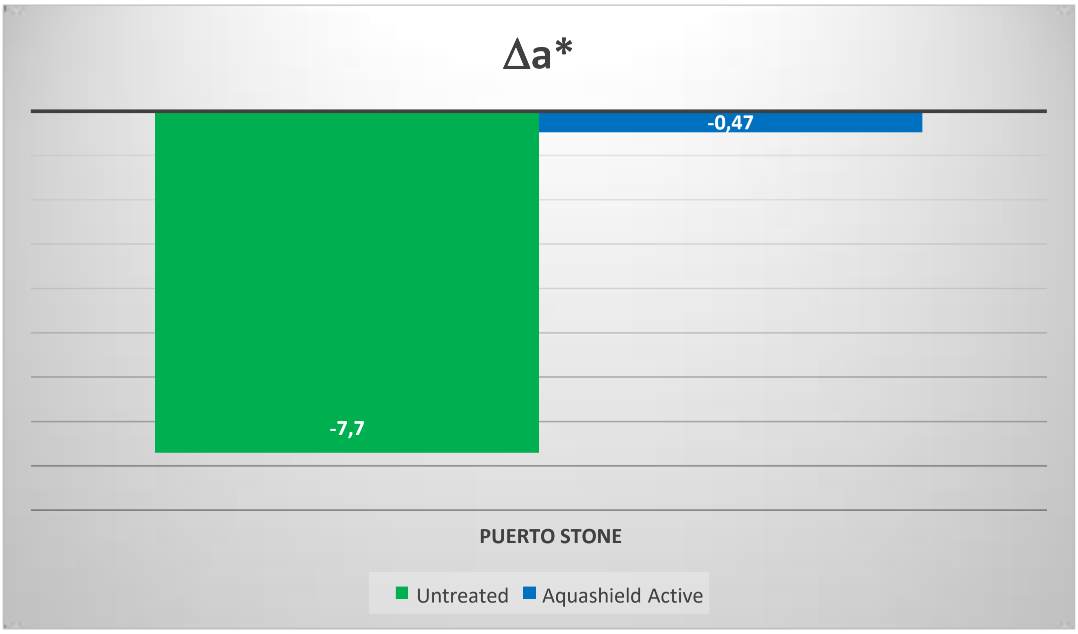
Quantification of photosynthetic growth.

The graph bellow shows the calculated percentage of the surface area covered by photosynthetic colonisation for the untreated substrate compared to the treated one with Aquashield Active.



Colourimetry.

The graph below shows the average values of the difference of the parameter a* (Δa*) compared to the pre-test measurements.



CONCLUSIONS.

An artificial colonisation has been carried out on Puerto Stone, in which the antimicrobial capacity of the hydrophobic product Aquashield Active has been tested.

At the end of the test, the final colonised surface area was almost 100% in the case of the untreated substrate with any product. On the other hand, in the case of the treated with Aquashield Active, the surface area colonised by photosynthetic microorganisms has decreased by an average of 84,45%.

The colourimetric data are in line with those of the colonised surface. The chromatic change towards green, due to the development of chlorophyll, is on average 16 times lower on the treated surface than on the untreated surface.

Although Aquashield Active reduces the growth of photosynthetic microorganisms, the vitality of the organisms developed is the same as in the untreated areas, so the product is not a biocide, but it does delay microbial growth due to its capability to repel water, which is a limiting factor for the development of these microorganisms.

The manufacturer of the product guarantees its durability for 10 years. This test has been carried out for one year, and although colonisation has been artificially accelerated by intensified exposure to both humidity and photosynthetic microorganisms, it cannot be extrapolated to real time and fully natural conditions; however, patterns or trends that also occur in natural conditions can be extracted. Therefore, if over one year of constant moistening and inoculation of the material treated with Aquashield Active, the results are as expected, we can consider that its functionality can be guaranteed for 10 years.

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