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DEVELOPING A METHODOLOGY TO EVALUATE THE EFFECTIVENESS OF A BIOCIDE

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Abstract

The present study develops an improved technique for evaluating the effectiveness of different methods of application of a commercially available biocide containing a quaternary ammonium formulation to reduce biocolonization on building stone. In anticipation of cleaning biocolonization from the Smithsonian's National Museum of the American Indian, the biocide was test applied to surplus blocks of dolomitic limestone left from the building's construction. The new technique avoids subjective visual judgments by comparing areas based on digital photography using a special set up, with data processed using commercially available software. Statistical analysis quantifies differences between application methods.

Keywords: quaternary ammonium compound, biocide, photography, statistical evaluation, digital imaging

1. Introduction

The study originated in 2010 when the Smithsonian's National Museum of the American Indian (NMAI) requested assistance from the Museum Conservation Institute (MCI) in elucidating the source of blackening of stonework on its six-year-old building on the National Mall as well as determining a course for its remediation. Douglas Cardinale, a Native American architect, designed the building to suggest a natural rock formation. In order to achieve this appearance the building's buff-colored dolomitic limestone (quarried at Kasota, Minnesota) curves around vertical axes with more than 150 different radii. Blocks were fashioned using hydraulic chisels in a curved array, which broke the stone to create highly irregular 'natural' surfaces. Cleaning to remove localized black deposits was done only three years after the building's dedication in 2004, but disturbing dark streaks steadily increased in some areas, as well as more localized disfigurement. Preliminary studies determined that the black deposits resulted from biocolonization (Capitelli *et al.* 2012) on areas preferentially wetted by rainfall related to the building's non-traditional architecture (Grissom and Charola n.d.a).

Initially, two commercially available biocides approved for use on buildings in the USA were chosen. Both are based on unspecified quaternary ammonium salt formulations (Cathedral Stone Products' D/2 Biological Solution and PROSOCO's Enviro Klean BioWash). Laboratory testing with a Minolta CR-300 Chroma Meter showed no recognizable color changes for areas treated with either biocide compared to untreated areas three days after application. D/2 was chosen for testing on the building, however, and a contractor cleaned designated black areas on the building with the biocide in April 2011 (Grissom and Charola n.d.b). The preliminary testing described

below was carried out on spare blocks from the construction stored outdoors at the Smithsonian's Paul E. Garber facility in Suitland, Maryland, to determine the effectiveness of various cleaning protocols using the D/2 biocide.

2. Testing Protocol

The biocide D/2 was tested on three slightly curved spare blocks of Kasota limestone measuring approximately 76 x 19 x 10 cm. The undiluted biocide was applied to some test areas according to the manufacturer's recommendation, which includes scrubbing after application of the biocide followed by rinsing. Other test areas were treated with two additional variables: prewetting the stone before the recommended application of the biocide and application of the biocide without scrubbing and rinsing.

About one year after the first block was treated, 2.5 cm-diameter cores were drilled from the three blocks: ten on biocide treated areas and seven on untreated areas. The cores were examined with an optical microscope in cross section as well as on exterior surfaces (tops of cores). Cyanobacteria and green algae were identified microscopically, and the presence of chlorophyll was confirmed by alcohol extraction of scrapings from untreated surfaces. Biocolonization was observed to reach 0.1 mm depth on some cross-sections, confirmed by scanning electron microscopy. Comparison of representative exterior surfaces in Figure 1 shows the effectiveness of the biocide both with and without scrubbing (c and d) as opposed to a water scrubbing (b) or no treatment (a).

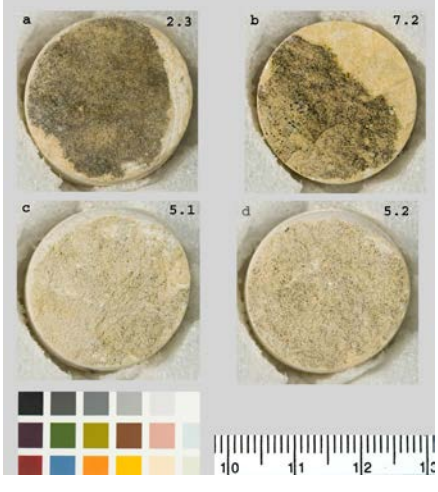

Figure 1. Examples of core tops. (a) A representative control surface. (b) A surface 10 months after water scrubbing. (c) A surface 13 months after biocide application without scrubbing. (d) A surface 13 months after biocide application with scrubbing. The scale at the bottom of the figure is in millimeters. Numbers in upper right corners of the photographs are sample designations.

To illustrate the new technique developed in this study for evaluation of biocide treated surfaces, the example of one test block is presented (Figure 2). The block was partitioned into six segments, each measuring approximately 13 cm wide; areas not

being treated were masked off with plastic during treatment of other areas. Half of the block (three areas) was prewet about 20 minutes before application of the biocide. D/2 was spray applied to two areas on each half of the stone. One biocide-treated area on each half of the stone was scrubbed after application of the biocide and rinsed, while the other biocide-treated areas on each half of the stone were not scrubbed or rinsed. Finally, one prewet area was scrubbed with water to evaluate the effect of mechanical removal alone, and one area was left untouched as a control. Left to right in Figure 2, the areas are untreated (U); biocide treated without scrubbing or rinsing (B); biocide treated with scrubbing and rinsing (B-S); prewetted, biocide treated, scrubbed and rinsed (P-B-S); prewetted and biocide treated without scrubbing or rinsing (P-B); and prewetted, water scrubbed and rinsed (P-S).

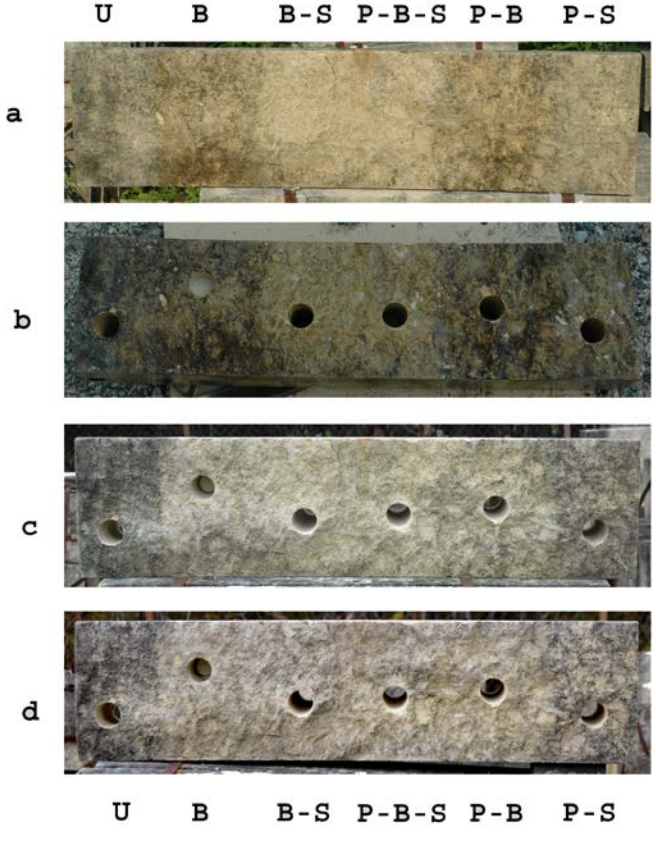

Figure 2. Photographs of the test block: (a) One day after treatment and just before coring; note slightly darker surfaces on the second (B) and fifth (P-B) areas where biocide was not rinsed off. (b) Just after coring with the block still wet from water used in the operation. (c) One year after application. (d) Two years and five months after application.

3. Visual Assessment Results

Table 1 gives a visual assessment for the block based on photographs taken at four points in time (Figure 2). The photographs show that the biocide was effective, but it is not possible to discern the contribution of scrubbing or prewetting on account of differences in light, humidity and camera angle during the photography. Thus, visual assessment provided at best a semiquantitative evaluation, which did not allow for a rigorous and unbiased assessment of changes.

Table 1. Visual assessment of changes in the block over time. Biocidal effect was assessed by cleaner appearance where + was the dirtiest appearance and +++++ the cleanest. BT= before treatment; AT= one day after treatment and (*) indicates greening on the surface.

	BT	AT	7 months	11 months
U	+	+	+	+
B	++	+++	+++	++++
B-S	++	++++	+++++ (*)	+++++
P-B-S	++	++++	+++++ (*)	++++++
P-B	++	+++	+++	+++++
P-S	+	+	+	++

4. Improved Evaluation Methodology

The new method of evaluation quantifies changes on the stone surfaces with the various treatments using information from a reproducible photographic setup. High quality close up photographs were taken of each of the six areas of the block 11 months after treatment application (Figure 3). The photography was carried out as follows: the camera (5D Mark II with Canon with 100mm f2.8 Macro @ f9, exposure 1/100 second, ISO 100) fitted with a ring flash (Canon MR-14EX Macro Ring Flash at 1/8 power) and reflector, was mounted on a tripod having an articulated arm to support the reflector. A black velvet shroud was used to cover both the object and camera lens to block out any ambient light. Photographs were taken without flash to demonstrate that no light penetrated the cover so that in the absence of the photographic flash the image was uniformly black. Photographs with flash of a grey card that filled the image area were taken to determine uniform lighting across the image, and they demonstrated that the light level was essentially the same brightness throughout (see Figure 3a at right).

The focus was set for the initial image, corresponding to one of the areas to be photographed, and was kept constant throughout the other five areas. The camera setup was manually moved laterally for each new area to be photographed. A ruler was included in each photograph as a reference and to determine comparative pixel dimensions in each of the six areas. A color calibration target (X-Rite ColorChecker Passport) was photographed in the same conditions to create a DNG (Adobe digital negative) profile for images. The Canon RAW format was used for the acquired images and converted to DNG. Adobe Photoshop CS4 from Lightroom 3 was used to place the six images in a single document. For each image area, a representative uniform subarea was chosen, measuring approximately 100 x 70 mm (see Figure 3a). RGB color and red channel data for the subareas were converted to grayscale and can be seen in Figure 3b and c with corresponding histograms. The subareas were saved into a new document in RGB format. Individual color channels gave similar histograms for the six treatment areas, but the red channel series showed greater differences (see Figure 4). For this

reason, the red channel histograms were selected for image analysis. The 14-bit raw color image file was converted into an 8-bit grayscale tiff image, and a new rectangular selection [104 x 73 mm (1275 x 900 pixels)], nearly equal to the original one, was used in ImageJ software to obtain data from the histogram (Rasband 1997-2008; Abramoff *et al.* 2004). These data were imported into MS Excel, and histograms for the six areas were plotted together as a function of the red channel grayscale values (Figure 5).

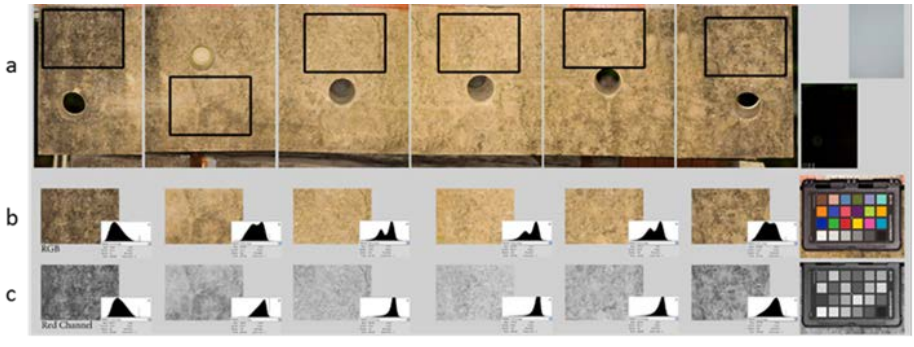

Figure 3. Photographs and histograms of the six areas on the block 11 months after treatment. (a) The six areas with subareas outlined; photographs taken of a grey card with flash (grey rectangle) and without light (black rectangle) are at right. (b) Subareas with histograms for RBG color. (c) Subareas with histograms for red channels. Color targets with greyscales in the bottom row are at right in b and c.

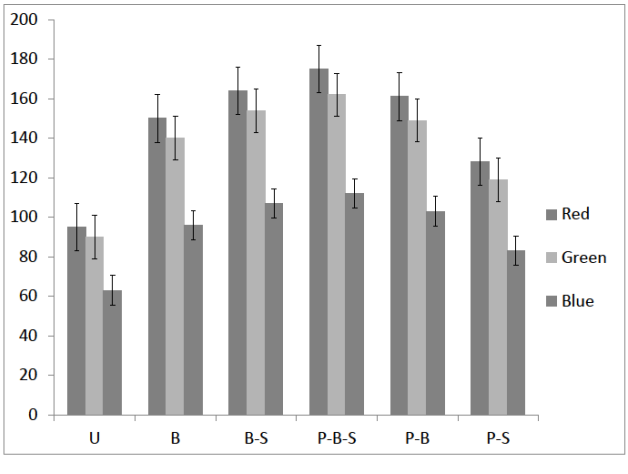

Figure 4. Means from histograms for the three RGB color channels in greyscale with standard deviations from photographs taken on the block 11 months after treatment.

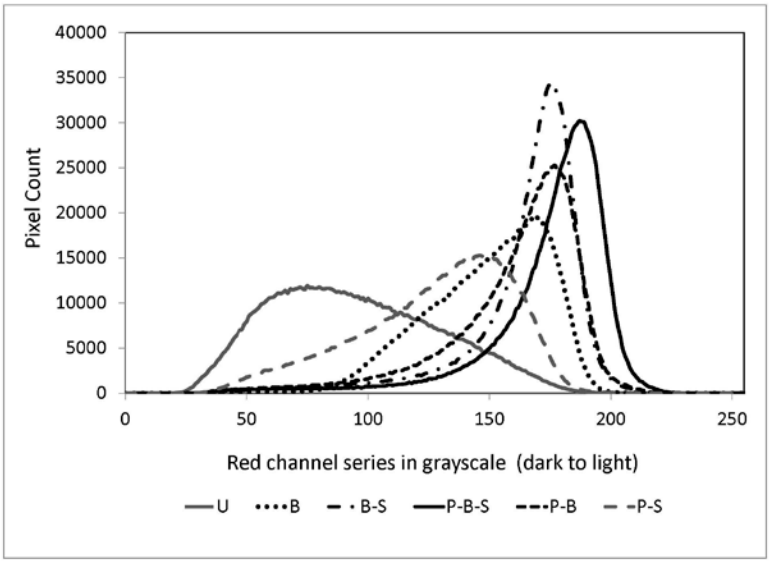

Figure 5. Histograms of pixel counts from the red channel values in grayscale for the six areas.

5. Results and Data Processing

The unimodal histograms for the red channels shown in Figure 5 were characterized by classical statistical measures, reported in Table 2. The mode (highest pixel count for a given curve) is at the lowest greygrayscale value for the untreated area (U) and is lower than the value of its mean, while the mode is at a value greater than the mean for all treated areas. The standard deviations are lower for all areas treated with the biocide than for the untreated (U) and prewetted scrubbed (P-S) areas. The standard error of measurement (SEM), also referred to as standard error of the mean, quantifies the uncertainty in the estimate of the mean (Glantz 1977). All biocide treated areas have lower SEMs and are better estimates of their means.

One of the problems encountered in the application of this method was that biocolonization was not homogeneously distributed between upper and lower areas of the block on account of non-uniform micro-environmental factors, such as incident light angle and water run-off, related to recession of the lower part. This would not have been a problem had subarea B been measured on the upper half like other subareas, but drilling of its core on the upper half precluded a large measurement area there. To test whether this made any difference, two smaller subareas (b) were measured (104 x 41 mm corresponding to 1275 x 500 pixels), one from for the upper part of the area (b_{up}) and one from the lower part (b_{low}). The latter falls within the larger subarea previously measured and serves as a control that data are reproducible.

Table 2. Values obtained from the histograms shown in Figure 5 through classical statistical analysis. In this table upper case letter designations are used to indicate original measurement areas; the lower case letter 'b,' the smaller subareas. The subscript 'up' indicates that the measurement area was on the upper portion of the stone; the subscript 'low', the lower portion.

	U_{up}	B_{low}	b_{up}	b_{low}	B-S_{up}	P-B-S_{up}	P-B_{up}	P-S_{up}
Mode	75	174	150	159	176	187	177	146
Mean	95	149	142	142	165	175	160	127
SEM	0.032	0.024	0.026	0.023	0.024	0.025	0.026	0.030
Stand. Dev.	34	24	20	24	25	26	27	31
Variance	1.2E+03	5.9E+02	4.2E+02	5.9E+02	6.3E+02	7.0E+02	7.6E+02	9.9E+02
Skewness	0.33	-0.58	-0.99	-0.33	-2.1	-2.2	-1.5	-0.62
Exc.Kurtosis	-0.65	-0.22	1.5	-0.58	5.3	6.6	2.7	-0.25

The histograms do not exhibit normal distributions. Thus, skewness and kurtosis were calculated to evaluate their lack of symmetry. Skewness refers to the third moment of a distribution, where the first and second moments are the mean and variance respectively. Skewness values are defined as:

$$skewness = \frac{\sum_{i=1}^{N}(Y_i - \bar{Y})^3}{(N-1)s^3}$$

where: $\bar{Y}$ is the mean, s the standard deviation, and N the number of data points.

The polarity of the skewness value indicates whether data are skewed positively or negatively with respect to a normal distribution that has a skewness of zero. All histograms for the treatment subareas are skewed negatively, that is, the trailing end is toward black, while only the untreated area (U) is skewed positively. The two red channel data sets most strongly skewed are the two biocide scrubbed areas (P-B-S and B-S). The prewetted biocide treated area without scrubbing (P-B) is less skewed.

Kurtosis is the fourth moment of a distribution of data and is a measure of the how peaked or flat a distribution is. Kurtosis values are defined as:

$$kurtosis = \frac{\sum_{i=1}^{N}(Y_i - \bar{Y})^4}{(N-1)s^4}$$

Because the kurtosis of a normal distribution is 3, *excess kurtosis* is reported, which is the quantity above minus 3. Positive excess kurtosis values represent a peaked histogram shape whereas negative values represent a relatively flat shape. Histograms of the untreated (U), biocide without scrubbing (B) and prewetted water scrubbed (P-S) areas have negative excess kurtosis values. Conversely, the prewetted biocide-scrubbed (P-B-S), biocide scrubbed (B-S) and prewetted biocide (P-B) treatments all have positive values in a descending order of magnitude. As with skewness, excess kurtosis increased as the treatment's mean and mode increased toward the lighter end of the grayscale.

Data culled from the histograms show that there is a small difference (about 5%) in the means of the original larger area (B_{low}) and the smaller area (b_{low}) within it, while the standard deviations and variance for both distributions are the same. A simple *F* test (the ratio of their variances) results equal to 1, the critical *F* value for infinite degrees of freedom (or N-1) with a confidence level of 99% or 0.01 probability level, suggesting that these two samples are not significantly different (Box *et al.* 1978; Snedecor and

Cochran 1983). On the other hand, the ratios of either bottom area (B_{low} or b_{low}) with the top area (b_{up}) show that the upper and lower areas are significantly different. Both skewness (-0.99) and kurtosis (1.54) for the upper area (b_{up}) fall in line with those of the other treated subareas.

6. Discussion and Conclusions

The methodology developed here utilizes a substantial quantity of greyscale image data—ranging from nearly 1,100,000 to over 600,000 pixels of red channel values for larger and smaller areas respectively—to analyze the effectiveness of various cleaning protocols using D/2 biocide. On the basis of visual assessment 11 months after treatment it appeared that the prewetted, biocide treated, scrubbed and rinsed (P-B-S) protocol was the most effective; furthermore, that biocide treatment with scrubbing (B-S) and with prewetting (P-B) produced nearly equivalent cleaning. Analysis of greyscale image data allowed us to compare treatments using statistical methods. For the example presented here, the most important comparisons were gleaned from unimodal image histograms and their shapes. Effective cleaning is positively correlated with larger mean grayscale and excess kurtosis of histograms for the areas and negatively correlated with skewness (Figure 6). P-B-S, B-S, and P-B had the greatest means and the most sharply pointed and narrow histograms (also apparent in Figure 5), as cleaning apparently pushed values closer to that of the light colored dolomitic limestone.

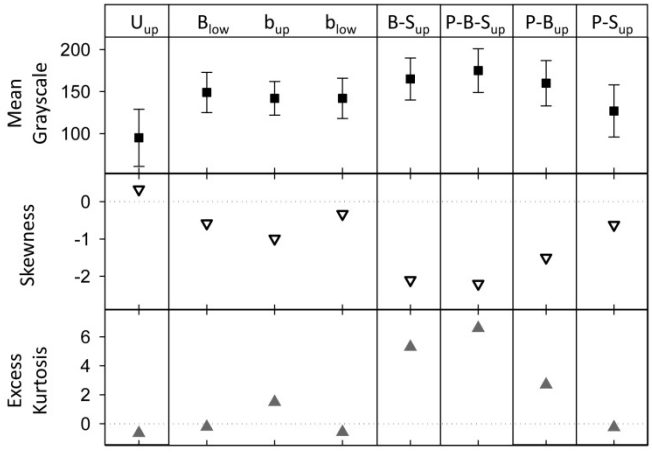

Figure 6. Statistical moments of red channel histograms for stone treatments. Mean grayscale (first moment) where error bars represent the standard deviation of the population is represented by filled squares. Skewness (third moment0 is represented by open inverted triangles. Excess kurtosis (fourth moment) is represented by grey filled triangles.

All areas were significantly different, as confirmed by the analysis of variance (ANOVA), Student-Newman-Keuls and chi-square statistical tests. In all of these statistical tests the largest significant differences were found between the untreated (U) and prewetted, biocide treated, scrubbed and rinsed (P-B-S) areas. To test if prewetting and scrubbing contributed to the action of the biocide, Bonferroni's ***t*** test, which allows

for comparison between samples with differing quantities of data, was applied the results used to rank the significance of these actions (Table 3). Scrubbing had a higher rank that prewetting, and these results suggest that the former has a greater influence than the latter. Scrubbing and prewetting did not have simple additive effects, however; on prewetted areas, scrubbing showed a smaller influence.

Table 3. Results for pairwise comparison of biocide treated area following Bonferrati's *t* test. The critical value for t at 99% confidence level (P= 0.01) is 2.58 (considering the area under the two tails of the ***t***-curve).

	Comparison of one vs two factors	Calculated ***t***	Comparison of two vs three factors	Calculated ***t***
Scrubbing	B_{up} and B-S	643	P-B and P-B-S	385
Prewetting	B_{up} and P-B	498	B-S and P-B-S	284

The study has developed a relatively simple methodology that is both rigorous and unbiased for evaluating changes resulting from the application of a biocide in various protocols. The method outlined here requires only a digital camera to obtain good photographs under controlled conditions and image processing/simple statistical programs needed to output the data. Our study shows that this technique is easily applied to light colored stones such as dolomitic limestone. Its application to more irregularly colored stones may require more advanced techniques to normalize image data, such as comparing data to those of freshly fractured stones. Two key points for future work would be (1) to determine the size of the smallest representative area given the heterogeneity of the surfaces and (2) to normalize image data for treatments with that of the stone substrate.

In summary, this preliminary study supports that application of the quaternary ammonium biocide is effective at removing biocolonization and that its application in conjunction with scrubbing and prewetting is even more effective. In addition, application of the biocide to a prewetted surface proved significantly more effective than application to a dry surface, as has been suggested by some researchers (Andrew *et al*. 1994: 22; Nugari and Salvadori 2002). Finally, although there were statistically significant differences, the degree of cleanliness achieved for a biocide treated and scrubbed area (B-S) and a prewetted biocide treated area without scrubbing (P-B) appeared similar, especially at almost one year after the biocide was applied. If this result is supported in further studies, it could have important implications for conservation treatments, given damage from repeated cleanings that incorporate mechanical operations such as scrubbing (Lazzarini and Laurenzi Tabasso 1986, Andrew *et al*. 1994: 68; Charola *et al*. 2007). A simple application of a biocide without scrubbing on surfaces mainly colonized by cyanobacteria and green algae has previously been shown to be effective and practically feasible (Charola *et al*. 2007, Delgado Rodrigues *et al*. 2011). While this approach is simple and cost effective, it requires time for results to be evident. Although this is a disadvantage for its application to buildings, it is an option that should be considered for buildings of great cultural significance, such as the National Museum of the American Indian.

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