Comparative Study of Commercially Available Cleaners for use on Federally-issued Headstones

National Cemetery Administration
Progress Update as of March 10, 2007
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This report provides information and progress on the comparative study of commercially available cleaners for federally issued headstones undertaken by the National Center for Preservation Technology and Training and the National Cemetery Administration through March 10, 2007.

1. Executive Summary

In 2004, the Department of Veterans' Affairs, National Cemetery Administration, and the National Center for Preservation Technology and Training entered into an interagency agreement to compare the effectiveness of commercially available cleaners for the removal of soiling and biological growth from Federally-issued headstones. The project goal was to test cleaning products for effectiveness and appropriateness and to make recommendations of products and methods best suited to both clean and preserve the headstones. Main tasks associated with the project were outlined in a project proposal and include both field and laboratory testing over a two-year period.

This study incorporates five national cemeteries that are distributed both geographically and climatically. Cemeteries included in this study are Alexandria National Cemetery in Pineville, LA; Bath National Cemetery in Bath, NY; Jefferson Barracks National Cemetery in St. Louis, MO; San Francisco National Cemetery, in San Francisco, CA; and Santa Fe National Cemetery, in Santa Fe, NM. Cemeteries were chosen to represent various regions of the National Cemetery Administration as well as different climatic zones. The cemeteries include sub-tropical, temperate, continental, semi-arid, and oceanic climates.

Water and five commercially available cleaners, including D/2Antimicrobial cleaner, Daybreak cleaner, World Environmental Group Marble cleaner, H2Orange Grout Safe cleaner, and Kodak Photo-Flo were evaluated at each test cemetery. Cleaners were applied to test patches on headstones carved from Colorado Yule marble and White Cherokee Georgia marble. Testing also included sunny and shady locations to help account for possible differences arising from local environmental variations.

Phase one of the study focused on field trials and ran from April 2005 to November 2006. Changes to headstone test patches as a result of cleaning with test cleaners were evaluated by appearance change and biological activity. Appearance changes were documented using photography and color measurements. Biological activity was documented initially and at six and twelve months after cleaning by enumerating bacteria, fungi and algae taken with BBL culture swabs from a three cm^2 area from each test patch. The color measurement data was evaluated by calculating the frequency of color changes where ΔE was greater than 5 and where ΔE was greater than 10. These values represent changes that may be perceived by the human eye. Biological activity was presumed to reflect the re-growth of micro-organisms six months after cleaning

(June 2006). The performance of test cleaners was evaluated based on biological activity and was ranked from one to six, with lower numbers indicating poorer performance. Biological activity was again evaluated twelve months after cleaning (February 2007) and the performance of four cleaners were evaluated and ranked.

Based on appearance change data and biological activity data, Kodak Photo-Flo was eliminated from further testing after six months. The greatest number of appearance changes for ΔE greater than 5 and ΔE greater than 10 was seen on test patches cleaned with Kodak Photo-Flo. This product was a poor performer at controlling bacteria in both sunny and shady locations in all cemeteries. It also ranked the lowest of all cleaners in limiting biological activity overall.

H_2Orange Grout Safe cleaner seemed to perform well based on color measurements and performance rankings of biological activity after six months. However, closer inspection of photographs taken from Jefferson Barracks National Cemetery indicated that biological staining was present on the edges of the headstone patches cleaned with H_2Orange Grout Safe cleaner. The location of the staining was not near the location of color measurements and was not reflected in the biological activity. Twelve months after the cleaning, all stains had disappeared. Researchers hypothesize that the H_2Orange Grout Safe cleaner did not kill all microbes initially and it took some time for all growth to die. Use of H_2Orange Grout Safe cleaner left an undesirable surface appearance for a period of time and thus was eliminated from the study.

Water and three cleaners remained – D/2 Antimicrobial cleaner, Daybreak cleaner, and World Monument Group Marble cleaner – as the project moved to phase two of the study. Appearance and biological activity continued to be documented for these cleaners in November/December 2006. Since further cleaners were being evaluated and the data from biological activity was more variable, few differences were noted. D/2 and Daybreak performed similarly in controlling overall biological activity. Researchers are concerned about possible chemical and physical changes from these cleaners, which are still under investigation.

Laboratory studies, including two accelerated weathering studies, were initiated. The first weathering study involved all six cleaners and two marble types. Lab test stones were cleaned on a daily basis for 33 days while being exposed to UV light, temperature cycles, and condensation cycles. These results were later considered to be too harsh, and a second accelerated weathering study was performed. In the second study, lab samples of Colorado Yule marble were cleaned and rinsed four times throughout the 33 day exposure.

Accelerated weathering samples are currently being evaluated for physical and chemical changes. Physical changes are being documented by changes in surface texture, color, and porosity. Chemical changes are being examined by optical microscopy, X-ray fluorescence spectroscopy, scanning electron microscopy, and determination of total soluble salts using gravimetric and conductivity methods. Accelerated weathering

samples cleaned with D/2 and Daybreak show some evidence of efflorescence which is being investigated.

Lab test samples were placed beside field test headstones during phase one of the study. They have been retrieved and were received at NCPTT on April 2, 2007. They will undergo evaluation similar to that described for accelerated weathering samples above. Phase two of the project will continue through fall 2007. A student intern will be assigned to assist in the analysis of lab and field test stones.

Researchers associated with the project, including scientists at NCPTT and biologists at the Laboratory of Applied Microbiology, Harvard University, are concerned than an eighteen month time period may not have been sufficient to document significant visual changes or to allow for the growth of algae and photosynthetic bacteria. The absence of algae and photosynthetic bacteria is significant. These organisms typically provide the most visual evidence of growth on headstones. Their absence, even from stones treated with water, suggest it is still too early to determine the effectiveness of any biocidal properties of the cleaners.

While some results can be obtained by the expected completion date of October 2007, continuation of the study for two additional years is recommended. NCPTT staff present four possible options for continuation and the study in this report, and other options are available. It is advisable that some decision regarding extending the study be made prior to June 2007 (the date of the final field trip to the cemetery test sites).

2. Background

The Department of Veteran Affairs provides patient care and veteran's benefits – including burial-related entitlements – to 70 million veterans and eligible family members. An agency of the Department of Veteran Affairs, the National Cemetery Administration maintains 3.6 million occupied gravesites in its 120 national cemeteries and 33 soldiers lots, which total more than 14, 250 acres.

Visitors to national cemeteries expect to find the burial grounds well-cared-for and looked after. Part of this expectation is that the headstones are well-aligned and display a pristine, white appearance. These beliefs lead to relatively frequent cleaning of federally issued headstones, particularly compared to cleaning efforts undertaken in private cemeteries. Over time national cemetery staff and visitors have noticed a deterioration of stones from weathering. When headstones show significant loss of legibility or deteriorating conditions, the headstones are replaced.

One contributing factor to the weathering of stones may be the selection and use of chemical cleaners on a regular basis. C. Price notes that cleaning is one of the first steps in the conservation of stone and leads to improved appearance. However, cleaning with unsuitable cleaning methods can damage the stone by the loss of surface, staining,

deposition of soluble salts, or making the stone more vulnerable to pollution or biological growths.[1]

Within the fields of conservation and historic preservation, guidelines for the care of cultural resources, such as cemetery headstones, have been established based on ethical considerations.[2,3] First and foremost, a conservation treatment, such as cleaning, should do no harm. Staff and volunteers undertaking the cleaning should choose the gentlest and least invasive methods. Guidelines also recommend that those undertaking the work should not use chemicals without thorough understanding of how those chemicals react to the materials of the artifact and any material that may have been applied later.

On December 16, 2003, the National Cemetery Administration took the lead to organize an interagency task force to develop solutions to shared issues of interagency responsibility for historic government-provided headstones in an effort to supply consistent service to the American public in keeping with agency policy and mission. Topics included definitions of what is "historic," the science and technology of appropriate cleaning, and when to repair or replace. One outcome of this task force was the identification of the need for scientific research on cleaning methods for headstones.

Based on observations in national cemeteries, documentation in conservation literature, ethical considerations, and recommendations of the Interagency Task Force on Government-Issued Headstones, this research study was devised through collaborative efforts of the National Cemetery Administration and the National Center for Preservation Technology and Training.

2.1. Purpose of Study

On September 13, 2004, the National Cemetery Administration and the National Center for Preservation Technology and Training entered into an agreement to study the effectiveness of commercially available cleaners to remove biological growth from federally-issued headstones. The project goal was to test cleaning products for effectiveness and appropriateness and to make recommendations of products and methods best suited to both clean and preserve the headstones.

Cleaners in this study are evaluated based on multiple criteria to include:

- Appearance immediately after cleaning and over time,
- Physical changes to the stone, such as surface roughness or porosity,
- Chemical changes to the stone, such as chemical interactions with the cleaners or residual chemicals left on the stone,
- Biological activity after cleaning and over time, and

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[1] Price, C.A., 1996, *Stone Conservation, an Overview of Current Research.* Santa Monica, CA: Getty Conservation Institute, J. Paul Getty Trust, pp 7-14.

[2] *Code of Ethics and Guidelines for Practice of the American Institute for Conservation of Historic and Artistic Works*, revised 1994, Washington, DC: AIC. http://aic.stanford.edu/about/coredocs/coe/index.html

[3] *The Secretary of the Interior's Standards for Rehabilitation & Illustrated Guidelines for Applying the Standards (1992),* National Park Service, Washington, DC. http://www.cr.nps.gov/hps/tps/tax/rhb/index.htm

- Ease of use and suitability for large-scale cleaning projects.

Main tasks associated with the project include both field and laboratory testing over a two-year period. The project is designed as a two phase project.

Phase one of the study includes the selection of cleaners and national cemetery test sites within five NCA regions or Memorial Service Networks. One aspect of the research looks at chemical cleaners that represent a variety of cleaning actions, for example basic versus acidic cleaning or ionic versus non-ionic cleaning. The research includes different geographic and climatic regions, such as a semi-tropical versus a dry or temperate climatic zone. Finally, cleaners are tested on two different types of marble – Colorado Yule marble from Marble, Colorado and White Cherokee marble from Tate, Georgia.

Five cleaning products are tested in side-by-side test patches on headstones in sunny and shady areas of each cemetery. Concurrent with the test patch studies, a series of cut marble samples are treated with each of the five products and exposed beside the test patch stones. These samples are used in both non-destructive and destructive laboratory testing. These laboratory samples help detect residual cleaning products on the stone and aide in evaluating potential stone deterioration.

Phase two of the study is based on results of the test patch evaluations after at least nine months of study. Based on phase one, three cleaning products are further tested on whole headstones. Whole stone studies allow for further evaluation based on visual appearance and ease of use.

3. Phase One of Study

Phase One of the study can be described in terms of planning and implementation and has distinct tasks associated with each activity. Planning activities included the choice of:

- Cleaners,
- Cemeteries,
- Headstones, and
- Evaluation methods for change in appearance and biological activity.

Implementation of the plan included

- visiting each cemetery,
- identifying and documenting headstones in sunny and shady locations within each cemetery,
- taking selected headstones out of regular maintenance cycles,
- making initial biological swabs for each headstone to establish baseline biological activity,
- making color measurements at each test patch to establish initial appearance,
- cleaning test patches on each headstone with each of the cleaners,
- monitoring the change in appearance through photographs and color measurements over time,
- monitoring biological re-growth through biological testing over time, and

- evaluating the data.

Phase One of the study began April 13, 2005 with a meeting of the partners held in the ASAE building at 1575 Eye Street, Washington, DC. Attendees at the meeting included Sarah Amy Leach, Karen Ashton, and Dave Schettler from NCA and Mary Striegel from NCPTT. Jason Church (NCPTT) and ElizaBeth Guin (Northwestern State University) participated by conference call. The purpose of the meeting was the selection of cleaning products and the identification of cemeteries to include in the study.

3.1. Choice of Cleaners

The most important variable in the study was the choice of cleaners. The team investigated fifteen possible cleaners for inclusion in the study. Possible cleaners are shown in Table 1. The chemical action of these cleaners includes acids, bases, alcohols, chelating agents, solvents, surfactants, and bactericides.

Acids and bases are strong chemical cleaners that work on the basis of the pH of the product. Products such as the Stone Kleen contain ammonium bifloride that easily converts to hydrofluoric acid, a strong acid that can chemically etch and potentially damage the surface of a stone. On the other end of the spectrum is the Kandu product, a low foaming cleaner containing sodium hydroxide, a basic compound.

Alcohols work on the basis of dissolving dirt and grease. They tend to evaporate quickly and are less likely to leave chemical residues. They may be one component of a multi-component cleaning system. Four of the products, including World Environmental group Marble Cleaner, incorporate alcohols into their formulas.

Chelating agents work on the premise that the cleaner will bind the dirt or grime to itself in order to remove the soiling. Some products containing chelating agents include Stone Quest, Zep-A-One, and World Environmental Group Multi Surface Cleaner 1000.

Solvents work similarly to alcohols in that they dissolve the soiling and may be a component of a more complex cleaning system. Only the product GK125 is listed as a solvent-based cleaner. Solvents are incorporated into some of the other cleaning systems.

Surfactants are wetting agents that lower the surface tension on a liquid allowing for easier spreading. Surfactants also allow grease and oils to be diluted and mixed into water and washed away. They are commonly found in cleaning detergents. Stone Quest, Multi Surface Cleaner 1000, Zep-A-One, World Environmental Group Marble Cleaner, D/2, Kodak Photo-Flo, and Kodak Hypo Clear all contain surfactants.

Bactericides are chemicals that kill bacteria and are commonly found in disinfectants, antiseptics, or antibiotics. One group of bactericides contain cationic surfactants such as quarternary ammonium cations. The D/2 product is an example of a bactericide containing quaternary ammonium cations. Another group of bactericides contain strong acids. Stone-Kleen is an example of a strong acid bactericide.

Product Name							
	acidic	basic	alcohol	chelate	solvent	surfactant	bactericide
Stone Quest Stone Care International				X		X	
GK125 Geokleen Inc.					X		
Multi Surface Cleaner 1000 World Environmental Group, Inc.			X	X		X	
Omni-Green National Plastics and Chemical Corp.							
Stone-Kleen Mid Atlantic Chemical	X						X
$H_2Orange_2$ Grout Safe Proven Solutions	X						
Hurricane Intensive Stone Cleaner National Chemical Laboratories			X		X		
Zep-A-One Zep Manufacturing, Co.				X	X	X	
Marble Cleaner World Environmental Group, Inc		X	X	X		X	
Kandu #110 SpaceAge Coating Concepts, Inc.		X					
Daybreak NCH Corporation, Certified Labs		X					
D/2 Sunshine Makers, Inc.						X	X
Sodium Bicarbonate							
Kodak Photo-Flo Kodak Corporation			X		X	X	
Hypo Clear Kodak Corporation				X		X	X

Table 1. A listing of chemical cleaners considered for testing, including main

The five cleaners chosen for inclusion in the study are shown in Table 2. The team wished to include cleaners that were environmentally friendly, user friendly, and were unlikely to damage the stone. Cleaners containing strong acids and bases, such as Stone-Kleen and Kandu, were eliminated on this basis. Daybreak was the most commonly used cleaner within the NCA, and thus was included in the study. $H_2Orange$ Grout Safe was chosen to represent an acidic cleaner containing citric acid. D/2 Antimicrobial cleaner was chosen as a bactericide and cleaner. The team felt that the two products by the

World Environmental Group were very similar in nature and relied on surfactants and chelating agents. The World Environmental Group Marble (WEG Marble Cleaner) was chosen for inclusion in the study. The final cleaner selected was the Kodak Photo-Flo because of its common use in the cemetery cleaning world. Table 3 shows the range of pH values found for the products chosen for the study.

	pH	Acidic	Basic	Alcohol	Chelate	Solvent	Surfactant	Bactericide
D-2	9.5						**X**	**X**
Daybreak	12.1		**X**					
H2Orange2 Grout Safe	3.81	**X**						
Kodak Photo-Flo	7			**X**		**X**	**X**	
Marble Cleaner	10.5		**X**	**X**	**X**		**X**	

Table 2. A listing of chemical cleaners chosen for the study, including published pH and component ingredients.

Cleaner	H2Orange2 Grout Safe	Kodak Photo-Flo	D-2	Marble Cleaner	Daybreak
pH	3.81	7	9.5	10.5	12.1

Table 3. Chosen Cleaners are ordered from Acidic to Basic.

3.2. Choice of Cemeteries

The second major variable in the study was the choice of locations for testing the cleaners. Did the biological growth found on headstones differ by location? Would bacteriai, algaes, or fungi dominate in some locations and not in others? How would climatic differences affect cleaning decisions? Would some cleaners perform better in some geographic areas and worse in others? To look at these issues, the team felt it was important to choose cemeteries that were geographically and climatically distinct.

Climate is the trends in weather patterns over an extended period of time. Two of the most important factors determining an area's climate are air temperature and precipitation. One way to classify climatic zones is using the Köppen Climate classification system. Within this system, five major climate types are classified based on average temperatures and precipitation, and designated by a capital letter. Subgroups are designated by a second, lower case letter which distinguish specific seasonal characteristics of temperature and precipitation. Further variations are noted by additional subgroups.[4]

In addition to climatic zones, NCA cemeteries are assigned to Memorial Service Networks (MSNs) based on their geographic location. The MSN offices are located in

[4] Köppen Climate Classification System, see http://www.blueplanetbiomes.org/climate.htm, and http://en.wikipedia.org/wiki/Koppen_climate_classification.

Philadelphia (MSN 1), Atlanta (MSN 2), Denver (MSN 3), Indianapolis (MSN 4) and Oakland (MSN 5).

Cemetery	History	Climate Zone	Climate Description
San Francisco National Cemetery, San Francisco, CA MSN 5 – Oakland, CA	First burial: 1850 The site was formerly part of an military post established by the Spanish, continued by Mexico, and seized by the United States Forces during the Mexican War.	Zone Csb, using the Köppen Climate classification system, Mediterranean Climate	This region is characterized by temperate wet winters contrasting with warm or hot summers. The average annual rainfall is between 15 and 55 inches and occurs between November and April.
Santa Fe National Cemetery, Santa Fe, NM MSN 3 – Denver, CO	First burial:1868 Original interments are the remains of 265 United States Soldiers for the battlefields of Glorieta, Koslouskys, and the Old Fort March (General Kearney's Camp of 1847).	Zone Bsk, Semi-arid steppe climate	The steppe climate is characterized by hot summers and cold winters with 10 to 20 inches of rain or snowfall a year. It is similar to a praire.
Jefferson Barracks National Cemetery St. Louis, MO MSN 4 – Indianapolis, IN	First burial: 1827 The national cemetery included the old Post Cemetery containing burials made as early as 1827 from the Garrison of Jefferson barracks.	Zone Dfa, Humid continental	This region is characterized by a humid, cold climate with harsh winters and year-round precipitation.
Alexandria National Cemetery, Pineville, LA MSN 2 – Atlanta, GA	First burial: 1867 The cemetery contains burials from the civil war through the present.	Zone Cfa Humid Sub-tropical	This region is characterized as a mild climate with no dry season, and a hot summer
Bath National Cemetery, Bath, NY MSN 1 – Philadelphia, PA	First burial: 1879 The cemetery was originally a part of the New York State Soldiers and Sailors Home, which was established in 1877	Zone Dfb Humid continental	This region is characterized as a humid climate with severe winter, no dry season, and a warm summer.

Table 4. Cemeteries chosen for this study, assigned to a typical climatic zone.

Based on climatic and geographic distribution, five cemeteries were chosen for the study (see Table 4.) They include San Francisco National Cemetery, Santa Fe National

Cemetery, Jefferson Barracks National Cemetery, Alexandria National Cemetery, and Bath National Cemetery.

3.3. Selection of Headstones

The next step in the study was the selection of headstones from each cemetery to be included in the research. In this process, the team considered the following questions:
1) Will the type of marble make a difference in the removal or regrowth of microorganisms and biological activity?
2) Will localized environmental conditions such as sun or shade, or orientation in the cemetery affect the regrowth?
3) Are there seasonal effects for cleaning? For example, is it better to clean in the spring or fall?

There are three main stone types commonly used to create federally-issued headstones. These stone types include 1) Imperial or Royal Danby, a white or bluish white marble form Danby , VT; 2) White Cherokee, a white-grayish marble from Tate, Georgia; and 3)Colorado Yule, a white-creamy marble from Marble, CO. Of the three stone types, the White Cherokee is the most easily recognizable based on its color and large grain size. The Royal Danby and the Colorado Yule are less easily distinguished. The team recommended where possible that testing be performed on two types of stone in each cemetery. One set of tests should include the White Cherokee Georgia Marble. The second set of tests should include Royal Danby or Colorado Yule marble.

Testing also included sunny and shady locations to help account for possible differences arising from local environmental variations. Thus, half of the White Cherokee Georgia Marble headstones included in the study should be located in predominantly shady locations while the other half should be located in predominantly sunny locations within each cemetery. The same criteria also applied to the second set of Royal Danby/Colorado Yule headstones.

Finally, in order to determine if seasons affected cleaning and biological regrowth, one set of headstones were cleaned in the spring and on set of headstones were cleaned in the fall.

Once the testing criteria were established, Sarah Amy Leach and Karen Ashton contacted each cemetery director and informed them about the testing program in June 2005. They created a one page briefing sheet and an informational Q&A document for the project in order to educate VA staff and visitors to the cemetery about the study. Additionally, informational signs were installed at each cemetery.

Figure 1. Example of the informational sign placed in each cemetery for the duration of the study.

3.4. Evaluation Methods for Change in Appearance and Biological Activity

The emphasis during phase one was on the visual appearance of the stone before and after cleaning and on the amount of biological re-growth over time. The five cleaners were evaluated based on patches tests on stones in sunny and shady locations within each cemetery. These evaluations were used to eliminate or retain cleaners for phase two of the study.

3.4.1. Visual Appearance

The appearance of the headstone is defined as the outward or visual aspect of the stone and is considered a subjective judgment of the viewer. Since the appearance of the stone is particular to a given individual, it is influenced by cultural bias. In general, military headstones are expected to be clean, white, legible, and well-aligned. Thus unacceptable appearance includes gray, yellow, black, or mottled coloring from either biological growth, dirt, or chemical changes. In all phases of the study, two methods for documentation and analysis of appearance are proposed – use of photo-documentation coupled with visual ranking and color measurement.

3.4.1.1. Photodocumentation/Visual Ranking

Photography is the way information in the form of light documented from a subject, in this case a headstone. It is an easy way to document information from a site or location and covey it to others. However, the environmental variables and instrumental variables can affect the way the light is captured in the photograph. Thus, when documenting the headstones and cleaning patches with photography, some variability in the images will be due to the time of day in which the photograph was taken, and the camera settings (e.g. aperture, focal length, shutter speed, etc.). Also, the way in which the photograph is perceived varies from viewer to viewer.

Because of these considerations, photo documentation is a qualitative method for studying appearance. One way to deal with this qualitative information is to have viewers rank what they see in photographs based on a set scale. The viewers should know little about the subject prior to the ranking, making them unbiased viewers. This produces a way to evaluate the information recorded in a photograph which is semi-quantitative.

3.4.1.2. Color Measurement

Being able to quantify the color and surface appearance of stones is a crucial factor in this study. Color is a physiological process by which the human eye translates electromagnetic radiation. It is generally dependent on the observer, the object, and the environment in which the object is viewed.

A colorimeter is an instrument that measures red, blue, and green color components of light and is used to determine a specific color reflected from a surface. The color is specified in numeric terms using the CIELAB color system. Colors are specified in terms of L*, a*, and b*. The L* values represent lightness and can range from 0 to 100, with 0 designating black, and 100 designating white. The a* values represent the red-green chromatic component. Values of a* range from -100, designating green to 100, designating red. The b* values represent the yellow-blue chromatic component, with values ranging from -100 to 100. A pure yellow is represented by 100 and a pure blue is represented as -100 on the B* scale.

The CIELab system lends itself well to measuring change sin color over time. The total color difference, ΔE*, can be calculated from:

$$\Delta E^* = (\Delta L^{*2} + \Delta a^{*2} + \Delta b^{*2})^{1/2}$$

ΔL^* is the lightness value difference between color 1 and color 2, $= L^*_1 - L^*_2$
Δa^* is the red-green value difference between color 1 and color 2, $= a^*_1 - a^*_2$
Δb^* is the yellow-blue value difference between color 1 and color 2, $= b^*_1 - b^*_2$

Equation 1. The total color difference between two Lab color measurements.

A total color difference of less than 2 ΔE* is imperceptible to the human eye.

Color measurements of L*, a*, and b* are taken of the headstones prior to cleaning and documented. Since the surface of the stone is not completely smooth, three measurements are taken at each location then averaged. Measurements are repeated at each cleaning test site on regular intervals throughout the study.

3.4.1.3. Biological Testing

A general overview of the biological testing is presented here. Details of the biological testing can be found in Appendix D, Appendix E, and Appendix F. The team determined the biological testing scheme for the study in consultation with Dr. Ralph Mitchell, Department of Engineering and Applied Science, Harvard University. Initially, NCPTT

scientists proposed the identification of biological species present on a large number of headstones. However the actual number of samples to be taken and the time and effort to complete the biological analyses would have resulted in over 63,000 hours of work and was dismissed as untenable. Mitchell recommended general identification of bacteria, fungi, and photosynthetic microorganisms (algae) found on headstone prior to cleaning. Then, over time, counts of bacteria, fungi, and algae would be determined for test patches each cleaner in sunny and shady locations.

To determine the baseline biological activity, swabs are taken from a three cm^2 area of each test patch using BBL Culture Swabs (Becton-Dickinson, Sparks, MD). Bacteria and fungi are enumerated by plating samples on solid media. Plates are incubated at room temperature for two days and colonies are counted. Photosynthetic microorganisms (algae) are analyzed using a hemocytometer. The numbers of algae in at least 10 fields of view are counted at 40X magnification.

3.5. Field Test Trials on Headstones

Once the planning activities of phase one were complete, implementation tasks were begun. Jason Church initiated the first of a field trip series beginning in June 2005[5] to each of the test cemeteries. The purpose of these first trips was to initiate contact with each cemetery staff, to identify headstones for inclusion, to set field test samples for phase two of the study, and to take overview photographs of each cemetery for the study.

All stones selected for inclusion in phase one were taped in a grid system that created six test patch sites.

3.5.1.Documentation of Headstones

Headstones were photographed before cleaning and at six month intervals after cleaning throughout phase one of the study. All photographs were taken digitally and saved in JPEG format. Photographs were taken in October 2005, April 2006, and November 2006.

In October 2005, photographs were taken using a Sony DSC-S85 digital camera. The camera has a built-in 34 mm-102 mm zoom lens. All images were taken at 2272 x 1704 pixel resolution on auto-exposure and auto-focus settings.

All subsequent photographs of headstones, taken in April 2006 and November 2006, were taken with a Nikon D50 digital camera body fitted with an AF-S 18-55 mm zoom lens. Images were captured at 3008 x 2000 pixel resolution (Large, JPEG Fine) at an approximate 45 mm lens focal length.

Appendix A, *Photographic Documentation of Field Trials*, contains a series of photographs taken in six month time intervals each headstone in phase one of the study. An overview shot and details of each test patch are found for each headstone prior to cleaning and every six months as the study progressed.

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[5] Phase one field trips included Jason Church's site visits on June 5-11, 2005 to San Francisco CA, Santa Fe NM, St. Louis MO, and Bath NY. He visited Alexandria LA on April 25, 2005 and June 22, 2005.

Figure 2. Jason Church positions the head of the Minolta colorimeter for measurements on a headstone in Alexandria National Cemetery.

Upon completion of the photo-documentation, color measurements were taken using a Minolta Colorimeter, CR-400. Each measurement was repeated three times on each stone sample and averaged in order to compensate for slight variations in surface texture. Color measurements were consistently taken at the same location– the lowest point on the inside corner – within each grid (see Illustration 1).

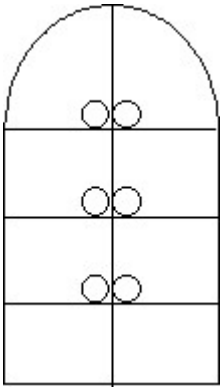

Illustration 1. The circles indicate the location where color measurements were taken within each test grid.

Appendix B, Color *Measurements on Field Trials*, provides measurement data for all color measurements taken on headstones in the field.

3.5.2. Initial Biological Activity

Initial biological activities were determined by culturing swabs taken from selected headstones within each cemetery. The purpose of these analyses was to establish the level of biological activity prior to any cleaning performed in this study.

Samples were collected by Jason Church from the five chosen cemeteries. Within each cemetery, samples were collected from 20 locations. A three cm^2 area of the tombstones were sampled for microorganisms using BBL Culture Swabs (Becton-Dickinson, Sparks, MD). Samples were shipped overnight to Harvard University.

Figure 3. Photographic detail showing an acetate template being used as a guide for swabbing the headstone.

Results from this study are found in Appendix D. *Analysis of Microorganisms on headstones in VA Cemeteries, First Report: December 2005* and are summarized here. Bacteria and/or fungi were found in most samples in all five cemeteries. Algae, which are photosynthetic organisms capable of darkening or staining the headstones, were not found in samples taken during this phase of the study. The decreasing order of biological activities was:

Santa Fe > Jefferson Barracks > Alexandria > San Francisco > Bath

Santa Fe National Cemetery displayed the largest amount of bacterial and fungal activity of the five cemeteries, which was five times greater than any other location. Jefferson Barracks results showed small quantities of fungal growth on all but one headstone. Fungi were found on headstones in both sunny and shady locations. Bacterial counts were limited to a few headstones in Jefferson Barracks. In Alexandria, more bacterial and fungal activity was seen on headstones in shady locations over sunny locations. Bacteria were not detected in many samples from San Francisco National Cemetery, but when found, were more likely to be seen in sunny locations. In contrast, bacteria and fungi were detected in few samples from Bath National Cemetery.

Initially, the presence of higher biological activity at Santa Fe National Cemetery seemed counter-intuitive. Santa Fe is a drier climate and little biological soiling had been observed in the cemetery. Locations such as Jefferson Barracks or Alexandria would be expected to have richer environments for biological growth due to their climates and higher relative humidities. Additionally, a visual survey of private cemeteries in these regions showed typical biological growth, see Figure 4.

Figure 4. Example of biological growth found on a grave marker in the Jewish Cemetery, Pineville, Louisiana which is located four blocks from Alexandria National Cemetery.

It is important to note before evaluating results from initial biological analyses that each cemetery has its own regular maintenance schedule which will influence the nature of the biological activity on headstones from that cemetery. Jason Church documented the cleaning activities of each cemetery by interviewing staff and maintenance crews (see Table 5).

Cemetery	Cleaner Used	Periodic Schedule	Methods
Santa Fe National Cemetery	Zep Ring Master All Purpose Bathroom Cleaner	Spot cleaning as needed	Applied with portable sprayer and rinsed thoroughly
Jefferson Barracks National Cemetery	50% Clorox and 50% water	Annually	Applied with pump sprayer, or Backpack sprayer. Left un-rinsed.
Bath National Cemetery	50% Clorox and 50% water	Annually, with spot cleaning as necessary	Applied with pump sprayer, or Backpack sprayer. Left un-rinsed.
San Francisco National Cemetery	40% Clorox Outdoor and 60% water	Total cleaning once a year with pressure washing as needed	Applied with portable sprayer. Left un-rinsed.
Alexandria National Cemetery	HTH Granular, mixed with water to an unknown concentration	Annually, with spot cleaning usually 8 months after	Applied with portable sprayer. Left un-rinsed.

Table 5. Cleaning schedules and use for Santa Fe, Jefferson Barricks, Bath, San Francisco, and Alexandria National Cemeteries.

Santa Fe National Cemetery cleans headstones infrequently using a highly acidic product, Zep Ring Master Bathroom Cleaner[6] for spot cleaning. Since the cleaner is a green liquid Santa Fe maintenance workers rinse thoroughly after cleaning. Jefferson Barracks National Cemetery cleans headstones annually using a 50/50 mixture of Clorox and water. The cleaner is applied with a backpack sprayer and left un-rinsed. Bath National Cemetery follows a similar cleaning regiment, cleaning once a year with the 50/50 Clorox mixture and following with spot cleaning as necessary. The cleaner is applied by sprayer and not rinsed after cleaning. A 40/60 mixture of Clorox Outdoor and water is used by the San Francisco National Cemetery to clean headstones using a portable sprayer. Headstones are not rinsed after cleaning. Alexandria National Cemetery uses HTH Granular, a calcium hypochlorite product commonly used for swimming pool treatments, to clean headstones.

Upon closer consideration of the data and the cyclic maintenance undertaken at each cemetery, logical conclusions could be drawn. This study did not begin with sterile stones inoculated with similar bacteria, fungi, and algae. The biological activity is a complex system influenced by seasonal changes, a variety of biota, the nature of the stone, and the history of headstone cleaning at each cemetery.

Santa Fe National Cemetery staff rarely cleans its headstones and then they undertake only spot cleaning as needed. Thus, a rich bio-film has developed over time on headstones in Santa Fe. Despite this biofilm, the stones appear clean because there is a lack of algae – the photosynthesizing organisms that can produce staining – or low numbers fungi.

In contrast, those cemeteries whose environments are likely to promote biological growth, such as Alexandria National Cemetery or Jefferson Barracks National Cemetery, are cleaned much more frequently in order to keep the stones white. In these places, HTH Granular (calcium hypochlorite) or bleach (sodium hypochlorite) is used for cleaning and left on the surface. After several cleaning cycles, the stones show much less biological activity.

The following general conclusions can be drawn:

- Bacteria and/or fungi were found in most samples.
- Numbers of bacteria were generally greater than numbers of fungi.
- Algae were not detected in the samples.
- Analysis of microbial growth showed wide variability in the size of the microbial community.
- Numbers of bacteria and fungi were low in most samples and may be due to the historical cleaning cycles the stone has seen.
- The presence of high numbers of bacteria and fungi at Santa Fe National Cemetery is likely due to its infrequent cleaning.

[6] According to the published Materials Data Safety Sheet, Zep Ring Master has a pH of less than 1.0 and contains phosphoric, hydrochloric, and sulfuric acids (which can cause sugaring and loss of binder in marbles and limestones.)

3.5.3. Cleaning Headstones

Jason Church began cleaning headstones after (1) documenting their visual appearance using digital photography and colorimetry and (2) sampling their surfaces for biological activity.

Figure 5. Jason Church applies WEG Marble Cleaner to a taped test patch. He holds a piece of acetate to the stone surface to prevent runoff below the patch. All cleaners were applied following manufacturer's recommendations for dwell time, etc. The stones were subsequently rinsed with water, again using the acetate to prevent runoff.

A grid was taped onto each stone using one inch wide 3M blue tape. Cleaners were applied to the surface of the headstone following manufacturers' recommendations. An 8.5 x 11 inch acetate sheet was used to insure that cleaner did not run to a second test grid. If the stone had raised or engraved lettering, the acetate was taped to the irregular surface. Cleaners were applied in the same test grid on each stone, as shown in Illustration 1 After each test patch was cleaned, the area was thoroughly rinsed with tap water.

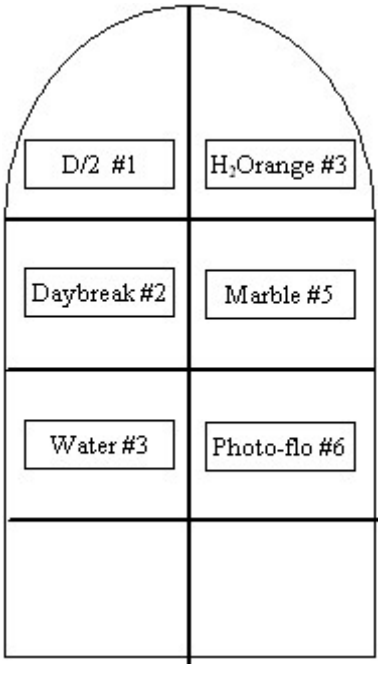

Illustration 2. This illustration shows the position of each cleaner on a headstone.

3.5.4. Appearance Changes

Based on visual observations, all cleaners effectively removed soiling and biological growth from the stone. Water removed soiling and to a much lesser extent staining from micro-organisms. Changes in appearance were recorded by photographs and color measurements. In general, there was natural variability in the results. NCPTT staff evaluated color change and visual appearance between April 2006 and November 2006, representing changes from six months to twelve months after cleaning. Most changes in appearance during this time were subtle.

Changes in color measurements were calculated from CIElab coordinates as ΔL^* (changes in lightness or darkness), Δa^* (changes towards red or green), Δb^* (changes towards blue or yellow), and ΔE (total Color change). These results are reported in Appendix B. *Color Measurements on Field Trials*.

Further evaluation of the data focused on establishing color change trends by counting the frequency of color changes at ΔE greater than 5, and ΔE greater than 10. Again, any changes of color observed were subtle to the human eye. Researchers looked at frequency trends compared by cemetery, by cleaners, and by sunny or shady location. The results are reported in Appendix C. *Color Analyses by Cemetery, Test Patch, and Location*. Once the frequency tables were created, photographs of each headstone were carefully examined to determine if measured color changes could be observed in the photographs. Two important points should be noted about the data. First, additional data regarding color changes will be measured in forthcoming field trips, thus data for some headstones continues to be collected. Second, some data from Bath National Cemetery is missing. These analyses exclude data from Bath National Cemetery at this time.

By looking at the frequency of ΔE color changes, researchers were able to initially identify headstones that displayed some subtle appearance changes. For example, data from headstones in Jefferson Barracks is shown in Table 6.

	Delta E for Jefferson Barracks					
Patch #	**32 2904-A**	**32 2898-A**	**72 1273**	**72 1370**	Freq dE> 5	Freq dE> 10
1	3.25	1.36	2.90	14.05	1.00	1.00
2	1.09	0.87	3.79	3.75	0.00	0.00
3	3.44	1.44	7.83	9.44	2.00	0.00
4	3.39	15.31	2.59	8.89	2.00	1.00
5	2.42	3.98	12.92	6.53	2.00	1.00
6	5.16	1.07	8.98	11.37	3.00	1.00
					10.00	4.00

Table 6. Frequency of color change greater than 5 (in yellow) and color change greater than 10 (in gold) for headstones at Jefferson Barracks National Cemetery, St. Louis, Mo.

By looking at this data, headstones 72 1273 and 72 1370 were identified as displaying color changes from April 2006 to November 2006. The same analyses were undertaken for data found in Appendix C from each cemetery. Based on color measurement changes

headstones at Jefferson Barracks displayed the most occurrences of color change in patch 6 (Kodak Photo-Flo). Next, researchers closely inspected photographs of headstones that displayed color changes to see if further visual changes could be noted. For example, upon closer inspection of headstone 72-1273, biological re-growth was noted in patch 4 (H_2Orange Cleaner) along the inset center cross, see Figure 6. Further review of the color measurement data indicated that the stone had darkened slightly (based on negative ΔL^* values for all patches).

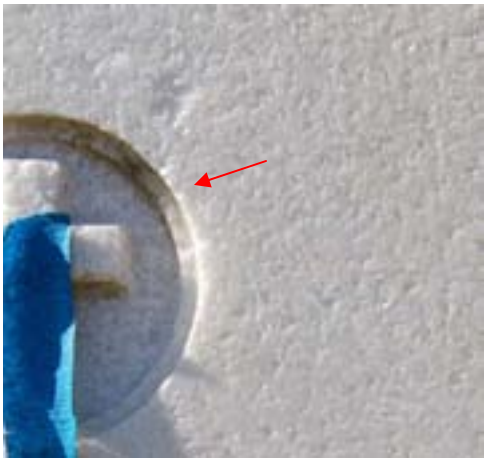

Figure 6. Close-up detail of headstone 72 1273, showing signs of biological growth on inset.

On closer inspection of headstone 72-1370 in Jefferson Barracks, scientists noted a mottling appearance that could be seen in most patches, see Figure 7. The headstone is darkening. There may be biological re-growth associated with the veining seen in the stone.

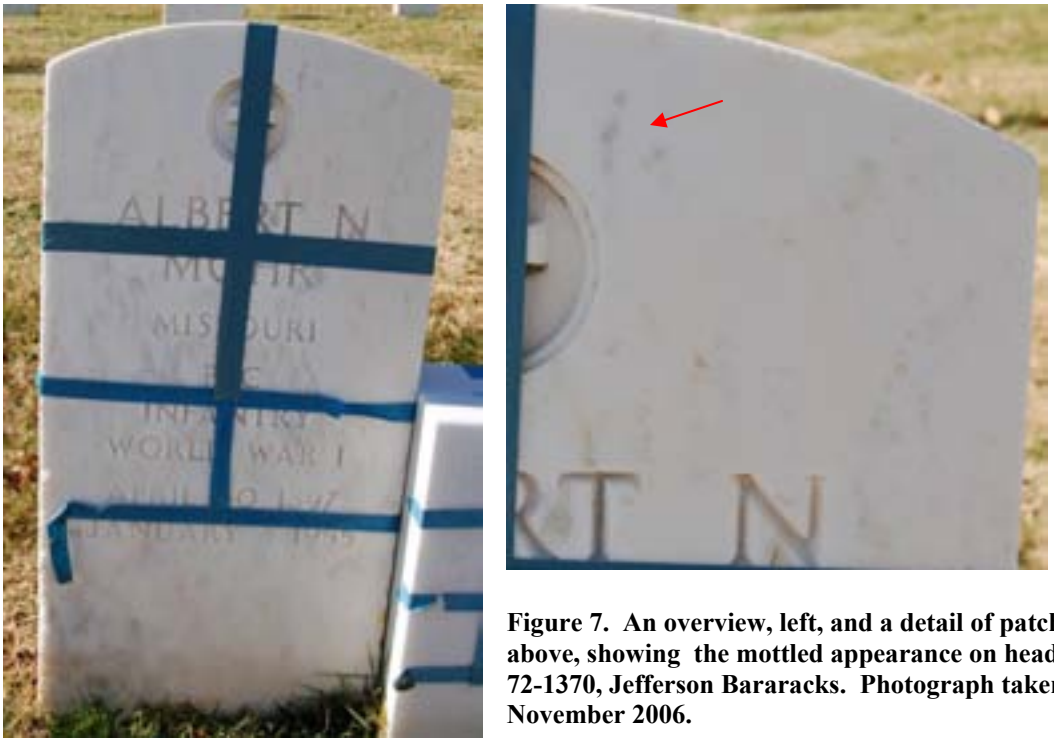

Figure 7. An overview, left, and a detail of patch 4, above, showing the mottled appearance on headstone 72-1370, Jefferson Bararacks. Photograph taken November 2006.

Similar observations can be made at other cemeteries. For example, color change was noted in several headstones in San Francisco, including WS 1032 B shown in Figure 8. Based on color measurements, five of the six test patches have changed color by ΔE greater that 5. Most of this color change comes from the darkening of the headstone (negative ΔL^* values). Visual observation supports these measurements. Biological growth can be seen on patch 2 (D2 cleaner) patch 4 (H2Orange cleaner) and patch 5 (WEG marble cleaner). Also, the brown staining seen on the lower portion of this stone is frequently found in the cemetery. Researchers hypothesize that the staining is due to the use of iron fortified fertilizer used by contractors in San Francisco National Cemetery.

Figure 8. Overview, left, of WS 1032B, San Francisco National Cemetery taken November 2006. Biological re-growth is evidenced on the stone, particularly in patches 2, 4 (above) and 6 (below).

Next, frequency data of ΔE color change was analyzed by cleaner, see Appendix C. The frequency of color change is given in Table 7.

Cleaner	Freq dE> 5	Freq dE> 10
D/2	5.00	1.00
Daybreak	7.00	2.00
Water	7.00	2.00
H2Orange Cleaner	5.00	2.00
WEG Marble Cleaner	8.00	1.00
Kodak Photo-flo	11.00	3.00

Table 7. This table shows the number of color changes greater than 5 and greater than 10 for each cleaner found on headstones at Alexandria, Jefferson Barracks, San Francisco, and Santa Fe National Cemeteries.

From this data, Kodak Photo-flo exhibited the greatest number of color changes both greater than 5 and 10. Based on this frequency analysis, the worst performer was likely Kodak Photo-Flo. Although Photo-flo test patches, location #6 (Illustration 2), were lower on the headstones it is unlikely that these changes were a result of rain water backsplash since water, in adjacent location #3, did not show the same frequency trend.

Finally, frequency data of ΔE color change was analyzed based on the location of the headstone in sunny or shady locations within the cemetery. The frequency analysis of this data is given in Table 8. The frequency of color changes greater than 5 is equal in sunny and shady locations (n = 22), indicating that there is a equal chance of seeing a color change in a sunny location or a shady location on the headstones. However, there is a greater chance of seeing a color change greater than 10 in a shady location than in a sunny location. Fungi and algae tend to grow in shady locations and may lead to greater visual appearance changes.

Shady Cleaner	Freq dE> 5	Freq dE> 10
D/2	2.00	0.00
Daybreak	4.00	2.00
Water	3.00	2.00
H2Orange Cleaner	3.00	1.00
WEG Marble Cleaner	4.00	1.00
Kodak Photo-flo	6.00	2.00
	22.00	8.00

Sunny Cleaner	Freq dE> 5	Freq dE> 10
D/2	3.00	1.00
Daybreak	3.00	0.00
Water	4.00	0.00
H2Orange Cleaner	3.00	1.00
WEG Marble Cleaner	4.00	0.00
Kodak Photo-flo	5.00	1.00
	22.00	3.00

Table 8. This table shows the number of color changes greater than 5 and greater than 10 headstones in sunny locations and shady locations at Alexandria, Jefferson Barracks, San Francisco, and Santa Fe National Cemeteries.

Some unusual visual observations were made at Jefferson Barracks National Cemetery. It was at this cemetery that Church inadvertently cleaned more stones than were needed for the study. In follow-up visits, biological activity was observed on these headstones as

well as headstones included in the study. Eight headstones were cleaned at Jefferson Barracks in October 2005 – six headstones were in the shade and two headstones were in the sun. On all shady headstones, the reoccurrence of biological growth was seen predominantly on patches cleaned with H_2Orange Cleaner (#4). The re-growth was sometimes seen on other patches #5 and #6 below the H_2Orange Cleaner. Figure 9 shows examples of biological re-growth on four of the stones. Sample and swabs and biological analysis did not indicate any significant differences between patch 4, cleaned with H_2Orange cleaner, and other test patches. Interestingly, six months after these photographs were taken the dark biological growth had disappeared on all samples! It may be possible that H_2Orange cleaner did not kill all microbes initially, and it took some time for all growth to die. Alternately, the growth seen on these headstones was seasonal and may return again in the future. In any case, the reoccurrence of microbial activity left an undesirable surface appearance for a period of time, thus NCPTT staff recommended the exclusion of H_2Orange cleaner from Phase Two of the study.

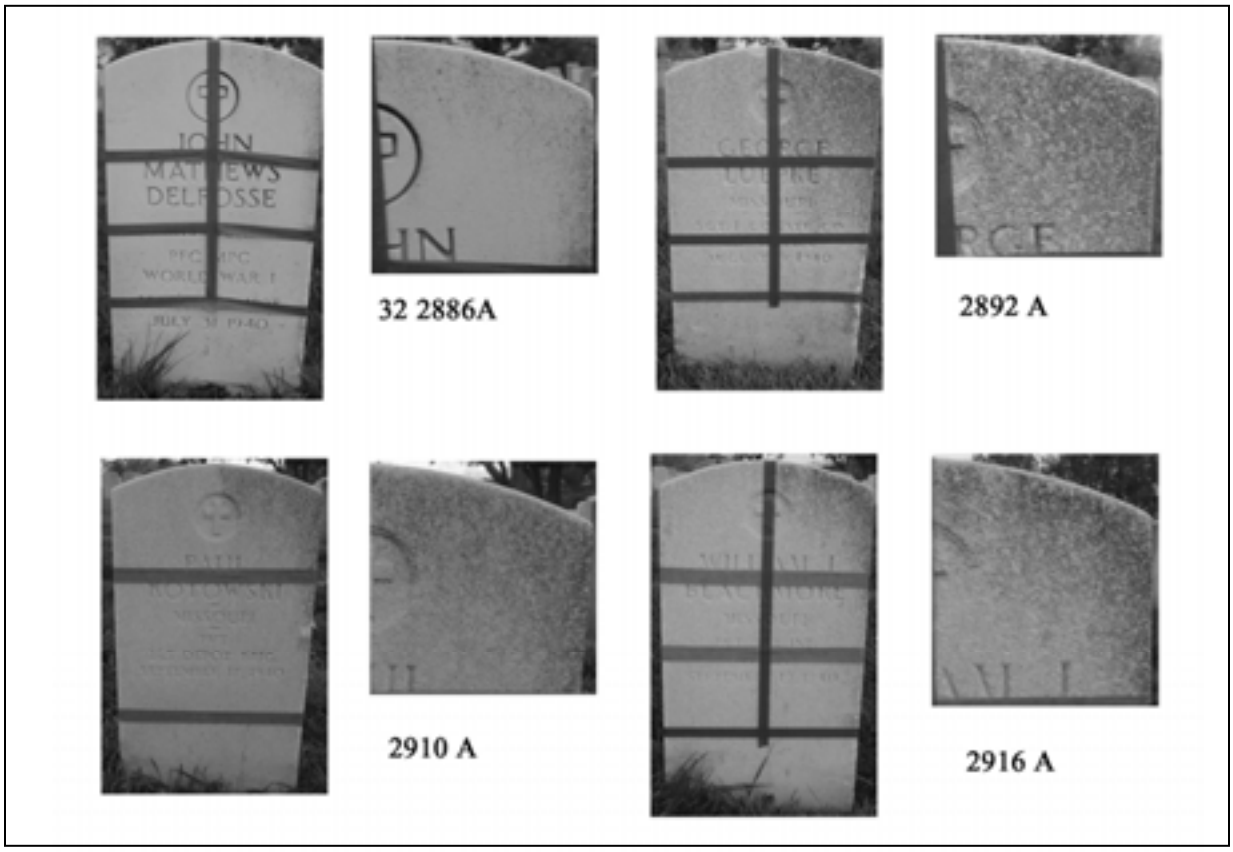

Figure 9. Overview and detail photographs of four headstones found in shady locations of Jefferson Barracks National Cemetery are shown. Microbial activity is found predominantly on patches cleaned with H_2Orange cleaner.

3.5.5.Follow-up Biological Activity, June 2006 Report

Headstones that were cleaned in October 2005 were swabbed again in April 2006 to determine biological activity. These results are reported in Appendix E. *Analysis of*

Microorganisms on Headstones in VA Cemeteries,
Second Report: June 2006 and are summarized here. The work here involved looking at many of the samples, but only a select number of samples were enumerated in this round of analyses.

No algae were detected in samples from any of the five cemeteries. Green coloration in some samples was due to the presence of fungi. In general, the numbers of bacteria were greater than the numbers of fungi found on the stones.

The decreasing order of biological activities was:

Bath > Jefferson Barracks > San Francisco > Alexandria > Santa Fe

Bath had five times more enumerated bacteria than bacteria found in Santa Fe, but both counts are in the same order of magnitude. In Santa Fe, the biological growth of both bacteria and fungi is greater in shady areas. Higher fungal counts were found in Bath and Jefferson Barracks. At Jefferson Barracks, fungal growth was higher in the shade than in sunny areas. Lower biological activity in Santa Fe may be expected because of the drier, hotter climate.

The enumerated biological activity does not fully account for visual changes observed on headstones. This is partly due to the fact that only one stone displaying discoloration, from Jefferson Barracks, was enumerated in the biological activity study. Also, the biological analyses did not attempt to identify specific fungi or bacteria genus present. One hypothesis is that some cleaners are not full spectrum and thus don't fully kill all fungi or bacteria. If most micro-organisms are eradicated, but a few are left behind, then those left behind may grow freely. Resistant fungi that continue to grow may cause discoloration but be limited to a select species.

Further analysis of the biological activity regarding biocidal effectiveness of each cleaner becomes more complex. NCPTT researchers chose to rank the biological activity for each cleaner to determine performance.

3.5.6. Ranking Biocidal Performance of Cleaners
Dr. Tye Botting prepared a ranking of the performance of the cleaners to inhibit biological growth based on the enumerated microbial activities determined from the June report. Botting ranked activities from 1 to 6 with lower numbers indicating higher biological activity found in the count. He ranked the activity by cemetery site, sunny or shady location, bacterial count, fungal count, and cleaner. Once her ranked each cleaner, he averaged the results for overall activity in sunny locations, overall activity in shady locations, and by total growth. He also looked only at bacterial activity and fungal activity. Results are found in Appendix G. *Biological Performance Based on June 2006 Report*. In this appendix, Botting highlighted those cleaners with average rankings below 3.0. The following observations can be made based on the rankings:

- Photo-flo was a poor performer at controlling bacteria in both sunny and shady locations at all cemetery sites. It also ranked lowest of all cleaners in limiting biological activity overall.
- D2 performed well in controlling bacteria and fungi in sunny locations and in controlling bacteria in the shade. Greater activity of fungi was seen on test patches cleaned with D2 in shady locations.
- D2 and Daybreak performed similarly in controlling overall biological activity.
- WEG Marble cleaner was a consistently high performer based on this analysis.

3.5.7. Follow-up Biological Activity, January 2007

A second analysis of biological activity focused on headstones that had been previously swabbed in October 2005 and April 2006. Swabs were collected at the designated cemeteries in October/November 2006. Swabs of patches cleaned with D2, Daybreak, WEG Marble Cleaner, and water were analyzed for biological activity, and they are reported in Appendix F.

Trends in biological re-growth were not evident in the evaluation of this data. No consistent differences were found for bacteria or fungi for the remaining cleaners. In general, numbers of bacteria were greater than fungi at all cemeteries. The greatest difference was seen between sunny and shady locations. Greater numbers of bacteria and fungi were found in shady areas. This is most likely due to drier conditions and more intense UV irradiation in sunny locations.

No algae were detected in samples from any of the five cemeteries sampled. Green coloration in some samples was due to the presence of fungi. Fungi and bacteria were enumerated by plating on solid media and counting colonies after incubation. Numbers of bacteria and fungi in samples were variable.

The absence of algae or photosynthetic bacteria is significant. These organisms typically provide the most visual evidence of growth on headstones. Their absence, even from the stones treated with water, suggests it is still too early to determine the effectiveness of the biocides.

Further ranking of this data, similar to the performance rankings discussed in Section 3.5.6, is shown in Appendix H. In this ranking, the performance of D2, Daybreak, WEG Marble Cleaner, was evaluated based on the numbers of bacteria, fungi, or total re-growth enumerated approximately twelve months after cleaning. Rankings were from one to four, with higher numbers indicating better performance. Ties were allowed. Few trends in biological activity were identified based on this analysis. Cleaners performed about equally in the shady areas. Water was the worst performer in slowing bacterial re-growth in shady areas. WEG Marble Cleaner was a poor performer for slowing bacterial re-growth in sunny locations.

Thus, all indications from the biological activity analysis is that more time is needed to allow significant re-growth to better distinguish between cleaners.

4. Phase Two of the Study

The primary concern in phase one was the biological aspects of the cleaners being tested. The end of phase one was concluded with the removal of two cleaners due to their poor biocidal properties. Phase two is primarily concerned with testing to see if the remaining cleaners have any adverse physical or chemical effects on the marble. This will be tested in three studies; the first will be on whole headstones cleaned in the field, the second is testing on the sample stones that were treated and weathered in the cemeteries and the third is a the continuation of the accelerated weathering studies done at NCPTT's laboratories.

4.1. Laboratory Testing

Laboratory testing in phase two of the study evaluated two groups of samples. The first group to be tested is laboratory samples that were treated with the select cleaners while going through an accelerated weathering phase in a QUV weatherometer. The second group to be tested is sample stones that were treated and weathered in the select cemeteries for the past 18 months. These stones were recently removed from the cemeteries and sent to NCPTT for testing.

Samples from both the laboratory study and the field test will undergo similar treatments. The first series of tests will look for any severe deterioration of the stones structure (such as surface loss) or discoloration of the surface. This includes photographic comparison, colorimetery measurements and laser profilometry among others. The next series of tests will look for soluble salts or other chemical residues left on the marble due to the cleaners. The marble will undergo a series of destructive and non-destructive testing. Preliminary destructive methods of testing for soluble salts include electrical conductivity and various gravimetric methods. The presents of soluble salts inside the marble changes the stone's pore structure. This change has negative effects on the way the stone will weather overtime. Methods used to test the two marble types for pore change include; mercury intrusion porosimetry and nitrogen absorption porosimetry. X-ray diffraction and XRF spectrometry will also me performance on the samples to help determine any chemical contamination to the stones. The level of testing will be determined by the amount of information found in the preliminary tests.

4.2 Whole Headstone Cleaning

Phase two of the study began by cleaning whole headstones in each of the five test cemeteries. In the fall of 2006 Jason Church traveled to each of the sites beginning with Bath National Cemetery on November 7th and ending with Alexandria National Cemetery on January 16th.

To begin this phase of the study, the remaining three cleaners – D/2, Daybreak and WEG Marble Cleaner – were used evenly to clean a total of 24 whole headstones in each cemetery. Of the 24 markers half are Colorado Yule marble and the remaining half are Georgia marble. For comparison purposes half of the stones were sprayed with the cleaners and the other half was physically agitated. Before any cleaning was done the headstones were first photographed and colorimetry measurements were taken. This information will be used to compare the stones appearance over time.

When whole headstones were cleaned, each of the manufactures recommendations were followed. Headstones were always cleaned from the bottom to the top starting with the face and proceeding around the stone counter clockwise. After the cleaner was applied and had significant dwell time, headstones were rinsed thoroughly with water from the site. In the cases where headstones were only sprayed with the cleaner, a Cepia 1-touch motorized sprayer was used. This 32oz handheld powered sprayed helped to control the amount of cleaner used and regulated the force in which the cleaner was applied. Rremaining headstones were cleaned using agitation. The cleaner was first applied to the stone using the motorized sprayer. Then the cleaner was agitated in a small circular motion starting from the bottom and working up using a soft natural bristle brush that measures approximately 3" by 9". After the surface of the stone had been evenly scrubbed the entire stone was rinsed.

During the next round of cemetery visits which will begin in May of 2007 photographs and color measurements of the cleaned headstones will be taken. These will be used to compare any change over time. Also, during this visit a measurement will be taken of each of the headstone using the portable XRF spectrometer. This will help determine if any of the cleaners left behind residual chemicals.

5. Current Research Activities

NCPTT staff is currently undertaking phase two research activities that focus on understanding physical and chemical changes to the stone. These efforts include laboratory studies involving accelerated weathering and comparison of accelerated results will field experiments that have been undertaken simultaneously.

5.1. Laboratory Studies

Laboratory studies in phase two consist of accelerated weathering studies at NCPTT laboratories and analytical evaluation of the laboratory samples and field test samples placed in the five chosen cemetery sites.

5.1.1. Accelerated Weathering Studies

Figure 10. Georgette Lang cores a sample of Colorado Yule for use in the accelerated weathering study.

In June of 2006 NCPTT began the first of two accelerated weathering studies. The purpose of these studies was to

simulate the long term use and exposure of the five selected cleaners on two types of marble. Newly quarried Colorado Yule marble and Cherokee White Georgia marble were obtained from the NCA contracted quarries. “New” marble was selected for these studies so that any residual chemicals found on the stone after the accelerated study could be attributed to the cleaner used and not to any prior treatments on the marble. By doing a laboratory accelerated weathering experiment; factors could be controlled such as humidity and light and dark exposures. Thus the samples and cleaners were compared under the same controlled conditions.

All accelerated weathering studies used a Q- Panel Lab Products model QUV/ Spray Accelerated Weather Tester (weatherometer). This instrument uses panels of UVA-340 lamps to control a programmable cycle of light and dark. The bulbs irradiance level is calibrated to a constant level of 0.77 W/m2.

Figure 11. Marble samples being removed from the QUV weatherometer during a dark cycle to be treated with cleaner.

For both accelerated studies, the Weatherometer was programmed for a continuing cycle of UV exposure for 4 hours at 60 degrees C followed by 4 hours of condensation at 50 degrees C. Note that this step was in the dark (no UV light) to mimic the natural cycle of night and day, and the temperature drop encouraged condensation from the surrounding humid air inside the Weatherometer. The water that condensed inside the Weatherometer initially comes from a lower holding pan that was supplied from a filtered water system that generated 18 megohm-cm purity of water. These cycles repeat for a total of 800 hours.

Marble samples were prepared in the same manner for both accelerated weathering studies. Newly quarried marble was placed on a drill press and cored with a water jacketed diamond coring bit to a diameter of 1 5/8 inches. Then cores were sliced with a water cooled MK tile saw to a uniformed thickness of ½ inch. Once all of the samples were cut to size, they were placed on a Buhler Ecomet 4 fitted with an Automet 3 rotating head and polished to remove any remaining saw marks. The Colorado samples were polished for 5 minutes at 30 rpm with 7 lbs. of force using a 120 grit sanding disk. The Georgia samples were polished for 5 minutes at 30 rpm with 12 lbs. of force using 120 grit. These steps were then repeated using 220 grit paper.

5.1.1.1. Thirty-Three Day Cleaning Study

The first accelerated weathering study began on July 24, 2006. This study was conducted by Georgette Lang, a chemistry major at Centenary College of Shreveport, Louisiana under the supervision of Jason Church. For this study two types of marble were cored and prepared. Three replicate samples of marble were prepared for each type of cleaner. Along with these samples, three untreated samples of each marble type were readied as internal standards. Finally, three samples of each marble were prepared that would not be treated in the Weatherometer but remained untreated as control samples. This brought the total number to 48 samples. Each sample was given a unique number which encoded information about marble type, chemical cleaner used and the sample identification number. This unique 3 digit number was inscribed on the back of each sample.

Pre-existing conditions of the marble surface were recorded by using the Laser Profilometer (see section 4.3.2.1.1.) to map the surface of each sample prior to treatment. Each sample was photographed and color measurements were taken to check for any color change as a result of the application of the cleaners. The weight of each sample was recorded as a baseline to identify residual material deposited from the cleaning. Once the samples were documented and mounted into the Weatherometer sample holders, the 800 hour test was initiated.

The samples were sprayed with the select cleaner and rotated inside the Weatherometer on a daily bases. The marble was treated with the six cleaners D2, Daybreak, Kodak Photo-flo, H2Orange2, Marble Cleaner, and water (plus one set that was weathered but untreated). Each of the chemicals was mixed to the manufactures recommendation and applied to the sample using a 16 oz hand pump spray bottle. The samples were removed from the Weatherometer in their holder and sprayed to completely wet the surface at the end of a dark cycle at approximately the same time each day. The end of a dark cycle was chosen as a time for treatment so that the cleaner would have sufficient time to soak into the stone without evaporating at elevated temperatures during the UV exposure. After the sample was sprayed, it was placed back into the Weatherometer without being rinsed. The decision was made not to rinse the marble after it had been treated because 4 out of the 5 cemeteries involved in the study stated that they do not rinse their stones post cleaning.

On August 27, 2006 the Weatherometer run ended and the samples were left in the powered down Weatherometer for 48 hours to allow any moisture in the stone to evaporate. Testing began after the samples were removed from their holders. Once accelerated weathering was concluded, testing repeated using the same methods as pre-testing documentation. First, the weight measurement is taken. Second, samples were photographed, and third, colorimetry measurements were taken. Finally, surface texture on each sample was measured using the laser profilometry. Also, at this time each of the elemental composition of sample surfaces were analyzed using the Tracer III portable X-Ray Fluorescence Spectrometer. The XRF Spectrometer under the following conditions: to the Rhodium target Xray tube was set to 15kv and 15ma. A vacuum pack was connected to the Spectrometer and a vacuum of 2 torr was pulled. All spectra were collected for 180 seconds. Spectra were taken of the front and the back surface of samples from both marble types treated with each cleaner. This helped to determine if any chemical residue had migrated through the sample.

Figure 12. Jason Church uses the XRF spectrometer to analysis a marble sample after artificial weathering.

There were a variety of results from the first accelerated weathering test. Colorado Yule marble was more likely than Cherokee White marble to display deterioration or discoloration in the accelerated weathering test. NCPTT is currently looking into the

possible reasons that the Colorado Yule marble samples were affected at a greater rate than the Cherokee White marble samples.

There was no discernable change in the samples that were untreated or treated with water. The Colorado Yule marble samples treated with D/2 discolored and took on a slightly translucent appearance. The backs of the Colorado Yule samples also had a very fine powdery deposit on them. When the backside of one sample was examined with the XRF there was a slight Potassium peak. The Colorado Yule samples that were treated with Daybreak discolored to a yellow appearance and had a fine "sandy" coating on the backside. When the backside of one Colorado sample was examined with the XRF a large Chloride peak was detected. The remaining 3 cleaners (Photo-Flo, WEG Marble Cleaner and H2Orange2) had no obvious detectable deterioration.

The thirty three day study represents a worst case scenario where the marble was saturated with a cleaner on a regular base and was not rinsed after cleaning. Any physical change to the marble or chemical deposition on the marble would likely be scene in an extreme situation. Because of the severity represented in the first accelerated weathering study the decision was made to start a second accelerated weathering study.

5.1.1.2. Four Time Cleaning Study

The second accelerated cleaning study used the Q- Panel Lab Products model QUV/ Spray Accelerated Weather Tester preformed at NCPTT began on December 22, 2006 and ended its 800 hour cycle on January 19, 2007. The second accelerated weathering test was an abbreviated version of the first experiment. For the second study only Colorado Yule marble was selected for testing. This decision was made due to the fact that deterioration was evident on the Colorado marble in the first experiment. These samples were prepared from the same marble using the same procedure as in the first accelerated weathering experiment.

For the second experiment only 10 samples were prepared for Weatherometer exposure. These consisted of 2 Colorado Marble replicates for each cleaner. Each sample was sprayed with D/2, Daybreak, WEG Marble Cleaner, or water. Two cleaners, H2 Orange Cleaner and Kodak photo-Flo, were removed excluded from the accelerated weathering, based on results of phase one of the study. Two untreated samples were weathered in this experiment as internal controls. Each of these samples was given a unique 3 digit number that was inscribed on the back of the stone.

One key feature of this study was the decision to only clean the samples weekly, once at the beginning and then three more times at the same cycle on each following Friday. This cleaning schedule may have provided a more realistic approach to the accelerated weathering. Also in this study, the cleaner was sprayed onto the sample then rinsed shortly after being treated according to manufacturers' suggested cleaning directions.

After the 800 hours of weathering was completed, the samples were analyzed in the same steps as the first experiment including laser profilometry and colorimetry. A few additional tests were added to this study to try and get a more detailed view of the stones

reaction to the cleaner. Salt deposits were visible on both the back side of some samples and on the Teflon Weatherometer holder ring that surrounds the stone in place. Gravimetric measurements were taken of each of the stone samples while they were still in the holder. Crystalline grow was visible on the backside of the samples treated with both D/2 and Daybreak.

In addition to the usual photo documentation the samples were also photographed under magnification using both a Leica MZ8 boom microscope at a magnification range of 10x to 50x and a Leica DMRX polarized light microscope at a magnification range of 100x to 500x. Both microscopes were fitted with a Diagnostic Instruments Inc. Digital Spot Camera. Through this process the shape, appearance, growth pattern and relative size of the crystalline growth can be documented.

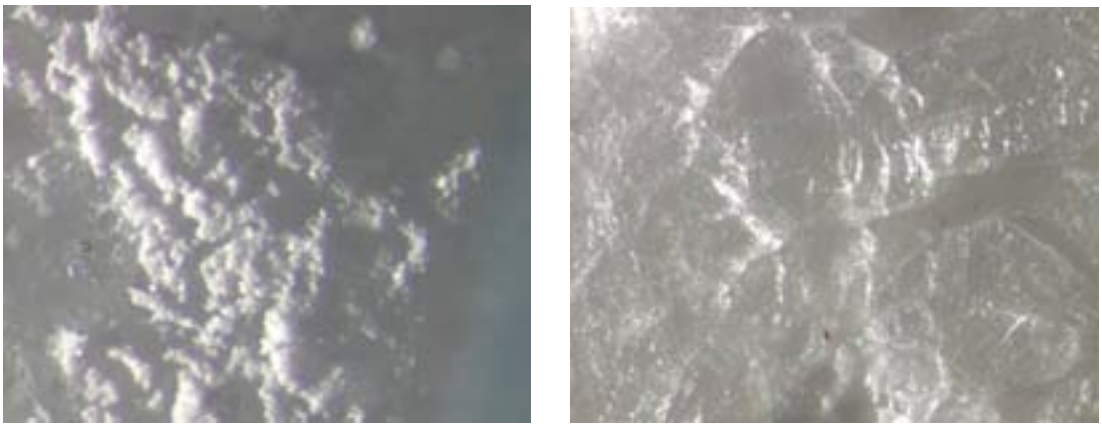

Figure 13. Examples of salt formation on marble samples treated with D/2 (left) and Daybreak (right) both viewed under 100x magnification.

Future research activities will investigate the chemical composition of the efflorescence found on the samples. Both the face and back of all of the samples will be analyzed using the portable XRF Spectrometer. Samples of the salt will be analyzed using X-ray Diffractometry. This will help to determine the bases of the visible salts and any other type of detectable chemical residue left on the stone after cleaning.

Treated and weathered Colorado Yule samples are still being tested and the data processed at this time. New testing methods are currently being considered to maximize the identification of the salt contents in the samples and to identify any other chemical or physical changes that may have taken place in the samples as a result of the cleaner's application.

5.2. Field Studies

Field studies generally consist of evaluating appearance and color changes in the field on field test stones and whole headstones. Additional laboratory analyses of field test stones are described in *Section 4.3, Methods of Analysis*, starting on page 38.

5.2.1. Field Stone Samples

Figure 14. NCA staff members Genaro Ocrato and Pat Meyer help set sample stones at San Francisco National Cemetery.

Early in the summer of 2005, 6”x 6” x 24” marble slabs of Colorado Yule and Cherokee White marble were procured from two National Cemetery Administration contracted quarries. These stones would serve NCPTT as the needed field sample stones. Each of the five project cemeteries and the NCPTT labs were shipped a pallet of 22 stones in early June 2005. Of the 22 stones 11 were Colorado Yule and the remaining 11 were Georgia marble. In June, Church visited each of the cemeteries as an initial contact visit. During this visit the sample stones were paired with existing grave markers of the same marble type. Each of the sample stones was set approximately 6” in the ground for stability, see Figure 14.

During the fall trip to each cemetery, ten of the stones were taped into a grid and treated with each of the five cleaners plus water. Of the ten stones half were Colorado and half were Georgia marble. This same process was repeated in the spring on the remaining sample stones, leaving one of each type untouched as a control sample.

In the fall of 2006, each of the stones were removed from the various cemeteries, cling wrapped and stacked on pallets. The pallets have recently been shipped to the laboratories and were received at NCPTT on April 2. They are awaiting testing. The tests preformed on the sample stones will be conducted to look for any physical or chemical changes to the marble itself or to identify any harmful residues left behind by the cleaners that may be harmful over time.

5.3. Methods of Analysis

Staff scientists have determined multiple ways to evaluate accelerated aging samples and field stone samples, based on the criteria established at the beginning of the study by the project team. Important questions to answer include:

- Is the stone appearance changed by the chemical cleaner? If so, is the appearance acceptable?
- Are there physical changes to the stone upon cleaning? Is the stone surface physically altered during the cleaning process with the cleaner? Is the surface rougher or smoother? Is the stone porosity altered during the cleaning process? Are the pores of the stone larger or smaller after cleaning?
- Are there chemical changes to the stone upon cleaning? Does the cleaner interact with the stone to produce chemical changes on the surface? Does the cleaner interact with the stone to produce salts or efflorescence?

5.3.1. Appearance Change

Appearance change is documented using photographic techniques and color measurements. Photographic images are taken of the lab test stones prior to accelerated weathering using the QUV weatherometer. Field test stones were photographed in each cemetery prior to cleaning with the test cleaners. The field tests stones were photographed again after cleaning.

5.3.1.1.Photo-Documentation/Visual Ranking (see section 3.4.1.1.)

NCPTT staff is photographing test samples from laboratory or field test stones under standard lighting on a Kodak gray card using a Polaroid copy stand and color balanced ECT incandescent bulbs. Digital photographs are taken using a Sony DSC-S85 digital camera at 2272 x 1704 pixel resolution. The photographs will be compared visually by at least ten unbiased observers and ranked on the basis of change in appearance.

5.3.1.2.Color Measurement (see section 3.4.1.2.)

Using a Minolta Colorimeter, CR-400, staff made color measurements on lab test samples prior to accelerated aging using the QUV weatherometer. Each measurement was repeated three times on each stone sample and averaged in order to compensate for slight variations in surface texture. The samples were then exposed to UV radiation, temperature cycling, and spray mist cycling as described in section 5.1.1.2. Samples were cleaned weekly for a total for four cleanings. Samples were cleaned either with D2, Daybreak or WEG Marble Cleaner.

Staff will make color measurements of the samples after exposure. The measurements will be taken following the procedures described in the above paragraph. Once the "after' pictures are taken, total color change, ΔE^*, will be calculated for each sample. Samples will be considered unchanged if the ΔE^* is 3 or less. When ΔE^* is 3 or greater, the samples will be considered to have undergone a color change. Shifts in lightness and chroma will be noted.

5.3.2. Physical Change

One aspect of stone deterioration is the change in physical properties of the stone. Physical changes commonly observed in the field include sugaring, blistering, and scaling, among others.[7] Visual observations allow researchers to determine the state of the condition at a moment in time. However, quantification, or putting numbers to the conditions, allows observations over a time range. The NCPTT staff chose two ways to characterize physical changes in the accelerated weathering tests and in the filed test samples. Laser profilometry can be used to quantify the surface of the stone samples. Changes in the pore structure can be measured by porosimetry.

5.3.2.1. Surface Texture

Surface texture is the local variations in the in the surface from its ideal shape. It can be characterized by a number of variables defined by international standards[8,9], including

S_a – Average Roughness for an Area,
S_p – Highest Peak Surface, the height of the highest peak in the roughness profile over the evaluation area,
S_v – Valley Depth from surface,
S_t – The total height of the surface, the sum of $S_p + S_v$,
S_{ku} – The Kurtosis, a measure of the randomness of heights and sharpness of a surface,
S_{vk} – The roughness of the valleys,
S_k – Roughness of the core
S_{fd} – the fractal dimension of the surface (complexity of the surface),
S_q – the root mean square of the roughness, and
V_v – Void volume of the valleys, among others.

In her doctoral work, ElizaBeth Bede Guin showed that surface texture and porosity can affect the deposition of air pollution on to surfaces.[10] Guin characterized the porosity and surface texture of four different types of high-calcium limestone including Salem, Cordova Cream, Cottonwood Top Ledge, and Monks Park limestones. Half of the stones were chemically etched to create rough surfaces while others were semi-polished to create smooth surfaces. Next the samples were exposed to a simulated polluted sulfur dioxide environment within the NCPTT environmental exposure chamber for 24 hours. The conditions were 50 ppb SO_2; 65% RH; 25°C; 4 m/s wind speed. Deposition velocities were calculated for each stone surface. While porosity was the dominant variable influencing pollution deposition, Guin's work showed that three texture

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[7] Price, C.A., 1996, *Stone Conservation, an Overview of Current Research.* Santa Monica, CA: Getty Conservation Institute, J. Paul Getty Trust, pp 1-4.
[8] International Organization for Standards, "Geometrical Product Specifications (GPS) - Surface texture: Profile method; Surfaces having stratified functional properties - Part 2: Height characterization using the linear material ratio curve," ISO 13565-2 1996.
[9] American Society of Mechanical Engineers, "Surface Texture, Surface Roughness Waviness and Lay," ASME B46.1, 1995.
[10] Bede, ElizaBeth Anne, "The surface morphology of limestone and its effect on sulfur dioxide deposition," Ph.D. dissertation, University of Delaware, 2001, 327 pp.

parameters including $S_{k,}$ $S_{vk,}$ and $S_{q,}$ did correlate to the deposition of sulfur dioxide on the stone.[11]

Cleaning the surface of the stone may affect both the porosity and the surface texture parameters. The changes may lead to additional soiling by atmospheric pollutants. Alternately, changes may increase moisture retention and lead to increased biological growth.

In this work, we will characterize several surface texture parameters of both laboratory stones and field test stone prior to and after cleaning/accelerated aging or field exposure. Changes in the surface texture from cleaning and/or aging will be noted. Surface texture will be analyzed using laser profilometry on small cut samples.

5.3.2.1.1. Laser Profilometry

Laser profilometery is based on the principle of optical triangulation. It employs a light source (a laser), imaging optics, and a photodetector. The laser is focused on to the surface of the sample. Reflected light is focused on to the photodetector, which generates a signal that is proportional to the position of the spot in its image plane. As the distance to the target surface changes, the imaged spot shifts due to parallax. To generate a three-dimensional image of the stone surface, the sensor is scanned in two dimensions, thus generating a set of distance data that represents the surface topography of the stone[12].

Figure 15. Researcher scans surface of stone using laser profilometer to determine texture parameters and 3-d profile.

NCPTT uses a Solarius LaserScan, a 3-d non-contact laser profilometer, to characterize stone sample surfaces. The instrument uses a class II diode laser (670 nm wavelength)

[11] Bede, Ibid., Chapter 6. (N.B. S parameters reflect area measurements, while R parameters reflect line measurements).

[12] "Introduction to Laser-based Profilometry," Laser Techniques Co., 14508 NE 20th St., Bellevue, WA, 98007 http://www.laser-ndt.com/LP_method.pdf (accessed 3/12/2007).

and a 2 μm spot size. The vertical resolution of this instrument is 0.1 μm. The maximum vertical range is 1 mm. This range allows for the measurement of surface peaks and valleys typically encountered on stone surfaces. The laser is scanned over an area of 31.07 mm (x-axis) by 23.02 mm (y-axis) at a scan speed of 5 mm/s and a resolution of 25 μm. [13] The estimated run time per sample is 111 minutes.

Laboratory samples chosen for accelerated weathering were documented by laser profilometry using the conditions described in the above paragraph. The samples will be analyzed after exposure and changes in parameters will be calculated.

Field test samples will be measured upon return from the field. This will require cutting small samples from each stone cleaning space. The surface texture will be compared to control samples which have not been exposed in the field, but have been carefully stored in the lab.

5.3.2.2. Stone Porosity

Porosity is the volume of void spaces found in the stone and is expressed as a fraction between 0 -1. The porosity of a stone is important consideration when determining how much water or liquid can be absorbed in a stone or how a stone might be affected by air pollution or long term weathering. Also, a more porous stone may absorb more and retain more cleaner, making it harder to rinse.

Increases in porosity may reflect erosion or material loss from the surface of the stone. This undesirable affect may come from mineral dissolution in water or cleaner. Alternately, decreases in porosity may reflect growth of salts or other residues within the stone pore system.

The voids within a stone have additional characteristics that can be described by size and shape. Large voids in the stone greater than 50 nm are considered macro-pores. Pores in the 50 nm to 2 nm range are considered meso-pores, while pores smaller than 2 nm are called micro-pores. The size of the pores affects the way fluids move through the stone.

5.3.2.2.1. Mercury Intrusion Porosimetry

One technique that can determine pore size in a material is called Mercury Intrusion Porosimetry (MIP). It can measure pore size in the range of meso-pores to macropores. Samples are submerged in a confined quantity of mercury and then the pressure of the mercury is hydraulically increased. This forces the mercury into the pores of the material. The results obtained from the instrument include

- pore size distribution (macro/meso range of porosity spectrum),
- hysteresis curve,
- specific surface,
- bulk density,
- total porosity (%), and
- particle size distribution.

[13] Other conditions include a row pitch of 85.95 and a column pitch of 88.33.

5.3.2.2.2. Nitrogen Absorption Porosimetry

Nitrogen gas absorption can be used to determine the micropores and the lower range of meso-pores in a material. The measurement of adsorption at the gas/solid interface is one of the most widely used techniques for the study of microporous and mesoporous solids. The gas molecule acts as a ruler for the measurement of features at the nanometric scale. Nitrogen is the gas most often used for this type of study. With this technique, a series of isotherms[14] are plotted for the absorption and desorption of nitrogen onto the surface of a stone. ASTM UOP821-81[15] describes a method of determining the distribution of surface area, pore volume (size) and length among the micropores, 60 nm (600 A) and smaller, as well as total surface area, total pore volume and average micropore diameter for porous substances using a Micromeritics Digisorb 2500 Analyzer.

5.3.3. Chemical Change

Criteria for effective cleaners included the ability to provide improved appearance, the efficient removal of biological growth, the deterrence of re-growth, and minimum to trace changes to the physical and chemical nature of the stone. The documentation of appearance change was described in section 4.3.1. Methods used to document physical changes were described in section 4.3.2. This section describes methods used to document chemical changes resulting from cleaning with the test cleaners and is a main focus of phase 2 of the study.

5.3.3.1. Optical Microscopy

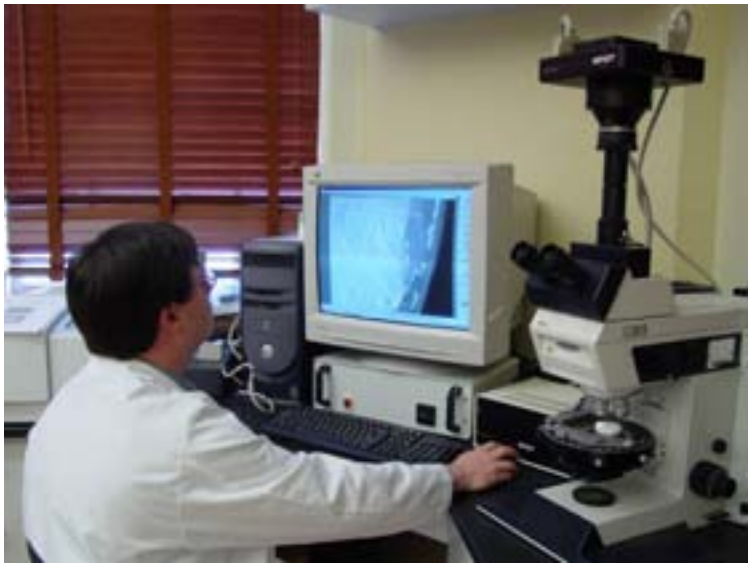

Figure 16. Jason Church uses an optical microscope fitted with a digital Spot camera to view salt deposits on the back of a sample

[14] Absorption isotherms are plots of the amount of gas absorbed at equilibrium as a function of the partial pressure at a constact temperature, usually nitrogen at its boiling point.

[15] "UOP821-81 Automated Micro Pore Size Distribution of Porous Substances by Nitrogen Adsorption and/or Desorption Using a Micromeritics Analyzer" ASTM International.

Optical microscopy is a simple but useful analytical method. Microscopy is the ability to view small areas in great detail by using magnification. This technique will be used to view the marble samples that have undergone accelerated weathering. This technique will help determine if there is any visible deposition on the stone. If efflorescence is present due to salt content it will be viewable using optical microscopy. Salt crystals grow in different patterns with varying shapes depending on their composition. The salt crystals shape will be viewable under magnification this will help to determine its composition. The surface of the field stones will be viewed with optical microscopy to check for any visible depositions or crystallization before further testing takes place.

For this analysis NCPTT will make use of its two in-house microscopes; a Leica MZ8 boom microscope with a total magnification range from 6.3X to 50X, and a Leica DMRX polarized light microscope with a magnification range from 50X to 500X. All samples under magnification can be photographed using the microscopes' digital Spot camera attachment. The photographs can provide important visual and comparative documentation.

5.3.3.2. X-ray Fluorescence Analysis

X-ray Fluorescence analysis is a non-destructive method used to determine the elemental composition of a sample. This is done by generating elections using an an X-ray tube. The generated electrons of specific energy bombard the sample. The x-rays can either be absorbed or scattered through the material. The way in which the atom absorbs the x-ray is by transferring the energy to its innermost electron. After this is done the electrons are pushed back from the inner shell causing vacancies. The atom to becomes unstable, and outer shell electrons cascade into the vacancies. This causes the release of energy in the form of X-rays of characteristic energy. Since each element produces x-rays that have a unique energy the elemental composition of the sample can be determined.

This process is accomplished with the use of an XRF Spectrometer and its supporting software. The XRF Spectrometer reads the characteristic energy levels and maps them into a spectrum chart where the elements can be labeled and compared. A comparison of the untreated marble and the marble sprayed with the selected cleaners may show chemical residue left behind due to the cleaners.
A handheld XRF Spectrometer will be used to analysis both marble samples in the laboratory from the accelerated weathering studies as well as the field stone samples that were treated in the cemeteries. Due to the handheld XRF Spectrometer's portability it will also be used to analyze chemical deposition on whole headstones cleaned in the field. NCPTT uses a Tracer III portable X-Ray Fluorescence Spectrometer with a Rhodium target.

5.3.3.3. Scanning Electron Microscopy

Scanning electron microscopy with electron microprobe capabilities permit the observation and characterization of materials.[16] Both techniques are based on irradiating the samples with a finely focused electron beam, which may be swept across the surface

[16] Goldstein, Joseph I., Dale E. Newbury, Patrick Echlin, David C. Joy, Charles Fiorl, and Eric Lifshin, 1981, *Scanning Electron Microscopy and X-ray Microanalysis*, New York, NY: Plenum Press, Chapter 1.

of a specimen. Different types of signals, including secondary electrons, back-scattered electrons, and characteristic x-rays are produced when the electron beam impinges on the surface of the sample.

The surface topography of a sample can be imaged by collecting secondary and back scattered electrons as the electron beam scans the surface of the sample. This rastered image produces a three dimensional appearance of the surface. Thus, the technique can help elucidate changes in surface texture such as pitting or sugaring.

Additionally, Scanning electron microscopy with energy dispersive spectrometry (EDS) permits the identification of elements present on the surface in major, minor, and trace concentrations. Identification is based on the specific energy of characteristic x-ray peaks for each element and is similar to x-ray fluorescence spectrometry. Also, the surface of the sample can be scanned for these characteristic x-rays, and maps of a specific element can be made on the surface of the sample.

Scanning electron microscopy may be used in this study to provide additional information about possible chemical and physical changes to the field test stones and the artificially weathered stone samples.

5.3.3.4. Total Soluble Salts

While the presence of soluble salts contributes to weathering and decay of porous stone, the decay mechanisms are complex.[17] Soluble salts, such as sodium chloride or calcium sulfate, may damage stone as a result of crystallization pressure or hydration pressure. Crystallization pressure can develop when a supersaturated solution occupies a smaller volume than the precipitating crystals and residual solution. This pressure pushes out on the pores of the stone and causes damage. Alternately, hydration pressure is developed when a salt collects water molecules around itself. Again, the volume needed for hydrated salts is larger than the restrictive pores. Salts push out against the walls of the pores and enlarge the pore space.

Clifford Price points out in his review on stone deterioration that salts represent one of the most important causes of stone decay.[18] Salts may be introduced into stone through rising damp, or blown by the wind. Use of deicing salts can be a problem in colder climates. Unsuitable cleaning may leave salts that ultimately damage the stone.

Based on this knowledge, it is important to determine if any of the cleaning test products leave significant soluble salts on the headstones. Tests to determine total soluble salts in stone include gravimetric and conductivity techniques. NCPTT staff will use one or both methods to evaluate the presence of soluble salts after cleaning in the field and after accelerated studies in the lab.

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[17]Charola, A. Elena, 2000, " Salts in the Deterioration of Porous Materials: An Overview," *Journal of the American Institute for Conservation*, Vol. 39, No. 3. (Autumn-Winter, 2000), pp 327-343.

[18] Price, C.A., 1996, *Stone Conservation, an Overview of Current Research.* Santa Monica, CA: Getty Conservation Institute, J. Paul Getty Trust, pp 7-9.

5.3.3.4.1. Gravimetric Methods

This test uses weight measurement to determine the soluble salts found in a stone sample. The test method is described in Boyer (1987) as:

> A crushed masonry sample of known weight is allowed to interact with distilled water for 24 hours. The sample is then filtered, dried and the precipitate weighed. Water soluble figures are then calculated based on the ratio of weight loss of the precipitate to the original sample. A high water-soluble content would indicate the masonry to be composed of highly water-soluble materials which would reduce its resistance to weathering.[19]

5.3.3.4.2. Electrical Conductivity Method

A second method that can be used to investigate salts and other soluble contents within the stone is the use of electrical conductivity measurements. Electrical conductivity is directly related to the concentration of dissolved ionized solids in a wash solution. Again, the sample is ground, then soaked in distilled water for 24 hours. The solution is filtered through a filter paper of 2 micrometer pores. Then an electrical conductivity meter is used to measure the conductivity of the solution in micro-siemens. Higher electrical conductivities indicate greater total dissolved solids.

6. Comments and Discussion

6.1. Appearance

Appearance changes of field trails were documented using photography and colorimetry throughout phase one of the study. Very subtle changes were seen on stones over time from six months to twelve months after cleaning. However these changes were often not noticeable to the viewer. None of the cleaners left obvious changes, such as yellowing, etc., from possible cleaning residues.

Color change trends were examined by determining the frequency of color changes at ΔE greater than 5 and ΔE greater than 10. Trends were evaluated by cemetery, by cleaners, and by sunny or shady locations. In most cases where color change occurred, headstones were darkening.

From frequency trend data associated with cleaners, Kodak Photo-Flo exhibited the greatest number of color changes greater than 5 ΔE and greater than 10 ΔE, and was likely the worst performer of the test cleaners. None of the other cleaners were readily distinguished based on changes in visual appearance.

It is important to note that, while H_2Orange cleaner seemed to perform well based on color measurements, significant visual changes were noted over a six month time period. The appearance of biological re-growth or staining was not always captured by color measurements, since changes often occurred at the outer edges of the headstone.

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[19] David W. Boyer, 1987, "A Field and Laboratory Testing Program: Determining the Suitability of Deteriorated Masonries for Chemical Consolidation," *APT Bulletin*, Vol. 19, No. 4, 1987, pp. 45-52.

Moreover, after twelve months, the visual changes had disappeared. Despite the fact that this phenomena was observed at only one cemetery, Jefferson Barracks National Cemetery, it was deemed to be an unacceptable short term appearance change.

Appearance changes were subtle during the six and twelve month time period. In general, more time is needed to see significant appearance changes to the headstones.

6.2. Biological Re-growth

Determination of biological re-growth in this study has offered some complex problems, from the sheer numbers of samples to be evaluated and enumerated, to how cleaning history of the stones affect the initial biological activity, to the length of time needed for observing visual biological re-growth.

Biological swabs were taken from many headstones and required considerable time and effort to enumerate in the course of this study. Initial estimates of the number of samples to be examined were 7,880 biological counts, taking over 63,000 hours of work to perform! This was an impossible task and in June 2005, we revised the number of samples to 600 swabs. Still the task was daunting and ultimately, fewer samples were evaluated.

All headstones started with a relatively small biofilm of bacteria and fungi at the beginning of the study, with the exception of headstones located in Santa Fe National Cemetery which displayed a larger biofilm. This is likely due to the fact that Santa Fe headstones are not regularly cleaned in the same manner as those located in the other test cemeteries. Importantly, no algaes or photosynthetic bacteria were observed in the samples. According to Dr. Ralph Mitchell,[20] it is likely that algaes or photosynthetic bacteria are the greatest source of visual appearance change found on headstones and thus are the most important to enumerate. Fungi are also sources of visual discoloration, but to a lesser extent.

As of November and December 2006, no algae were detected in samples from any of the five cemeteries sampled. Green coloration in some samples was due to the presence of fungi. Fungi and bacteria were enumerated by plating on solid media and counting colonies after incubation. Numbers of bacteria and fungi in samples were variable.

The absence of algae or photosynthetic bacteria is significant. These organisms typically provide the most visual evidence of growth on headstones. Their absence, even from the stones treated with water, suggests it is still too early to determine the effectiveness of the biocides.

NCPTT staff attempted to identify performance trends based on the biological activity documented over the course of twelve months. Performance of each cleaner was ranked based on data from swabs. Rankings from June 2006 results appeared to illuminate differences to a greater extent than rankings from February 2007. This is partly due to

[20] Mitchell, Ralph. Harvard University, Division of Engineering and Applied Sciences, personal communication, March 2007.

the fact that there were six cleaners to rank in June 2006 where as there were four cleaners to rank in February 2007. The latter rankings grouped more closely together thus making it more difficult to see significant differences.

Based on the June 2006 rankings, Kodak Photo-Flo was likely the worst performer of the six cleaners evaluated.

6.3. Physical Changes

Evaluation of physical changes is a significant task in phase 2 of the study. Physical changes will be evaluated for field test stones and for accelerated weathering laboratory samples. To date, NCPTT staff has identified methods to be used in evaluating physical changes to the stones. They include changes in appearance by colorimetry, changes in surface texture to be monitored by laser profilometry, and changes in porosity to be examined by mercury porosimetry and Nitrogen BET absorption porosimetry.

Laboratory samples were examined using colorimetry, laser profilometry, and weight measurements prior to any accelerated weathering studies as the baseline data. Field test stones will be compared to control samples kept in pristine conditions in the laboratory.

This work is on-going.

6.4. Chemical Changes

As with the evaluation of physical changes, chemical changes caused by cleaners will be evaluated in the laboratory as part of phase 2 of the study. The possible presence of soluble salts will be evaluated using optical microscopy and analysis of total soluble salts using both gravimetric and conductivity methods. The chemical nature of the efflorescence may be studied using X-ray Diffraction analysis. Detection of possible cleaning residues or minor chemical changes may be studied using Electron Microscopy-EDS, and X-ray Fluorescence Spectroscopy.

NCPTT staff has tested its new portable XRF analyzer for identifying chlorides on field test stones with success. The task of identifying chemical changes to the stones continues.

6.5. Issues Associated with Bath National Cemetery

Figure 17. Signage in Section F of Bath National Cemetery.

On November 7, 2006 Church arrived at the Bath National Cemetery to begin work on the first part of phase two of the project. The first noticeable thing in the cemetery was that sections A, B, D and F had recently gone through a section renovation which consists of the raising and realignment of each of the headstones in the section. This affected headstones involved in the project in several ways, including soiling, contamination and total loss of the stones themselves.

Section F is included in the test study as the "Sunny Section." There are 24 Headstones and 22 lab sample stones located in section F. Of these headstones and field test stones, all were affected by general soiling. This was caused when the headstone was dug out of the ground and laid aside during resetting. Ongoing monitoring of the visual appearances of stones have been altered by this soiling, see Figure 18. Also, in the renovation process two of the lab sample stones were moved and reset one row up from where they had been placed, thus losing their connection to the original headstone.

Figure 18. Headstone F712 in April 2006 and again after resetting in November 2006.

Through out the sections A, B, D and F, hydro-seeding was used to help with erosion and to replace the ground cover that was lost during renovations. Hydro-seeding is the process of planting mass quantities of grass seed by spraying a slurred mixture over a large area. The mixture for hydroseeding contains five basic components including – a recycled paper pulp, grass seed, tracking dye, fertilizer and a tackifier. The tackifier usually is a linear polyacrylamide polymer that electrochemically binds soil particles. This process could have contaminated the biological data as well as the colorimeter and visual inspection of the all the headstones involved.

Figure 19. Headstone F812, note that the tan spots are clumps of hydro-seed still attached to the marble.

The effects of the hydro-seeding are visible in section F where the headstones are directly beside the lab sample stones. In the hydro-seeding process the cemetery maintenance staff placed small plastic bags over the headstones to shield them from the grass slurry. This process was not photographed at the time the work was being done. This seams to have been adequate protection for most of the grave markers, with the exception of any headstone that was set beside a lab stone. These headstones and lab stones still retained a spotty coating of the hydro-seed.

Sections B and D on the cemetery are mostly Spanish American War markers. Church was told by Bath's maintenance staff that most of these were originally set with concrete around the base of the stone. During the renovation of sections B and D several stones were broken in attempts to raise and realign them. All historic stones broken in this section were replaced with newly carved Georgia marble that mimic the original markers' design and font. Four of the stones broken and replaced in this section were in the cleaning study. They were

- D 7 14 Albert McKinzie
- B 1 8 SE Catlin
- B 1 9 Peter Welch
- B 1 10 Adam Graf

Biological and colorimeter data had been collected on these stones. When asked about the stones the staff stated that they were unaware of which stones had been replaced and that it was believed only the lab samples and their corresponding headstones were still in the study.

Figure 20. Marker B18 in April 2006 and its replacement in November 2006.

7. Recommendations

The following recommendations are based on data taken in the field from June 2005 to March 2007, from analysis of biological activity performed at the Laboratory of Applied Microbiology at Harvard University, and NCPTT staff research experiences during the course of this study. There are three main recommendations – the elimination of two cleaners, the elimination of Bath National Cemetery from the study, and the continuation of the study for an additional time period.

7.1. Elimination of Cleaners

- Kodak Photo-Flo was eliminated from the first part of phase one based on the performance rankings of the biological activity seen in June 2006.
- Kodak Photo-Flo was likely the worst performer based on frequency of color change data as well.
- H_2Orange cleaner was eliminated from the first part of phase one based on visual examination of headstone test patches at Jefferson Barracks National Cemetery, as observed six months after cleaning.
- Three cleaners continue to be studied, including D/2 Antimicrobial cleaner, Daybreak, and WEG Marble cleaner.

7.2. Bath National Cemetery

- Unique problems are associated with phase one of the study at Bath National Cemetery as documented in section 5.5 of this report.
- It is unlikely that data from the cleaning test patches or field test stones will provide meaningful data, since they were likely contaminated during the recent

section renovation. Moreover, four headstones were broken and completely lost during the renovation.

- NCPTT staff recommends that the NCA consider eliminating the evaluation of whole headstones at Bath National Cemetery.

7.3. Continuation of Study

- NCPTT staff recommends extending the study for an additional time frame of two years.
- Subtle appearance changes, the variability of biological growth, and the absence of algae support this recommendation.
- NCPTT offers four options for consideration:
    - Option A is to continue the study at four cemeteries, including field trips annually, with additional funding for travel, salary, and evaluation of biological activity.
    - Option B is to continue the study at two cemeteries,[21] including field trips annually, with additional funding for travel, salary, and evaluation of biological activity.
    - Option C is to continue the study at Alexandria National Cemetery, including field trips every six months, with additional funding for salary, and evaluation of biological activity annually.
    - Option D is to continue the study at Alexandria National Cemetery, including field trips every six months, with minimal additional funding for salary only. Appearance changes would be examined; no biological testing would be planned.
- Cost considerations for the above recommendations are found in Appendix I.

8. Appendices

Appendix A. Photographic Documentation of Field Trials
Appendix B. Color Measurements on Field Trials
Appendix C. Color Analyses by Cemetery, Test Patch, and Location
Appendix D. Analysis of Microorganisms on headstones in VA Cemeteries, First Report: December 2005
Appendix E. Analysis of Microorganisms on Headstones in VA Cemeteries, Second Report: June 2006
Appendix F. Analysis of Microorganisms on Headstones in VA Cemeteries, Third Report: February 2007
Appendix G. Biological Performance Based on June 2006 Report
Appendix H. Biological Performance Based on February 2007 Report
Appendix I. Cost Estimates, Four Options for Continuing the Study for Two Years

[21] We recommend continuation of the study at Jefferson Barracks National Cemetery and Alexandria National Cemetery. We would eliminate San Francisco National Cemetery because cleaning maintenance is contracted out and Santa Fe National Cemetery may not show significant visual appearance change because of the hot dry climate.

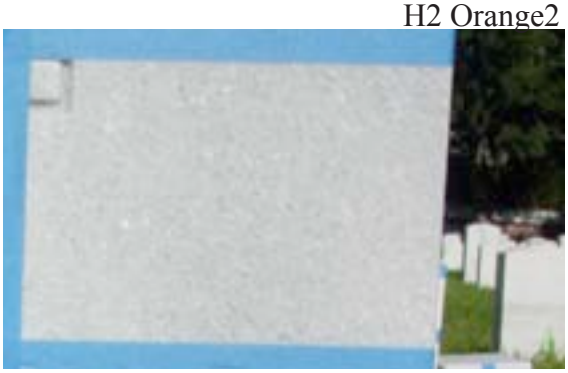
Appendix A.
Photographic Documentation of Field Trials

Alexandria National Cemetery

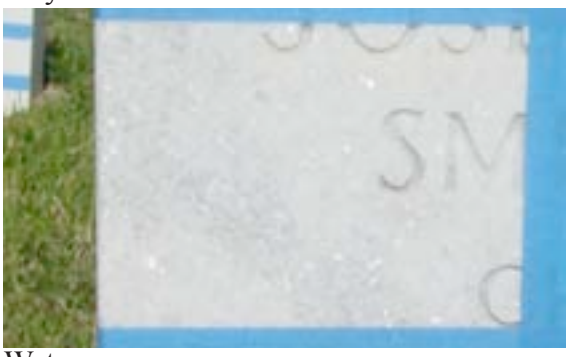
October 4, 2005
B 1200-A

D/2

H2 Orange2



Daybreak



Marble Cleaner Conc.

Water

Photo-flo

Alexandria National Cemetery

May 17, 2006
B 1200-A

D/2

H2 Orange2

Daybreak

Marble Cleaner Conc.

Water

Photo-flo

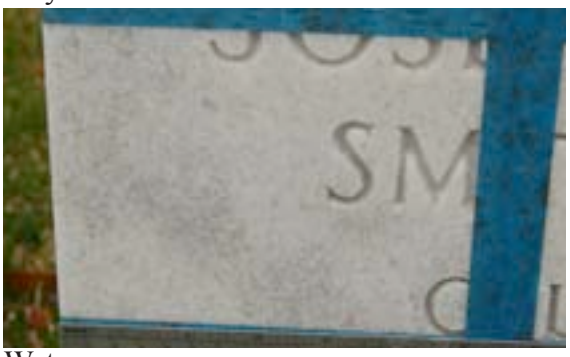
Alexandria National Cemetery

January 16, 2007
B 1200-A

D/2

H2 Orange2

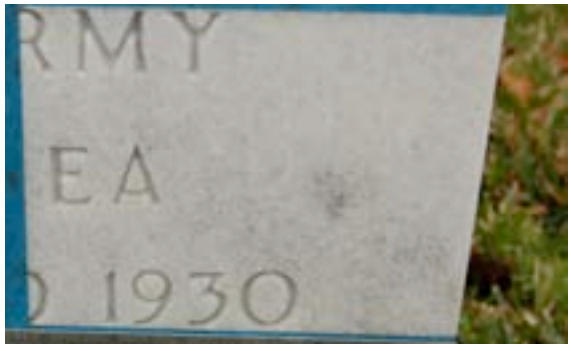
Daybreak



Marble Cleaner Conc.

Water

Photo-flo



Alexandria National Cemetery

October 4, 2005
B 1312

D/2

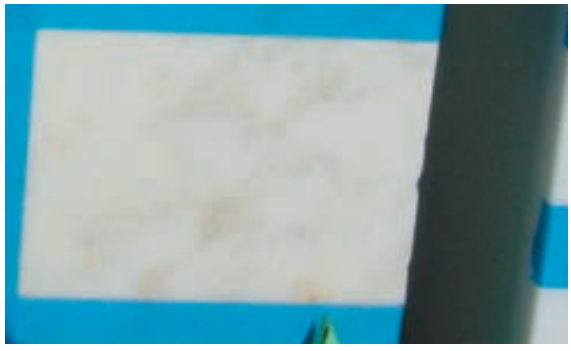
Daybreak

H2 Orange2

Marble Cleaner Conc.

Water

Photo-flo



Alexandria National Cemetery

May 17, 2006
B 1312

D/2

H2 Orange2

Daybreak

Marble Cleaner Conc.

Water

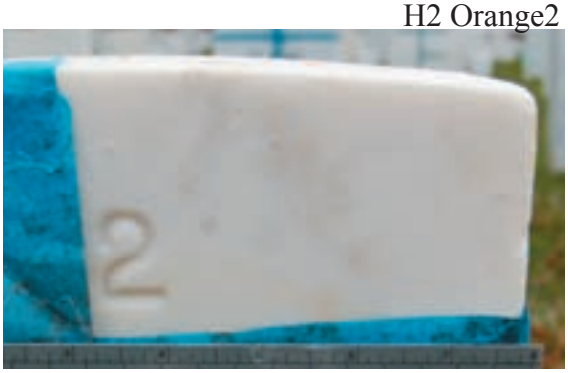
Photo-flo

Alexandria National Cemetery

January 16, 2007
B 1312

D/2

H2 Orange2



Daybreak

Marble Cleaner Conc.

Water

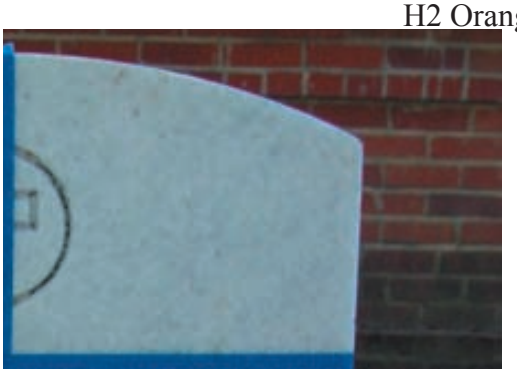
Photo-flo

Alexandria National Cemetery

October 4, 2005
C 418-A

D/2

H2 Orange2



Daybreak

Marble Cleaner Conc.

Water

Photo-flo

Alexandria National Cemetery

May 17, 2006
C 418-A

D/2

H2 Orange2

Daybreak

Marble Cleaner Conc.

Water

Photo-flo

Alexandria National Cemetery

January 16, 2007
C 418-A

D/2

Daybreak

H2 Orange2

Marble Cleaner Conc.

Water

Photo-flo

Alexandria National Cemetery

October 4, 2005
C 417-A

D/2

Daybreak

H2 Orange2

Marble Cleaner Conc.

Water

Photo-flo

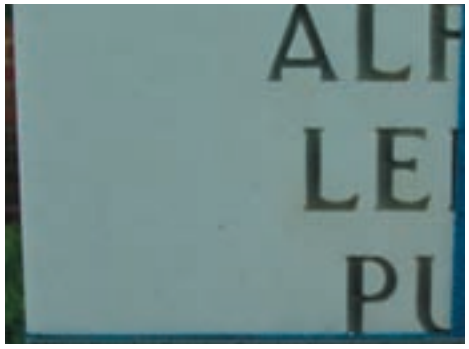
Alexandria National Cemetery

May 17, 2006
C 417-A

D/2

H2 Orange2

Daybreak



Marble Cleaner Conc.

Water

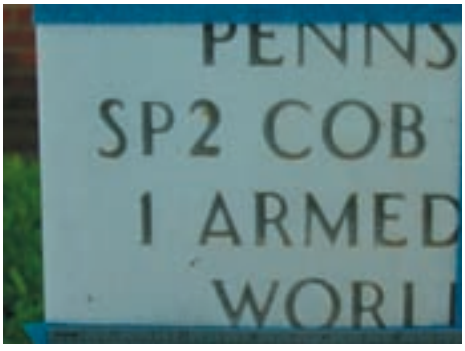


Photo-flo

Alexandria National Cemetery

January 16, 2007
C 417-A

D/2

Daybreak

H2 Orange2

Marble Cleaner Conc.

Water

Photo-flo

Alexandria National Cemetery

May 17, 2006
B 1201-A

D/2

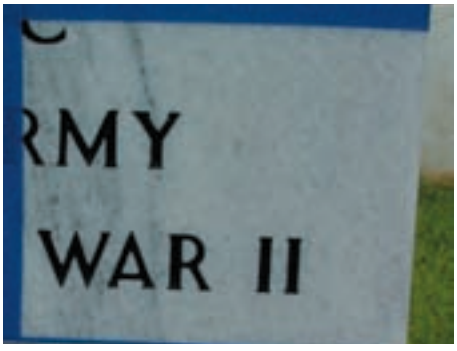
H2 Orange2

Daybreak

Marble Cleaner Conc.

Water

Photo-flo



Alexandria National Cemetery

January 16, 2007
B 1201-A

D/2

H2 Orange2

Daybreak

Marble Cleaner Conc.

Water

Photo-flo

Alexandria National Cemetery

May 17, 2006
B 1202

D/2

H2 Orange2

Daybreak

Marble Cleaner Conc.

Water

Photo-flo

Alexandria National Cemetery

January 16, 2007
B 1202

D/2

H2 Orange2

Daybreak

Marble Cleaner Conc.

Water

Photo-flo

Alexandria National Cemetery

May 17, 2006
C 419

D/2

H2 Orange2

Daybreak

Marble Cleaner Conc.

Water

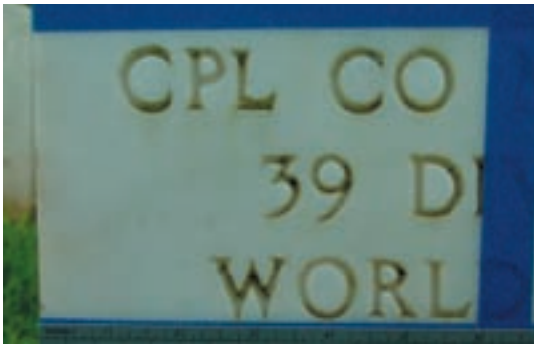
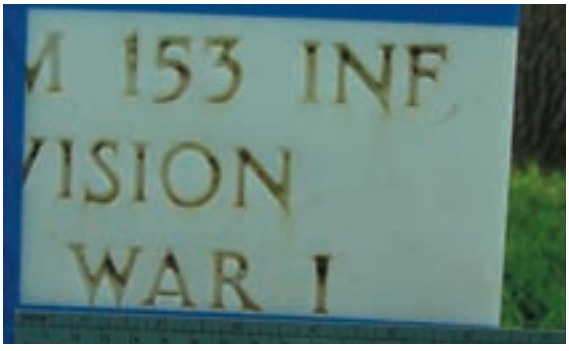


Photo-flo



Alexandria National Cemetery

January 16, 2007
C 419

D/2

H2 Orange2

Daybreak

Marble Cleaner Conc.

Water

Photo-flo

Alexandria National Cemetery

May 17, 2006

K 151

D/2

H2 Orange2

Daybreak

Marble Cleaner Conc.

Water

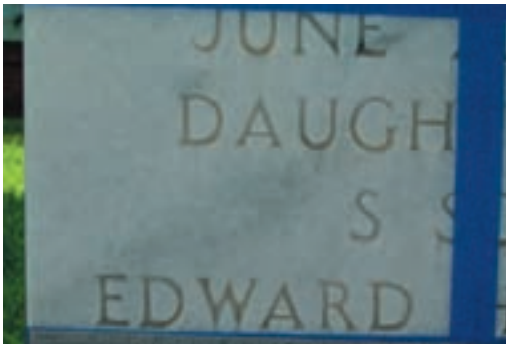


Photo-flo

Alexandria National Cemetery

January 16, 2007
K 151

D/2

H2 Orange2

Daybreak

Marble Cleaner Conc.

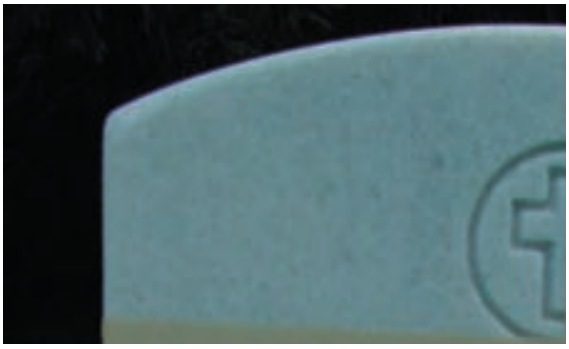
Water

Photo-flo

Bath National Cemetery

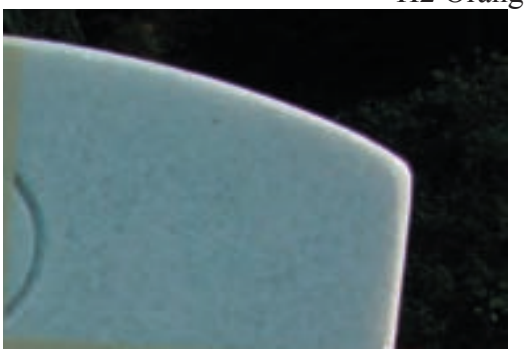
October 6, 2005
A2 1-B

D/2



Daybreak

H2 Orange2



Marble Cleaner Conc.

Water

Photo-flo

Bath National Cemetery

April 4, 2006
A2 1-B

D/2

Daybreak

H2 Orange2

Marble Cleaner Conc.

Water

Photo-flo

Bath National Cemetery

November 7, 2006

A2 1-B

D/2

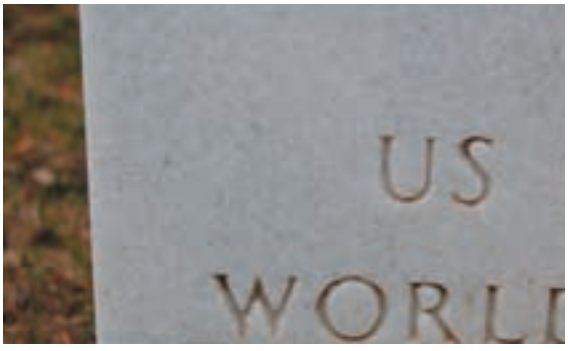
Daybreak

H2 Orange2

Marble Cleaner Conc.

Water

Photo-flo

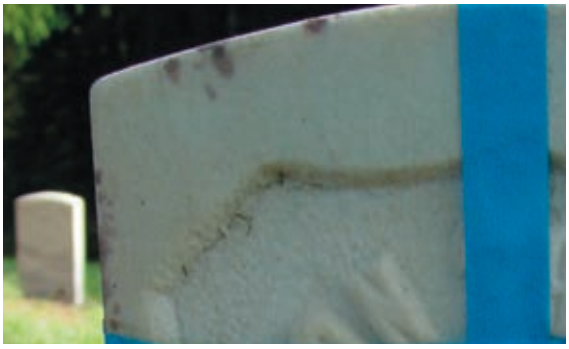


Bath National Cemetery

October 6, 2005

D 713

D/2



H2 Orange2

Daybreak

Marble Cleaner Conc.

Water

Photo-flo

Bath National Cemetery

April 4, 2006

D 713

D/2

H2 Orange2

Daybreak

Marble Cleaner Conc.

Water

Photo-flo

Bath National Cemetery

November 7, 2006

D 713

D/2

H2 Orange2

Daybreak

Marble Cleaner Conc.

Water

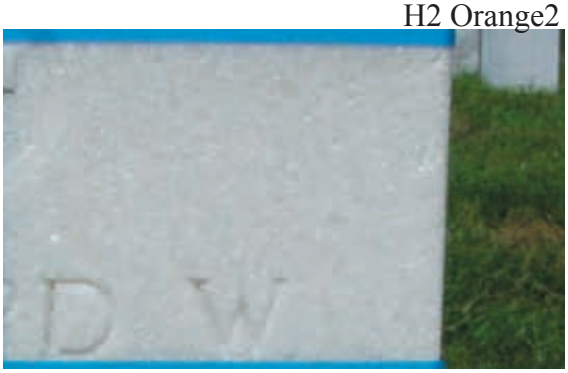
Photo-flo

Bath National Cemetery

October 6, 2005
F 215

D/2

H2 Orange2

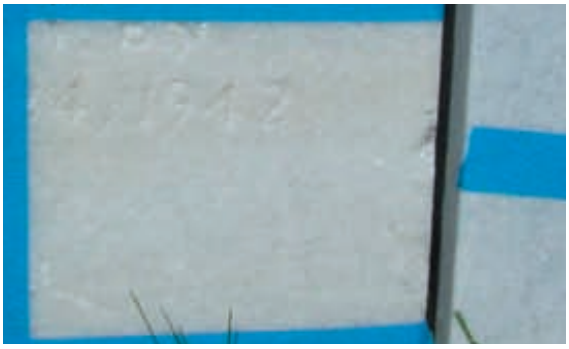


Daybreak

Marble Cleaner Conc.

Water

Photo-flo



Bath National Cemetery

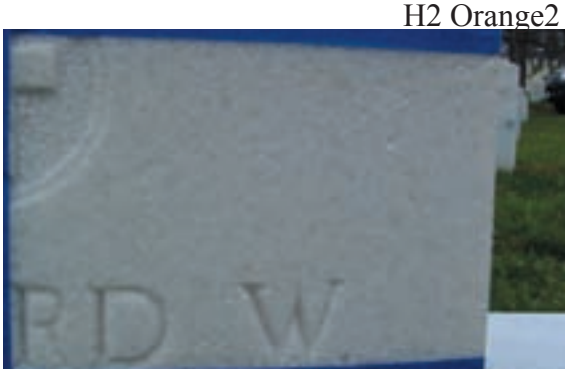
April 4, 2006

F 215

D/2

Daybreak

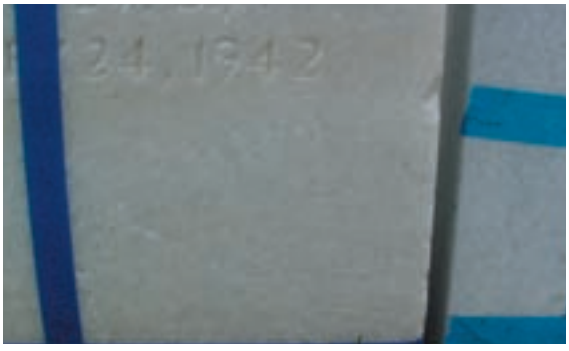
H2 Orange2



Marble Cleaner Conc.

Water

Photo-flo



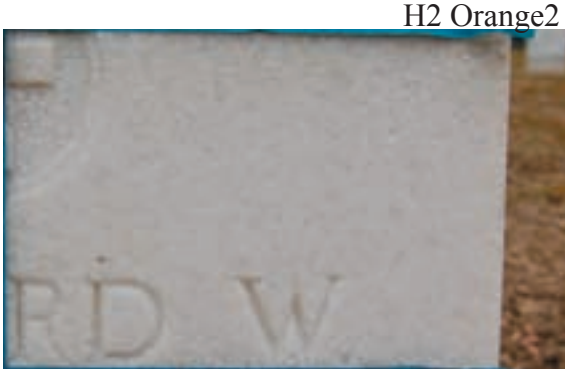
Bath National Cemetery

November 7, 2006

F 215

D/2

H2 Orange2

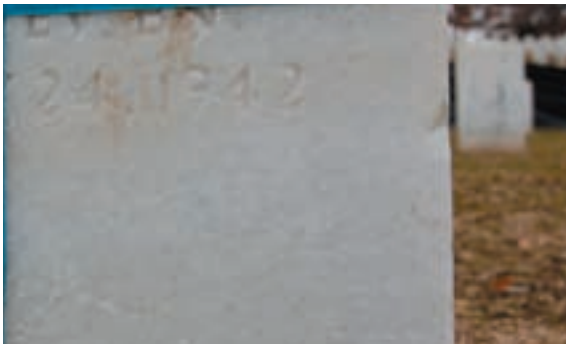


Daybreak

Marble Cleaner Conc.

Water

Photo-flo

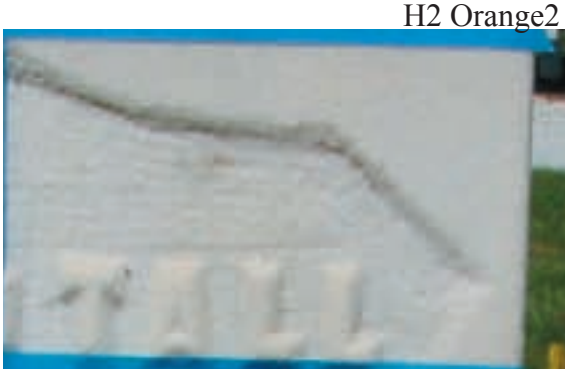


Bath National Cemetery

October 6, 2005
F 712

D/2

H2 Orange2



Daybreak

Marble Cleaner Conc.

Water

Photo-flo

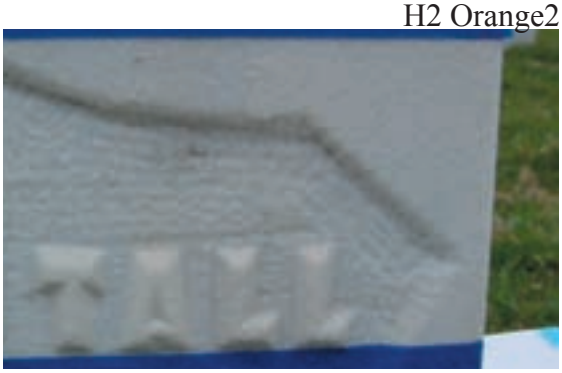
Bath National Cemetery

April 4, 2006

F 712

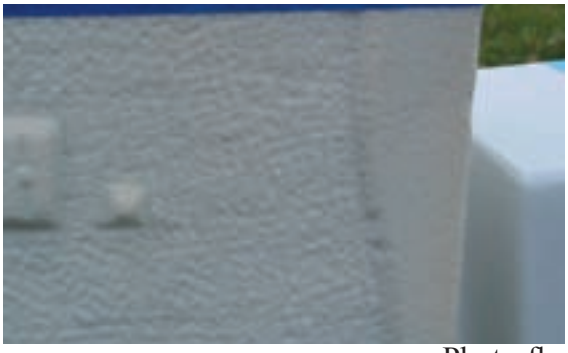
D/2

H2 Orange2



Daybreak

Marble Cleaner Conc.



Water

Photo-flo

Bath National Cemetery

November 7, 2006

F 712

D/2

H2 Orange2

Daybreak

Marble Cleaner Conc.

Water

Photo-flo

Bath National Cemetery

April 4, 2006

A 132

D/2

H2 Orange2

Daybreak

Marble Cleaner Conc.

Water

Photo-flo

Bath National Cemetery

November 7, 2006

A 132

D/2

Daybreak

H2 Orange2

Marble Cleaner Conc.

Water

Photo-flo

Bath National Cemetery

April 4, 2006

B 112

D/2

H2 Orange2

Daybreak

Marble Cleaner Conc.

Water

Photo-flo

Bath National Cemetery

November 7, 2006

B 112

D/2

H2 Orange2

Daybreak

Marble Cleaner Conc.

Water

Photo-flo

Bath National Cemetery

April 4, 2006

F 513

D/2

H2 Orange2

Daybreak

Marble Cleaner Conc.

Water

Photo-flo

Bath National Cemetery

November 7, 2006

F 513

D/2

H2 Orange2

Daybreak

Marble Cleaner Conc.

Water

Photo-flo

Bath National Cemetery

April 4, 2006

F 812

D/2

H2 Orange2

Daybreak

Marble Cleaner Conc.

Water

Photo-flo

Bath National Cemetery

November 7, 2006

F 812

D/2

Daybreak

H2 Orange2

Marble Cleaner Conc.

Water

Photo-flo

Jefferson Barracks National Cemetery

October 19, 2005
32 2898-A

D/2

H2 Orange2

Daybreak

Marble Cleaner Conc.

Water

Photo-flo

Jefferson Barracks National Cemetery

April 11, 2006
32 2898-A

D/2

H2 Orange2

Daybreak

Marble Cleaner Conc.

Water

Photo-flo

Jefferson Barracks National Cemetery

April 11, 2006
32 2898-A

D/2

H2 Orange2

Daybreak

Marble Cleaner Conc.

Water

Photo-flo

Jefferson Barracks National Cemetery

October 19, 2005

32 2904-A

D/2

H2 Orange2

Daybreak

Marble Cleaner Conc.

Water

Photo-flo

Jefferson Barracks National Cemetery

April 11, 2006
32 2904-A

D/2

H2 Orange2

Daybreak

Marble Cleaner Conc.

Water

Photo-flo

Jefferson Barracks National Cemetery

November 9, 2006
32 2904-A

D/2

Daybreak

H2 Orange2

Marble Cleaner Conc.

Water

Photo-flo

Jefferson Barracks National Cemetery

October 19, 2005
72 1273

D/2

H2 Orange2

Daybreak

Marble Cleaner Conc.

Water

Photo-flo

Jefferson Barracks National Cemetery

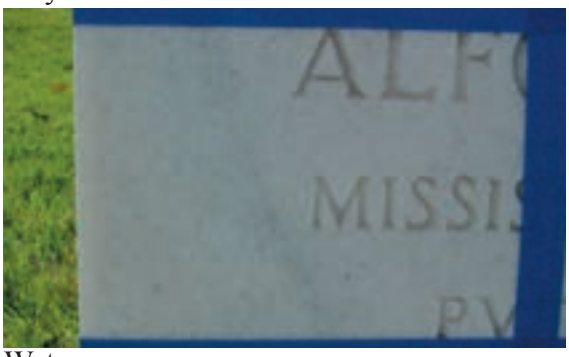
April 11, 2006
72 1273

D/2

Daybreak

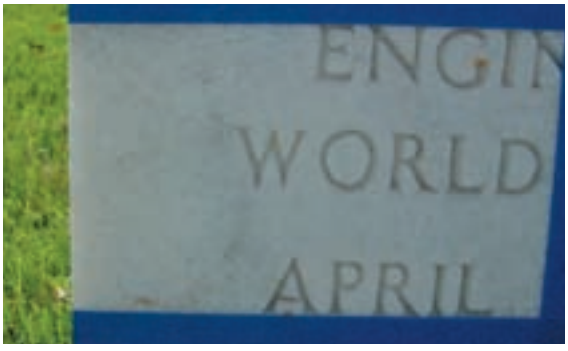
H2 Orange2

Marble Cleaner Conc.



Water

Photo-flo



Jefferson Barracks National Cemetery

November 9, 2006
72 1273

D/2

Daybreak

H2 Orange2

Marble Cleaner Conc.

Water

Photo-flo

Jefferson Barracks National Cemetery

October 19, 2005
72 1370

D/2

H2 Orange2

Daybreak

Marble Cleaner Conc.

Water

Photo-flo

Jefferson Barracks National Cemetery

April 11, 2006
72 1370

D/2

H2 Orange2

Daybreak

Marble Cleaner Conc.

Water

Photo-flo

Jefferson Barracks National Cemetery

November 9, 2006
72 1370

D/2

H2 Orange2

Daybreak

Marble Cleaner Conc.

Water

Photo-flo

Jefferson Barracks National Cemetery

April 11, 2006

3151

D/2

H2 Orange2

Daybreak

Marble Cleaner Conc.

Water

Photo-flo

Jefferson Barracks National Cemetery

November 9, 2006

3151

D/2

Daybreak

H2 Orange2

Marble Cleaner Conc.

Water

Photo-flo

Jefferson Barracks National Cemetery

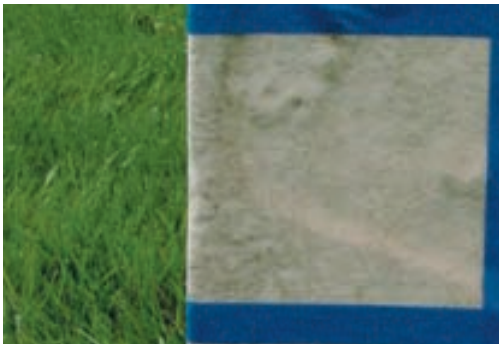
April 11, 2006

3187

D/2

H2 Orange2

Daybreak



Marble Cleaner Conc.

Water

Photo-flo

Jefferson Barracks National Cemetery

November 9, 2006

3187

D/2

H2 Orange2

Daybreak

Marble Cleaner Conc.

Water

Photo-flo

Jefferson Barracks National Cemetery

April 11, 2006
72 1164

D/2

H2 Orange2

Daybreak

Marble Cleaner Conc.

Water

Photo-flo

Jefferson Barracks National Cemetery

November 9, 2006
72 1164

D/2

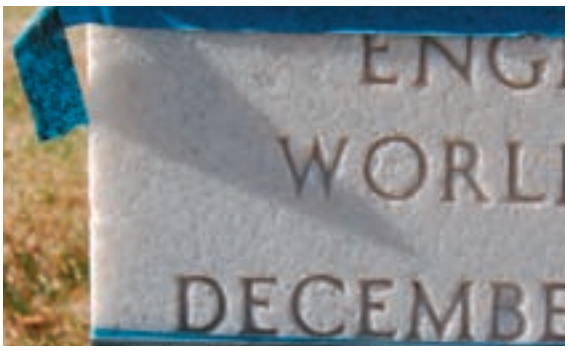
Daybreak

H2 Orange2

Marble Cleaner Conc.

Water

Photo-flo



Jefferson Barracks National Cemetery

April 11, 2006
72 1268

D/2

Daybreak

H2 Orange2

Marble Cleaner Conc.

Water

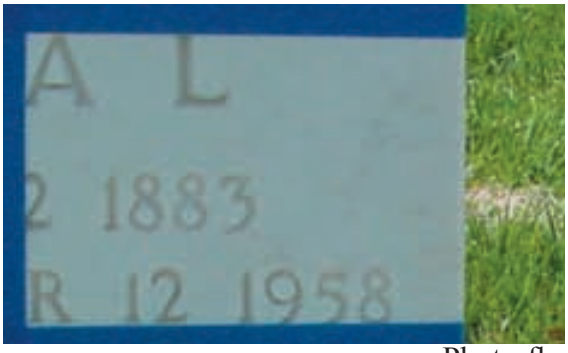


Photo-flo

Jefferson Barracks National Cemetery

November 9, 2006
72 1268

D/2

Daybreak

H2 Orange2

Marble Cleaner Conc.

Water

Photo-flo

San Francisco National Cemetery

November 2, 2005

NAWS 881B

D/2

H2 Orange2

Daybreak

Marble Cleaner Conc.

Water

Photo-flo

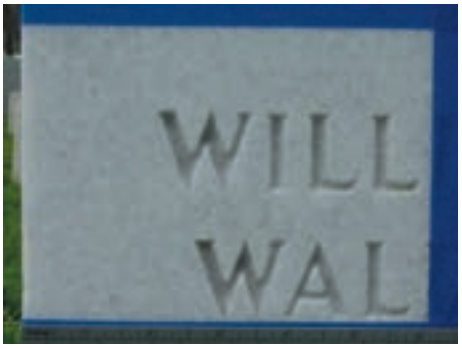
San Francisco National Cemetery

April 26, 2006
NAWS 881B

D/2

H2 Orange2

Daybreak



Marble Cleaner Conc.

Water

Photo-flo

San Francisco National Cemetery

December 5, 2006

NAWS 881B

D/2

Daybreak

H2 Orange2

Marble Cleaner Conc.

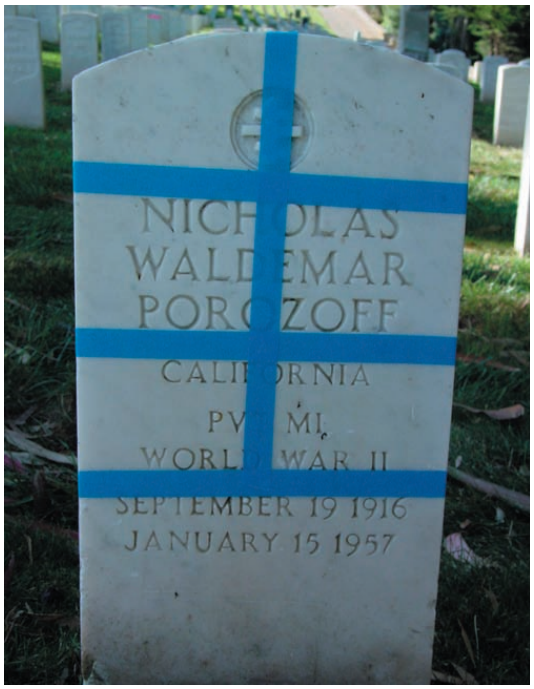
Water

Photo-flo

San Francisco National Cemetery

November 2, 2005

NAWS 886B



D/2

Daybreak

H2 Orange2

Marble Cleaner Conc.

Water

Photo-flo

San Francisco National Cemetery

April 26, 2006
NAWS 886 B

D/2

H2 Orange2

Daybreak

Marble Cleaner Conc.

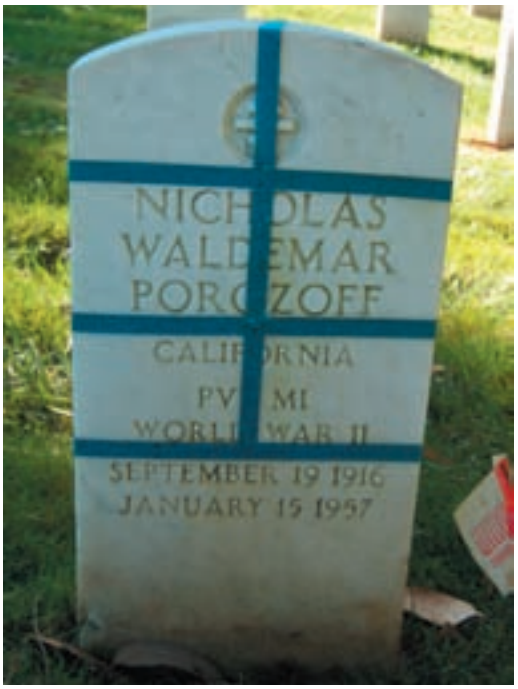
Water

Photo-flo

San Francisco National Cemetery

December 5, 2006

NAWS 886B



D/2

Daybreak

H2 Orange2

Marble Cleaner Conc.

Water

Photo-flo

San Francisco National Cemetery

November 2, 2005
WS 1032B

D/2

H2 Orange2

Daybreak

Marble Cleaner Conc.

Water

Photo-flo

San Francisco National Cemetery

April 26, 2006

WS 1032B

D/2

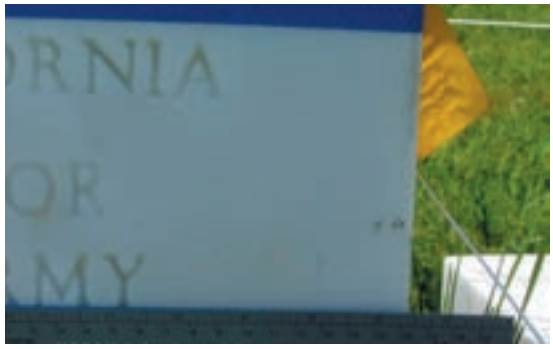
H2 Orange2

Daybreak

Marble Cleaner Conc.

Water

Photo-flo



San Francisco National Cemetery

December 5, 2006
WS 1032B

D/2

H2 Orange2

Daybreak

Marble Cleaner Conc.

Water

Photo-flo

San Francisco National Cemetery

November 2, 2005
WS 1033B

D/2

H2 Orange2

Daybreak

Marble Cleaner Conc.

Water

Photo-flo

San Francisco National Cemetery

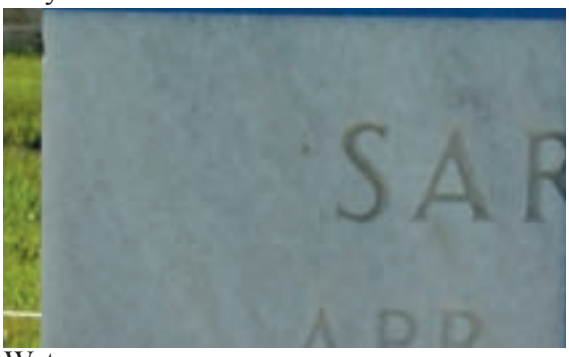
April 26, 2006

WS 1033B

D/2

H2 Orange2

Daybreak



Marble Cleaner Conc.

Water

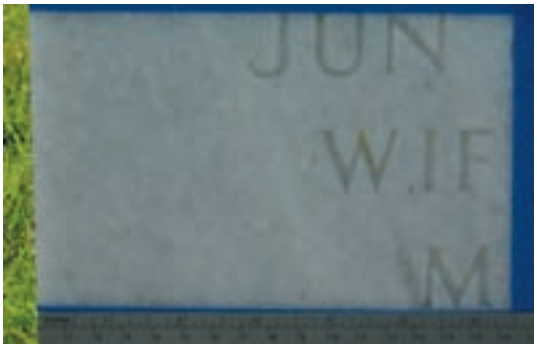


Photo-flo

San Francisco National Cemetery

December 5, 2006
WS 1033B

D/2

Daybreak

H2 Orange2

Marble Cleaner Conc.

Water

Photo-flo

San Francisco National Cemetery

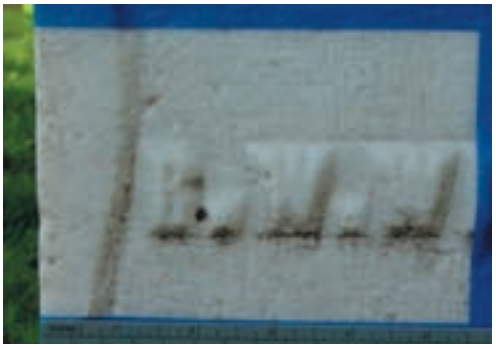
April 26, 2006

1075

D/2

H2 Orange2

Daybreak



Marble Cleaner Conc.

Water

Photo-flo

San Francisco National Cemetery

December 5, 2006

1075

D/2

H2 Orange2

Daybreak

Marble Cleaner Conc.

Water

Photo-flo

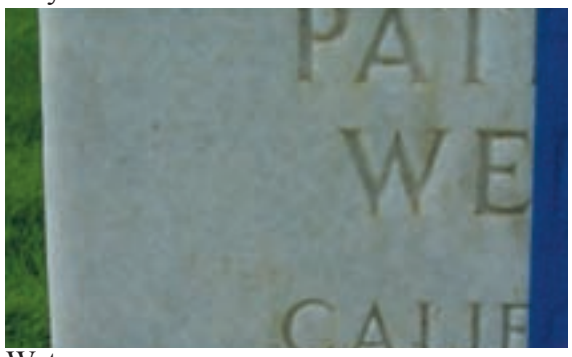
San Francisco National Cemetery

April 26, 2006
NAWS 739B

D/2

H2 Orange2

Daybreak



Marble Cleaner Conc.

Water

Photo-flo

San Francisco National Cemetery

December 5, 2006
NAWS 739B

D/2

Daybreak

H2 Orange2

Marble Cleaner Conc.

Water

Photo-flo

San Francisco National Cemetery

April 26, 2006

WS 862B

D/2

H2 Orange2

Daybreak

Marble Cleaner Conc.

Water

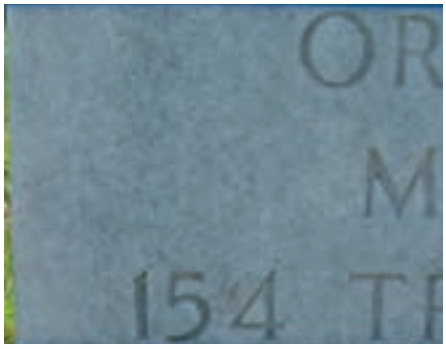


Photo-flo

San Francisco National Cemetery

December 5, 2006

WS 862 B

D/2

H2 Orange2

Daybreak

Marble Cleaner Conc.

Water

Photo-flo

San Francisco National Cemetery

April 26, 2006
WS 1038B

D/2

H2 Orange2

Daybreak

Marble Cleaner Conc.

Water

Photo-flo

San Francisco National Cemetery

December 5, 2006
WS 1038B

D/2

Daybreak

H2 Orange2

Marble Cleaner Conc.

Water

Photo-flo

Santa Fe National Cemetery

November 11, 2005
H 526 D

D/2

Daybreak

H2 Orange2

Marble Cleaner Conc.

Water

Photo-flo

Santa Fe National Cemetery

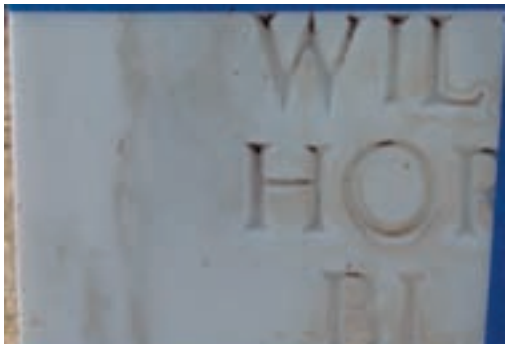
May 3, 2006

H 526 D

D/2

H2 Orange2

Daybreak



Marble Cleaner Conc.

Water

Photo-flo

Santa Fe National Cemetery

December 7, 2006

H 526 D

D/2

Daybreak

H2 Orange2

Marble Cleaner Conc.

Water

Photo-flo

Santa Fe National Cemetery

November 11, 2005
H 526 J

D/2

Daybreak

H2 Orange2

Marble Cleaner Conc.

Water

Photo-flo

Santa Fe National Cemetery

May 3, 2006
H 526 J

D/2

Daybreak

H2 Orange2

Marble Cleaner Conc.

Water

Photo-flo

Santa Fe National Cemetery

December 7, 2006
H 526 J

D/2

Daybreak

H2 Orange2

Marble Cleaner Conc.

Water

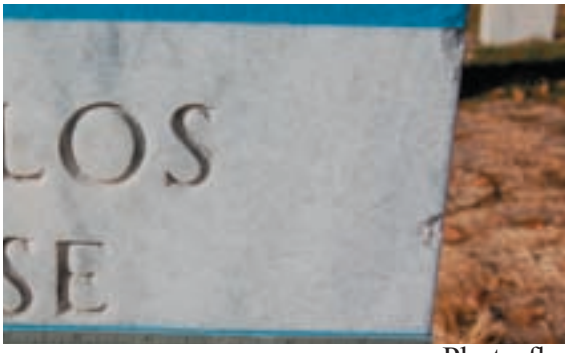


Photo-flo

Santa Fe National Cemetery

November 11, 2005
U 311-A

D/2

Daybreak

H2 Orange2

Marble Cleaner Conc.

Water

Photo-flo

Santa Fe National Cemetery

May 3, 2006

U 311-A

D/2

H2 Orange2

Daybreak

Marble Cleaner Conc.

Water

Photo-flo

Santa Fe National Cemetery

December 7, 2006

U 311-A

D/2

Daybreak

H2 Orange2

Marble Cleaner Conc.

Water

Photo-flo

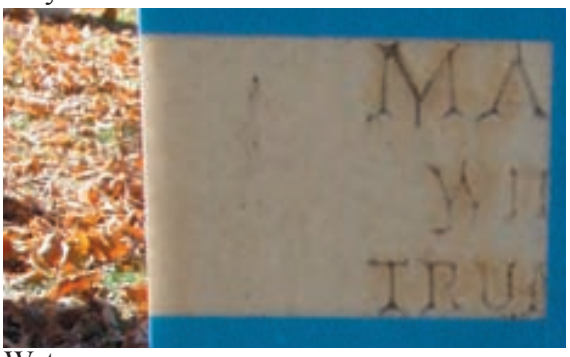
Santa Fe National Cemetery

November 11, 2005
U 343

D/2

H2 Orange2

Daybreak



Marble Cleaner Conc.

Water

Photo-flo

Santa Fe National Cemetery

May 3, 2006
U 343

D/2

H2 Orange2

Daybreak

Marble Cleaner Conc.

Water

Photo-flo

Santa Fe National Cemetery

December 7, 2006

U 343

D/2

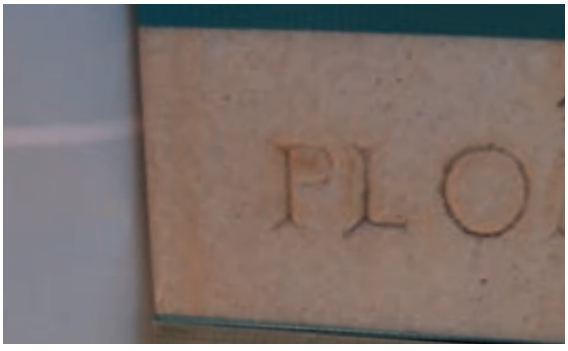
Daybreak

H2 Orange2

Marble Cleaner Conc.

Water

Photo-flo



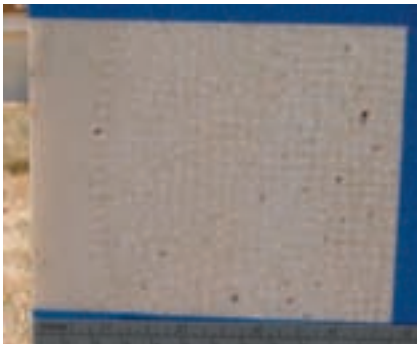
Santa Fe National Cemetery

May 3, 2006
H 530

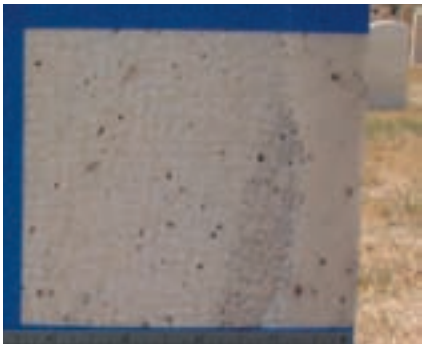
D/2

H2 Orange2

Daybreak



Marble Cleaner Conc.



Water

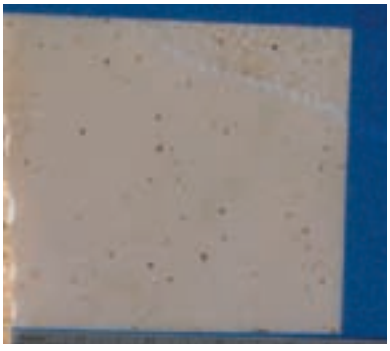


Photo-flo

Santa Fe National Cemetery

December 7, 2006
H 530

D/2

H2 Orange2

Daybreak

Marble Cleaner Conc.

Water

Photo-flo

Santa Fe National Cemetery

May 3, 2006
I 444

D/2

H2 Orange2

Daybreak

Marble Cleaner Conc.

Water

Photo-flo

Santa Fe National Cemetery

December 7, 2006

I 444

D/2

Daybreak

H2 Orange2

Marble Cleaner Conc.

Water

Photo-flo

Santa Fe National Cemetery

May 3, 2006
U 280

D/2

H2 Orange2

Daybreak

Marble Cleaner Conc.

Water

Photo-flo

Santa Fe National Cemetery

December 7, 2006

U 280

D/2

Daybreak

H2 Orange2

Marble Cleaner Conc.

Water

Photo-flo

Santa Fe National Cemetery

May 3, 2006
U 311

D/2

H2 Orange2

Daybreak

Marble Cleaner Conc.

Water

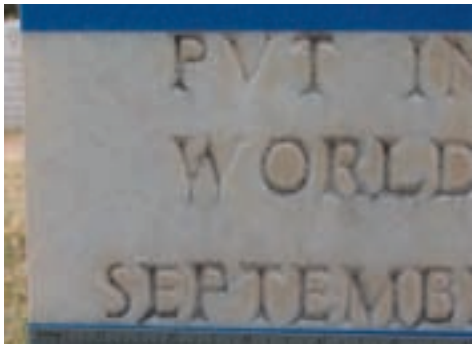


Photo-flo

Santa Fe National Cemetery

December 7, 2006

U 311

D/2

Daybreak

H2 Orange2

Marble Cleaner Conc.

Water

Photo-flo

Appendix B.
Color Measurements on Field Trials

Alexandria NC

	4-Oct-05				17-May-06				16-Jan-07				May 17, 2006 to January 16, 2007			
Grave	**B 1202**	Average L*	Average a*	Average b*	**B 1202**	Average L*	Average a*	Average b*	**B 1202**	Average L*	Average a*	Average b*	ΔL*	Δa*	Δb*	ΔE
Space	1				1	81.73	-0.05	2.62	1	82.49	-0.08	1.80	0.76	-0.03	-0.82	1.12
	2				2	76.26	-0.13	1.47	2	71.24	-0.49	1.89	-5.02	-0.36	0.42	5.05
	3				3	68.36	-0.52	2.38	3	65.47	-0.43	1.04	-2.89	0.09	-1.34	3.19
	4				4	82.20	0.00	2.83	4	82.17	-0.02	2.64	-0.03	-0.02	-0.19	0.19
	5				5	76.77	-0.10	1.52	5	70.24	-0.62	2.35	-6.53	-0.52	0.83	6.60
	6				6	68.01	-0.51	1.79	6	64.14	-0.58	1.60	-3.87	-0.07	-0.19	3.88
	1312				**1312**				**1312**							
	1	71.64	0.15	4.54	1	78.04	-0.46	6.59	1	79.65	-0.66	7.61	1.61	-0.20	1.02	1.92
	2	69.64	0.23	5.56	2	80.52	-0.51	6.94	2	81.14	-0.70	7.19	0.62	-0.19	0.25	0.69
	3	67.84	0.32	7.55	3	78.37	-0.49	5.94	3	76.47	-0.70	5.12	-1.90	-0.21	-0.82	2.08
	4	66.39	0.42	6.16	4	77.32	-0.37	6.79	4	77.13	-0.82	8.65	-0.19	-0.45	1.86	1.92
	5	68.21	0.20	6.69	5	78.70	-0.39	8.34	5	81.58	-0.63	6.91	2.88	-0.24	-1.43	3.22
	6	67.49	0.33	6.78	6	75.66	-0.40	5.41	6	71.53	-0.71	7.36	-4.13	-0.31	1.95	4.58
	B 1200 A				**B 1200 A**				**B 1200 A**							
	1	71.57	0.21	3.29	1	74.43	-0.04	0.75	1	74.39	-0.14	0.79	-0.04	-0.10	0.04	0.11
	2	68.69	0.25	2.74	2	73.63	0.03	0.19	2	74.29	-0.08	0.23	0.66	-0.11	0.04	0.67
	3	65.38	0.36	3.90	3	70.86	0.01	-0.44	3	69.18	-0.12	-0.32	-1.68	-0.13	0.12	1.69
	4	61.99	0.24	4.91	4	68.11	0.09	1.38	4	71.84	-0.02	1.18	3.73	-0.11	-0.20	3.74
	5	66.40	0.27	3.19	5	70.30	0.10	0.86	5	74.21	-0.12	0.54	3.91	-0.22	-0.32	3.93
	6	66.38	0.34	3.18	6	68.64	-0.09	-0.07	6	70.74	-0.23	-0.01	2.10	-0.14	0.06	2.11
	K 151				**K 151**				**K 151**							
	1				1	66.61	-0.15	2.16	1	66.72	-0.34	1.25	0.11	-0.19	-0.91	0.94
	2				2	65.80	-0.16	0.57	2	65.11	-0.38	0.49	-0.69	-0.22	-0.08	0.73
	3				3	62.26	-0.12	0.25	3	64.81	-0.59	2.25	2.55	-0.47	2.00	3.27
	4				4	66.80	-0.10	1.48	4	63.71	-0.31	1.38	-3.09	-0.21	-0.10	3.10
	5				5	67.03	-0.18	0.96	5	57.60	-0.26	-0.11	-9.43	-0.08	-1.07	9.49
	6				6	67.51	-0.11	1.17	6	68.20	-0.34	1.37	0.69	-0.23	0.20	0.75
	C 419				**C 419**				**C 419**							
	1				1	73.05	-4.01	12.32	1	82.13	-0.55	2.87	9.08	3.46	-9.45	13.55
	2				2	81.05	-1.50	6.44	2	74.27	-0.71	2.05	-6.78	0.79	-4.39	8.12
	3				3	72.80	-0.87	5.30	3	69.25	-0.78	2.48	-3.55	0.09	-2.82	4.53
	4				4	73.27	-3.99	13.50	4	80.35	-0.36	3.31	7.08	3.63	-10.19	12.93
	5				5	84.70	-1.26	7.97	5	78.36	-0.65	3.17	-6.34	0.61	-4.80	7.98
	6				6	73.89	-0.74	5.75	6	70.09	-0.84	3.59	-3.80	-0.10	-2.16	4.37
	B 1201 A				**B 1201 A**				**B 1201 A**							
	1				1	71.70	-0.02	-2.32	1	70.20	-0.17	-1.09	-1.50	-0.15	1.23	1.95
	2				2	73.23	0.07	-1.99	2	67.16	-0.21	-1.86	-6.07	-0.28	0.13	6.08
	3				3	73.78	0.06	-2.06	3	75.17	-0.25	-0.80	1.39	-0.31	1.26	1.90
	4				4	70.66	-0.01	-2.39	4	75.88	-0.33	0.09	5.22	-0.32	2.48	5.79
	5				5	71.64	0.03	-2.39	5	70.35	-0.28	-1.91	-1.29	-0.31	0.48	1.41
	6				6	70.83	-0.01	-2.30	6	71.32	-0.26	-0.86	0.49	-0.25	1.44	1.54
	C 417-A				**C 417-A**				**C 417-A**							
	1	84.02	0.02	2.01	1	82.40	-0.03	1.76	1	74.03	-0.38	1.75	-8.37	-0.35	-0.01	8.38
	2	83.21	0.00	2.03	2	78.78	-0.14	1.98	2	72.34	-0.53	3.00	-6.44	-0.39	1.02	6.53
	3	83.50	0.04	2.08	3	75.90	-0.25	3.86	3	70.69	-0.64	7.28	-5.21	-0.39	3.42	6.24
	4	83.39	0.04	2.08	4	81.53	-0.05	1.94	4	75.36	-0.41	2.54	-6.17	-0.36	0.60	6.21
	5	84.00	0.05	1.98	5	79.26	-0.07	1.79	5	73.26	-0.50	3.95	-6.00	-0.43	2.16	6.39
	6	83.21	0.03	2.02	6	75.68	-0.24	5.14	6	71.25	-0.69	8.19	-4.43	-0.45	3.05	5.40
	C 418-A				**C 418-A**				**C 418-A**							
	1	68.32	0.11	2.63	1	66.70	0.02	1.12	1	69.53	-0.15	1.25	2.83	-0.17	0.13	2.84
	2	69.78	0.16	3.12	2	68.43	-0.10	1.21	2	66.22	-0.38	2.03	-2.21	-0.28	0.82	2.37
	3	68.25	0.02	2.16	3	66.08	-0.12	1.98	3	67.17	-0.41	3.12	1.09	-0.29	1.14	1.60
	4	65.62	-0.03	0.75	4	68.60	0.01	1.27	4	68.57	-0.17	1.09	-0.03	-0.18	-0.18	0.26
	5	69.17	0.04	0.81	5	68.50	0.02	1.17	5	67.12	-0.31	1.86	-1.38	-0.33	0.69	1.58
	6	67.07	0.01	1.59	6	68.26	-0.09	2.57	6	65.25	-0.46	3.60	-3.01	-0.37	1.03	3.20

Jefferson Barracks National Cemetery

	10/1/2005				4/1/2006				11/1/2006				Difference from 4/1/06 to 11/1/06			
Grave	**32 2904-A**	Average L*	Average a*	Average b*	**32 2904-A**	Average L*	Average a*	Average b*	**32 2904-A**	Average L*	Average a*	Average b*	ΔL*	Δa*	Δb*	ΔE
Space	1	81.26	0.22	5.34	1	82.80	0.52	5.23	1	85.99	-0.02	5.52	3.19	-0.54	0.29	3.25
	2	79.04	0.24	5.11	2	84.05	0.41	5.78	2	83.72	-0.02	6.73	-0.33	-0.43	0.95	1.09
	3	80.97	0.55	5.99	3	81.84	0.12	4.33	3	78.70	-0.23	5.70	-3.14	-0.35	1.37	3.44
	4	80.28	0.22	5.49	4	72.26	-0.56	8.55	4	74.79	0.04	6.37	2.53	0.60	-2.18	3.39
	5	80.86	0.13	5.46	5	81.05	0.22	7.08	5	83.43	-0.09	6.75	2.38	-0.31	-0.33	2.42
	6	82.63	0.52	5.04	6	82.67	0.24	5.29	6	77.61	0.01	6.29	-5.06	-0.23	1.00	5.16
	32 2898-A				**32 2898-A**				**32 2898-A**							
	1	71.52	0.57	4.37	1	71.68	0.62	4.90	1	72.37	0.19	5.99	0.69	-0.43	1.09	1.36
	2	70.22	0.48	5.12	2	75.22	0.43	4.95	2	75.88	0.11	5.42	0.66	-0.32	0.47	0.87
	3	71.41	0.50	4.62	3	75.18	0.42	6.05	3	74.54	0.19	7.32	-0.64	-0.23	1.27	1.44
	4	73.69	0.65	4.63	4	57.47	-1.84	9.13	4	72.12	-0.08	5.03	14.65	1.76	-4.10	15.31
	5	68.76	0.98	5.86	5	71.35	-0.28	6.91	5	75.25	-0.07	6.13	3.90	0.21	-0.78	3.98
	6	70.10	0.63	5.08	6	74.78	0.25	6.21	6	74.85	-0.03	7.24	0.07	-0.28	1.03	1.07
	3151				**3151**				**3151**							
	1				1	57.70	-1.68	9.55	1	75.46	-0.13	4.69	17.76	1.55	-4.86	18.48
	2				2	74.16	0.26	6.29	2	59.21	0.08	5.15	-14.95	-0.18	-1.14	14.99
	3				3	70.88	0.16	4.05	3	73.26	-0.13	3.12	2.38	-0.29	-0.93	2.57
	4				4	54.48	-1.71	9.25	4	74.83	0.17	6.98	20.35	1.88	-2.27	20.56
	5				5	75.54	0.23	7.74	5	78.16	-0.13	7.59	2.62	-0.36	-0.15	2.65
	6				6	70.38	0.00	4.33	6	75.86	-0.14	4.27	5.48	-0.14	-0.06	5.48
	3187				**3187**				**3187**							
	1				1	69.29	0.41	11.12	1	78.25	0.16	9.74	8.96	-0.25	-1.38	9.07
	2				2	72.06	0.84	13.64	2	68.48	1.41	13.23	-3.58	0.57	-0.41	3.65
	3				3	78.33	0.15	7.77	3	75.25	-0.59	2.39	-3.08	-0.74	-5.38	6.24
	4				4	70.63	-0.20	10.72	4	75.61	0.08	8.68	4.98	0.28	-2.04	5.39
	5				5	73.43	0.98	13.64	5	72.40	1.51	13.90	-1.03	0.53	0.26	1.19
	6				6	76.44	0.07	9.66	6	73.52	-0.60	2.41	-2.92	-0.67	-7.25	7.84
	72 1273				**72 1273**				**72 1273**							
	1	70.86	1.28	8.53	1	77.97	0.30	5.59	1	75.15	0.12	6.23	-2.82	-0.18	0.64	2.90
	2	70.23	1.71	9.65	2	78.96	0.22	5.60	2	75.21	-0.06	5.13	-3.75	-0.28	-0.47	3.79
	3	71.92	1.32	8.81	3	78.89	0.12	5.43	3	71.14	-0.13	4.32	-7.75	-0.25	-1.11	7.83
	4	71.31	1.05	6.82	4	75.91	0.35	5.53	4	73.35	-0.02	5.62	-2.56	-0.37	0.09	2.59
	5	72.12	0.90	6.76	5	77.28	0.22	6.51	5	64.37	-0.34	6.50	-12.91	-0.56	-0.01	12.92
	6	71.76	0.96	6.72	6	77.40	0.22	5.81	6	68.46	0.33	6.64	-8.94	0.11	0.83	8.98
	72 1164				**72 1164**				**72 1164**							
	1				1	75.65	0.49	7.05	1	57.72	0.34	5.31	-17.93	-0.15	-1.74	18.01
	2				2	75.31	0.49	6.68	2	64.02	0.38	5.10	-11.29	-0.11	-1.58	11.40
	3				3	72.63	0.39	4.91	3	63.13	0.17	3.97	-9.50	-0.22	-0.94	9.55
	4				4	74.80	0.46	5.70	4	64.08	0.44	5.23	-10.72	-0.02	-0.47	10.73
	5				5	75.41	0.41	5.81	5	70.67	0.32	6.31	-4.74	-0.09	0.50	4.77
	6				6	72.70	0.44	5.41	6	64.58	0.17	3.17	-8.12	-0.27	-2.24	8.43
	72 1268				**72 1268**				**72 1268**							
	1				1	85.47	0.51	6.89	1	89.63	-0.12	4.89	4.16	-0.63	-2.00	4.66
	2				2	86.40	0.56	7.62	2	78.44	0.23	5.60	-7.96	-0.33	-2.02	8.22
	3				3	85.76	0.33	6.35	3	78.43	0.35	6.50	-7.33	0.02	0.15	7.33
	4				4	85.08	0.42	6.42	4	77.21	-0.20	7.26	-7.87	-0.62	0.84	7.94
	5				5	86.18	0.46	7.38	5	85.97	0.13	7.11	-0.21	-0.33	-0.27	0.48
	6				6	85.03	0.39	6.33	6	78.29	-0.13	4.26	-6.74	-0.52	-2.07	7.07
	72 1370				**72 1370**				**72 1370**							
	1	79.17	0.47	9.09	1	88.85	0.01	5.14	1	74.80	-0.15	5.01	-14.05	-0.16	-0.13	14.05
	2	78.95	0.78	9.77	2	88.64	0.11	3.82	2	85.12	-0.27	2.57	-3.52	-0.38	-1.25	3.75
	3	73.06	0.32	7.65	3	83.04	-0.13	3.07	3	73.64	-0.43	2.20	-9.40	-0.30	-0.87	9.44
	4	79.54	1.01	10.78	4	86.22	0.24	7.72	4	77.49	-0.15	6.10	-8.73	-0.39	-1.62	8.89
	5	78.67	0.50	9.18	5	87.30	0.34	6.59	5	81.61	-0.34	3.45	-5.69	-0.68	-3.14	6.53
	6	89.98	0.01	3.76	6	83.04	0.02	3.44	6	71.68	-0.37	3.57	-11.36	-0.39	0.13	11.37

San Francisco NC

	2-Nov-05				26-Apr-06				5-Dec-06				April 26, 2006 to December 5, 2006			
Grave	**1075**	Average L*	Average a*	Average b*	**1075**	Average L*	Average a*	Average b*	**1075**	Average L*	Average a*	Average b*	ΔL*	Δa*	Δb*	ΔE
Space	1				1	68.94	-0.09	7.05	1	69.08	-0.18	4.90	0.14	-0.09	-2.15	2.16
	2				2	68.30	0.01	7.05	2	62.42	-0.01	4.82	-5.88	-0.02	-2.23	6.29
	3				3	64.14	0.33	6.85	3	70.52	-0.15	6.51	6.38	-0.48	-0.34	6.41
	4				4	67.25	-1.07	9.21	4	69.71	-0.31	5.78	2.46	0.76	-3.43	4.29
	5				5	66.83	-0.04	5.84	5	61.68	0.01	4.94	-5.15	0.05	-0.90	5.23
	6				6	62.48	0.43	7.72	6	69.71	-0.19	5.25	7.23	-0.62	-2.47	7.67
	NAWS 881 B				**NAWS 881 B**				**NAWS 881 B**							
	1	69.77	0.06	0.68	1	74.13	0.20	-0.51	1	72.41	-0.58	0.79	-1.72	-0.78	1.30	2.29
	2	67.74	0.09	0.63	2	73.78	0.17	-0.76	2	56.21	-0.16	0.95	-17.57	-0.33	1.71	17.66
	3	66.79	0.08	0.94	3	73.24	0.18	-0.42	3	68.77	-0.23	0.29	-4.47	-0.41	0.71	4.54
	4	68.57	0.06	0.76	4	74.14	0.12	0.00	4	73.62	-0.01	-0.39	-0.52	-0.13	-0.39	0.66
	5	69.66	0.05	1.01	5	75.10	0.12	-0.45	5	64.99	-0.32	0.55	-10.11	-0.44	1.00	10.17
	6	67.43	0.03	0.98	6	74.42	0.16	-0.52	6	59.52	0.06	1.47	-14.90	-0.10	1.99	15.03
	WS 1032 B				**WS 1032 B**				**WS 1032 B**							
	1	82.57	-0.27	2.14	1	82.80	0.00	1.48	1	76.27	-0.24	0.49	-6.53	-0.24	-0.99	6.61
	2	72.40	-0.53	3.33	2	82.49	-0.24	0.43	2	74.81	-0.29	0.45	-7.68	-0.05	0.02	7.68
	3	71.92	0.01	3.61	3	78.07	-0.33	0.52	3	68.78	-0.94	1.41	-9.29	-0.61	0.89	9.35
	4	81.78	0.03	2.63	4	85.61	0.09	1.32	4	75.38	-0.29	-0.01	-10.23	-0.38	-1.33	10.32
	5	81.96	-0.03	3.17	5	82.42	-0.13	0.75	5	76.98	-0.55	1.77	-5.44	-0.42	1.02	5.55
	6	81.68	0.00	2.63	6	72.06	-0.20	-0.01	6	68.90	-0.85	1.69	-3.16	-0.65	1.70	3.65
	WS 1033B				**WS 1033B**				**WS 1033B**							
	1	67.65	0.00	1.03	1	68.13	0.03	0.06	1	62.13	-0.20	-1.28	-6.00	-0.23	-1.34	6.15
	2	66.00	0.02	1.49	2	65.83	-0.12	0.20	2	61.93	-0.18	-0.89	-3.90	-0.06	-1.09	4.05
	3	67.03	0.00	1.71	3	61.40	-0.01	-0.79	3	56.71	-0.19	-0.29	-4.69	-0.18	0.50	4.72
	4	65.04	-0.09	2.21	4	69.15	0.05	0.61	4	65.13	-0.35	0.88	-4.02	-0.40	0.27	4.05
	5	67.99	-0.08	2.63	5	67.33	0.01	0.03	5	60.23	-0.12	-0.39	-7.10	-0.13	-0.42	7.11
	6	67.35	0.09	1.04	6	66.88	-0.01	-0.33	6	63.98	-0.16	0.49	-2.90	-0.15	0.82	3.02
	WS 1038B				**WS 1038B**				**WS 1038B**							
	1				1	83.81	-0.32	1.69	1	74.47	-0.73	0.88	-9.34	-0.41	-0.81	9.38
	2				2	79.75	-0.72	1.53	2	71.66	-0.80	1.05	-8.09	-0.08	-0.48	8.10
	3				3	68.66	-0.76	2.97	3	64.47	-1.02	3.78	-4.19	-0.26	0.81	4.28
	4				4	81.98	-0.38	2.17	4	71.28	-0.74	1.92	-10.70	-0.36	-0.25	10.71
	5				5	78.38	-0.50	0.89	5	73.94	-0.76	1.92	-4.44	-0.26	1.03	4.57
	6				6	68.55	-0.82	2.30	6	65.53	-0.81	2.08	-3.02	0.01	-0.22	3.03
	WS 862B				**WS 862B**				**WS 862B**							
	1				1	62.63	0.17	-0.78	1	63.58	-0.12	-0.83	0.95	-0.29	-0.05	0.99
	2				2	64.93	0.23	-0.77	2	55.19	-0.28	-0.31	-9.74	-0.51	0.46	9.76
	3				3	61.33	0.16	-1.17	3	51.74	-0.41	0.23	-9.59	-0.57	1.40	9.71
	4				4	63.87	0.23	-0.75	4	59.32	-0.10	-1.03	-4.55	-0.33	-0.28	4.57
	5				5	61.49	0.19	-0.97	5	62.15	-0.15	-1.21	0.66	-0.34	-0.24	0.78
	6				6	60.74	0.02	-0.12	6	56.63	-0.06	0.38	-4.11	-0.08	0.50	4.14
	NAWS 886B				**NAWS 886B**				**NAWS 886B**							
	1	78.87	0.03	2.86	1	81.68	-0.19	2.53	1	77.51	-0.82	3.00	-4.17	-0.63	0.47	4.24
	2	78.36	0.00	3.40	2	78.00	-0.25	1.91	2	69.91	-2.51	6.47	-8.09	-2.26	4.56	9.56
	3	80.05	0.11	2.92	3	69.94	-0.23	2.22	3	60.55	-2.64	9.75	-9.39	-2.41	7.53	12.28
	4	80.61	-0.13	3.40	4	79.52	-0.61	4.19	4	73.48	-1.20	5.94	-6.04	-0.59	1.75	6.32
	5	79.70	-0.39	2.34	5	71.81	-3.39	9.96	5	64.32	-3.27	8.93	-7.49	0.12	-1.03	7.56
	6	80.68	0.12	2.86	6	68.85	-1.50	6.16	6	60.50	-3.69	11.02	-8.35	-2.19	4.86	9.91
	NAWS 739B				**NAWS 739B**				**NAWS 739B**							
	1				1	70.51	0.15	1.84	1	66.30	-2.68	10.29	-4.21	-2.83	8.45	9.86
	2				2	69.67	0.22	2.32	2	65.93	-1.08	3.68	-3.74	-1.30	1.36	4.19
	3				3	69.27	0.18	2.29	3	64.35	-1.04	3.75	-4.92	-1.22	1.46	5.28
	4				4	71.69	0.11	2.37	4	67.71	-2.60	9.55	-3.98	-2.71	7.18	8.65
	5				5	69.31	0.12	2.16	5	66.16	-1.20	4.48	-3.15	-1.32	2.32	4.13
	6				6	69.27	0.13	2.14	6	63.51	-1.35	5.10	-5.76	-1.48	2.96	6.64

Color Difference for Santa Fe National Cemetery

	14-Nov-05				3-May-06				7-Dec-06				May 3, 2006 to December 7, 2006			
Grave	**U 280**	Average L*	Average a*	Average b*	**U 280**	Average L*	Average a*	Average b*	**U 280**	Average L*	Average a*	Average b*	ΔL*	Δa*	Δb*	ΔE
Space	1				1	79.69	-0.45	4.04	1	74.96	-0.73	2.95	-4.73	-0.28	-1.09	4.86
	2				2	74.88	-0.59	4.04	2	66.69	-0.78	5.27	-8.19	-0.19	1.23	8.28
	3				3	72.45	-0.64	3.51	3	63.98	-0.32	5.48	-8.47	0.32	1.97	8.70
	4				4	80.77	-0.45	2.80	4	73.80	-0.98	2.81	-6.97	-0.53	0.01	6.99
	5				5	74.99	-0.53	2.71	5	65.98	-0.78	6.13	-9.01	-0.25	3.42	9.64
	6				6	72.91	-0.53	2.75	6	65.48	-0.65	4.49	-7.43	-0.12	1.74	7.63
	U 343				**U 343**				**U 343**							
	1	67.03	0.4	4.77	1	74.61	-0.31	6.35	1	69.57	-0.49	7.81	-5.04	-0.18	1.46	5.25
	2	66.86	0.53	4.98	2	74.42	-0.26	6.94	2	69.60	-0.42	6.30	-4.82	-0.16	-0.64	4.86
	3	67.96	0.41	3.72	3	73.05	-0.25	5.00	3	69.45	-0.48	4.05	-3.60	-0.23	-0.95	3.73
	4	67.58	0.57	5.22	4	72.92	-0.28	5.89	4	70.13	-0.57	6.96	-2.79	-0.29	1.07	3.00
	5	68.38	0.59	4.51	5	73.89	-0.29	5.91	5	67.16	-0.51	8.00	-6.73	-0.22	2.09	7.05
	6	67.53	0.53	4.28	6	73.80	-0.19	4.75	6	68.66	-0.50	4.23	-5.14	-0.31	-0.52	5.18
	U 311				**U 311**				**U 311**							
	1				1	71.69	-0.36	5.26	1	68.65	-0.58	4.04	-3.04	-0.22	-1.22	3.28
	2				2	71.44	-0.46	4.41	2	67.94	-0.75	5.00	-3.50	-0.29	0.59	3.56
	3				3	68.57	-0.44	4.44	3	63.59	-0.38	6.22	-4.98	0.06	1.78	5.29
	4				4	72.35	-0.27	4.68	4	71.23	-0.57	3.88	-1.12	-0.30	-0.80	1.41
	5				5	69.03	-0.35	5.29	5	63.77	-0.29	6.63	-5.26	0.06	1.34	5.43
	6				6	66.78	-0.33	6.10	6	62.21	-0.37	7.30	-4.57	-0.04	1.20	4.73
	H 530				**H 530**				**H 530**							
	1				1	76.64	0.28	9.09	1	75.34	-0.17	8.89	-1.30	-0.45	-0.20	1.39
	2				2	76.27	0.22	10.22	2	74.59	-0.13	10.35	-1.68	-0.35	0.13	1.72
	3				3	72.44	-0.11	9.62	3	70.81	-0.56	8.28	-1.63	-0.45	-1.34	2.16
	4				4	75.75	0.48	9.26	4	73.69	0.01	8.95	-2.06	-0.47	-0.31	2.14
	5				5	72.40	0.25	8.48	5	76.38	-0.13	9.63	3.98	-0.38	1.15	4.16
	6				6	69.38	-0.16	7.52	6	68.86	-0.60	7.51	-0.52	-0.44	-0.01	0.68
	H 526 D				**H 526 D**				**H 526 D**							
	1	85.93	0.15	7.27	1	82.78	-0.47	3.88	1	82.18	-0.53	2.44	-0.60	-0.06	-1.44	1.56
	2	83.52	0.04	6.49	2	81.44	-0.57	3.77	2	76.46	-0.64	2.16	-4.98	-0.07	-1.61	5.23
	3	76.88	-0.66	9.28	3	78.70	-0.51	3.66	3	76.68	-0.61	3.12	-2.02	-0.10	-0.54	2.09
	4	75.65	-0.59	8.37	4	88.39	-0.35	3.05	4	83.07	-0.44	3.09	-5.32	-0.09	0.04	5.32
	5	83.57	-1.23	8.19	5	81.97	-0.42	3.16	5	76.99	-0.61	2.89	-4.98	-0.19	-0.27	4.99
	6	83.76	-0.45	7.52	6	80.52	-0.50	3.06	6	74.40	-0.68	2.43	-6.12	-0.18	-0.63	6.15
	H 526 J				**H 526 J**				**H 526 J**							
	1	68.98	0.34	1.94	1	77.20	0.10	0.24	1	70.18	-0.26	-0.67	-7.02	-0.36	-0.91	7.09
	2	68.62	0.23	2.70	2	72.01	0.00	-0.45	2	70.59	-0.28	-0.53	-1.42	-0.28	-0.08	1.45
	3	67.75	0.11	3.43	3	69.87	-0.09	-0.90	3	66.26	-0.30	-0.09	-3.61	-0.21	0.81	3.71
	4	65.80	0.31	1.83	4	74.24	0.04	0.09	4	70.13	-0.19	0.63	-4.11	-0.23	0.54	4.15
	5	70.15	0.28	1.93	5	71.28	-0.03	-0.51	5	70.99	-0.17	-0.66	-0.29	-0.14	-0.15	0.36
	6	69.23	0.21	1.86	6	71.61	-0.01	-0.71	6	66.59	-0.15	0.67	-5.02	-0.14	1.38	5.21
	I 444				**I 444**				**I 444**							
	1				1	76.15	-0.49	4.59	1	73.70	-0.68	4.49	-2.45	-0.19	-0.10	2.46
	2				2	72.30	-0.46	3.79	2	65.56	-0.58	4.35	-6.74	-0.12	0.56	6.76
	3				3	70.14	-0.65	4.49	3	63.98	-0.58	2.94	-6.16	0.07	-1.55	6.35
	4				4	77.15	-0.52	4.48	4	68.79	-0.60	4.26	-8.36	-0.08	-0.22	8.36
	5				5	73.27	-0.51	3.27	5	65.28	-0.52	4.21	-7.99	-0.01	0.94	8.05
	6				6	71.21	-0.58	2.54	6	65.82	-0.81	2.77	-5.39	-0.23	0.23	5.40
	U 311-A				**U 311-A**				**U 311-A**							
	1	83.47	-0.18	1.82	1	81.63	-0.66	1.54	1	77.84	-1.27	2.06	-3.79	-0.61	0.52	3.87
	2	83.37	-0.09	2.25	2	78.58	-0.79	0.97	2	52.83	-0.75	1.87	-25.75	0.04	0.90	25.77
	3	84.39	-0.07	2.33	3	77.09	-0.65	0.21	3	66.41	-0.84	0.48	-10.68	-0.19	0.27	10.69
	4	82.98	0.02	3.68	4	79.14	-0.54	1.28	4	76.55	-0.75	1.82	-2.59	-0.21	0.54	2.65
	5	82.72	-0.22	2.12	5	78.21	-0.67	1.08	5	75.97	-0.94	1.98	-2.24	-0.27	0.90	2.43
	6	83.56	-0.04	2.21	6	76.50	-0.58	1.04	6	63.66	-0.72	0.90	-12.84	-0.14	-0.14	12.84

Appendix C.
Color Analyses by Cemetery, Test Patch, and Location

Frequency of color change by test patch for each cemetery

Delta E for San Francisco

Patch #	1075	NAWS 881 B	WS 1032 B	NAWS 886B	WS 1033B	WS 1038B	WS 862B	NAWS 739B	Freq dE> 5	Freq dE> 10
1	2.16	2.29	6.61	4.24					1.00	0.00
2	6.29	17.66	7.68	9.56					4.00	1.00
3	6.41	4.54	9.35	12.28		Data expected May 2007			3.00	1.00
4	4.29	0.66	10.32	6.32					2.00	1.00
5	5.23	10.17	5.55	7.56					4.00	1.00
6	7.67	15.03	3.65	9.91					3.00	1.00
									17.00	5.00

Delta E for Santa Fe

Patch #	U 343	H 526 D	H 526 J	U 311-A	U 280	U 311	H 530	I 444	Freq dE> 5	Freq dE> 10
1	5.25	1.56	7.09	3.87					2.00	0.00
2	4.86	5.23	1.45	25.77					2.00	1.00
3	3.73	2.09	3.71	10.69		Data expected May 2007			1.00	1.00
4	3.00	5.32	4.15	2.65					0.00	0.00
5	7.05	4.99	0.36	2.43					1.00	0.00
6	5.18	6.15	5.21	12.84					4.00	1.00
									10.00	3.00

Delta E for Jefferson Barracks

Patch #	32 2904-A	32 2898-A	72 1273	72 1370	3151	3187	72 1164	72 1268	Freq dE> 5	Freq dE> 10
1	3.25	1.36	2.90	14.05					1.00	1.00
2	1.09	0.87	3.79	3.75					0.00	0.00
3	3.44	1.44	7.83	9.44		Data expected May 2007			2.00	0.00
4	3.39	15.31	2.59	8.89					2.00	1.00
5	2.42	3.98	12.92	6.53					2.00	1.00
6	5.16	1.07	8.98	11.37					3.00	1.00
									10.00	4.00

Delta E for Alexandria

Patch #	1312	B 1200 A	C 417-A	C 418-A	B 1202	K 151	C 419	B 1201 A	Freq dE> 5	Freq dE> 10
1	1.92	0.11	8.38	2.84					1.00	0.00
2	0.69	0.67	6.53	2.37					1.00	0.00
3	2.08	1.69	6.24	1.60		Data expected May 2007			1.00	0.00
4	1.92	3.74	6.21	0.26					1.00	0.00
5	3.22	3.93	6.39	1.58					1.00	0.00
6	4.58	2.11	5.40	3.20					1.00	0.00
									6.00	0.00

Delta E for Bath

Patch #	F 712	F 215	A 21B	D 713	F 513	F 812	B 112	A1 32	Freq dE> 5	Freq dE> 10
1										
2										
3		Data Missing				Data expected May 2007				
4										
5										
6										

Frequency of color change by test patch for each cleaner

	Delta E for San Francisco				Delta E for Santa Fe			
Patch #	**1075**	**NAWS 881 B**	**WS 1032 B**	**NAWS 886B**	**U 343**	**H 526 D**	**H 526 J**	**U 311-A**
D/2	2.16	2.29	6.61	4.24	5.25	1.56	7.09	3.87
Daybreak	6.29	17.66	7.68	9.56	4.86	5.23	1.45	25.77
Water	6.41	4.54	9.35	12.28	3.73	2.09	3.71	10.69
H2Orange Cleaner	4.29	0.66	10.32	6.32	3.00	5.32	4.15	2.65
WEG Marble Cleaner	5.23	10.17	5.55	7.56	7.05	4.99	0.36	2.43
Kodak Photo-flo	7.67	15.03	3.65	9.91	5.18	6.15	5.21	12.84

Delta E for Jefferson Barracks				Delta E for Alexandria					
32 2904-A	**32 2898-A**	**72 1273**	**72 1370**	**1312**	**B 1200 A**	**C 417-A**	**C 418-A**	Freq dE> 5	req dE> 10
3.25	1.36	2.90	14.05	1.92	0.11	8.38	2.84	5.00	1.00
1.09	0.87	3.79	3.75	0.69	0.67	6.53	2.37	7.00	2.00
3.44	1.44	7.83	9.44	2.08	1.69	6.24	1.60	7.00	2.00
3.39	15.31	2.59	8.89	1.92	3.74	6.21	0.26	5.00	2.00
2.42	3.98	12.92	6.53	3.22	3.93	6.39	1.58	8.00	1.00
5.16	1.07	8.98	11.37	4.58	2.11	5.40	3.20	11.00	3.00

Frequency of color change by test patch for sunny and shady locations

Shady

	Delta E for San Francisco		Delta E for Santa Fe		Delta E for Jefferson Barracks		Delta E for Alexandria			
Patch #	**NAWS 881 B**	**NAWS 886B**	**U 343**	**U 311-A**	**32 2904-A**	**32 2898-A**	**C 417-A**	**C 418-A**	Freq dE> 5	Freq dE> 10
D/2	2.29	4.24	5.25	3.87	3.25	1.36	8.38	2.84	2.00	0.00
Daybreak	17.66	9.56	4.86	25.77	1.09	0.87	6.53	2.37	4.00	2.00
Water	4.54	12.28	3.73	10.69	3.44	1.44	6.24	1.60	3.00	2.00
H2Orange Cleaner	0.66	6.32	3.00	2.65	3.39	15.31	6.21	0.26	3.00	1.00
WEG Marble Cleaner	10.17	7.56	7.05	2.43	2.42	3.98	6.39	1.58	4.00	1.00
Kodak Photo-flo	15.03	9.91	5.18	12.84	5.16	1.07	5.40	3.20	6.00	2.00
									22.00	8.00

Sunny

	Delta E for San Francisco		Delta E for Santa Fe		Delta E for Jefferson Barracks		Delta E for Alexandria			
Patch #	**1075**	**WS 1032 B**	**H 526 D**	**H 526 J**	**72 1273**	**72 1370**	**1312**	**B 1200 A**	Freq dE> 5	Freq dE> 10
D/2	2.16	6.61	1.56	7.09	2.90	14.05	1.92	0.11	3.00	1.00
Daybreak	6.29	7.68	5.23	1.45	3.79	3.75	0.69	0.67	3.00	0.00
Water	6.41	9.35	2.09	3.71	7.83	9.44	2.08	1.69	4.00	0.00
H2Orange Cleaner	4.29	10.32	5.32	4.15	2.59	8.89	1.92	3.74	3.00	1.00
WEG Marble Cleaner	5.23	5.55	4.99	0.36	12.92	6.53	3.22	3.93	4.00	0.00
Kodak Photo-flo	7.67	3.65	6.15	5.21	8.98	11.37	4.58	2.11	5.00	1.00
									22.00	3.00

Appendix D.
Analysis of Microorganisms on headstones in VA Cemeteries,
First Report: December 2005

Analysis of Microorganisms on Headstones in VA Cemeteries

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December 2005

OBJECTIVES

The objective of this project is to test cleaning agents for use in cleaning headstones within national cemeteries overseen by the National Cemetery Administration. The purpose of the current work was to analyze of numbers of microorganisms in samples collected from tombstones in five Veterans Administration cemeteries to provide baseline data for future testing of the effectiveness of cleaning strategies.

METHODS

Sample Collection and Study Sites

Samples were collected by Jason Church from the five cemeteries described below. Within each cemetery, samples were collected from 20 locations. A three cm^2 area of the tombstones were sampled for microorganisms using BBL Culture Swabs (Becton-Dickinson, Sparks, MD). Samples were shipped overnight to Harvard University.

Alexandria National Cemetery

Alexandria National Cemetery is located in the community of Pineville, Rapides Parish, La. In 1804, under the new U.S. Territorial government, Rapides became one of the 12 parishes into which the Territory of New Orleans (later the State of Louisiana) was divided and, by 1805, a crude settlement had developed at the site below the rapids named Alexandria. When Abraham Lincoln was elected president in fall 1860, the people of Alexandria and Pineville saw the handwriting on the wall. On Jan. 26, 1861, the citizens of Louisiana voted for secession and swiftly committed to joining the Confederacy.

Ships appeared at the mouth of the Mississippi River determined to go upriver and capture New Orleans in May 1862. Within a year, Rapides Parish citizens were shocked when they realized their homes, the roads leading through Alexandria parish and other crossroads villages of the parish might become part of the battlefield.

Between 1863 and early 1864, the area was invaded twice. Plantations were laid waste, houses burned, fences torn down, trees cut for firewood and sugarhouses and barns burned. Both armies lived off the land, taking away food, livestock and poultry. The final destruction of Alexandria occurred on May 13, 1864, when Alexandria was burned to the ground by Union troops.

After the war, federal troops moved into the region to begin the process of reconstruction. In 1867, an eight-acre plot was appropriated from local resident François Poussin for the establishment of a national cemetery for deceased Union soldiers who died in the region. Approximately a decade later, a suit was filed by Poussin's heirs and the United States was ordered to pay his descendents $1,200 for title to the property. Bodies were removed from the surrounding towns such as Mount Pleasant, Cheneyville and Yellow Bayou and reinterred in Alexandria. Later, remains from Fort Brown, Texas, were reinterred at the national cemetery when the fort was no longer deemed necessary. Alexandria (LA) National Cemetery was placed on the National Register of Historic Places in 1997.

The 1911 granite Memorial to Unknowns marks the burial of 1,537 unknown Federal soldiers who were removed from the Brownsville National Cemetery and re-interred at Alexandria National Cemetery. Another 1911 granite Memorial to Unknowns marks the burial of 16 unknown federal soldiers who were removed from the Fort Ringgold Post Cemetery (Texas) and re-interred at Alexandria National Cemetery. The remains of 25 unknown soldiers from post and private cemeteries near Fort Jessup, La., are also interred in one grave and it's marked with a white government marker.

There are 57 Buffalo Soldiers interred at the Alexandria National Cemetery. They represent the following units: 24th Infantry, 10th Calvary, and the 9th Calvary and are interred in Sections A, B, C, and R. [1]

[1] http://www.cem.va.gov/nchp/alexandriala.htm

Bath National Cemetery

Bath National Cemetery is located in Steuben County, N.Y., adjacent to the Department of Veterans Affairs Medical Center.

The cemetery was originally a part of the New York State Soldiers and Sailors Home, which was established in 1877; the cemetery was dedicated in Dec. 25, 1879. In 1930, the Soldiers and Sailors Home and cemetery became two integrated components of the Veterans Administration Medical Center (VAMC). When 82 national cemeteries were transferred from the Department of Army to the Veterans Administration in 1973, the Bath VAMC cemetery became part of the National Cemetery System and was designated appropriately.

Bath is the final resting place of the "first and oldest" U.S. MIAs (Missing in Action). On Oct. 26, 1987, an archeologist discovered a skeleton during the construction of a house in Fort Erie, Canada. Scientists and military historians were subsequently sent to investigate the site and ultimately, they discovered 28 remains. The bones were initially believed to be remains of the area's indigenous population. The discovery of buttons, however, led authorities to believe that the men buried at the site were British soldiers.

The 28 soldiers had been interred in a traditional manner, lying east-west with hand crossed; this indicates that they had been buried during a lull in the fighting by fellow soldiers rather than the enemy. Further investigation by the military indicated that the men had fought during the Niagara Campaign with clashes at Chippaw and Lundy's Lane before they died at Snake Hill, a battery overlooking Fort Erie. The Department of the Army, working with Canadian officials, held a repatriation ceremony at Fort Erie, Canada, on June 30, 1988 and the soldiers were reinterred with full military honors.[2]

Jefferson Barracks National Cemetery

Jefferson Barracks, one of the National Cemetery Administrations oldest interment sites, has served as a burial place soldiers from all wars. The original military post was built south of St. Louis, Mo., on the banks of the Mississippi River to replace Fort Bellefontaine. Selected for its strategic geographic location, the post was opened in 1826. Jefferson Barracks became the army's first permanent base west of the Mississippi River. By the 1840s, it was the largest military establishment in the United States. During the Civil War, Jefferson Barracks served as a training post for the Union Army. There was also a hospital at the post for the Union army's sick and wounded.

Although Jefferson Barracks was formally established as a national cemetery in 1866 by passage of a join resolution, the first burial, at what is now Jefferson Barracks National Cemetery is believed to have occurred the year after the post's founding, on Aug. 5, 1827. On that date, Elizabeth Ann Lash, the infant daughter of an officer stationed at Jefferson Barracks was interred at the post cemetery. The Civil War initiated the beginnings of a formal network of military cemeteries. The first general U.S. cemetery legislation was an omnibus bill enacted July 17, 1862, authorizing President Lincoln "to purchase cemetery grounds, and cause them to be securely enclosed, to be used as a national cemetery for the soldiers who shall have died in the service of the country." By the end of the year, the first 14 national cemeteries were created. Jefferson Barracks was formally established as a national cemetery in 1866 by passage of a joint resolution authorizing the Secretary of War to take action to preserve graves from desecration and "secure suitable burial-places in which they may be properly interred…."

The original portion of the cemetery is located in the northeastern section of the present acreage, appropriately delineated by four roads designated as Old Post Drive—East, West, North and South, respectively—containing Sections 1-4, and OPS-1, OPS-2, and OPS-3. It was set aside for the burial of military and civilian personnel who died at the garrison. In 1869 the cemetery experienced enormous growth when more than 10,200 recovered remains of soldiers originally buried at other Missouri locations including Cape Girardeau, Pilot Knob, Warsaw, and Rolla were removed here. About 470 victims of smallpox at Arsenal Island were also reinterred here.

The old cemetery contains approximately 20,000 gravesites, including more than 1,000 Confederate dead. During this era, Union dead were interred in sections by state, as far as that could

[2] http://www.cem.va.gov/nchp/bath.htm

be determined, including: 7,536 Whites, 1,067 African Americans, 1,010 Confederate POWs, and 556 "not of military service." Within the original cemetery tract, Sections 5 through 53 were laid out; the sections currently numbered 54-66, and 88, contain older burials but are irregularly numbered because the ponds, sink holes and administrative open space was converted to burial areas.

In 1870, the cemetery "quadrangle" at Jefferson Barracks measured approximately 750' x 1,230', and was surrounded by a standardized wooden picket fence "recently whitewashed." Within two years this fence was replaced by a stonewall 4,269 feet long and 1'-6" wide. A 16'-wide drive lined the interior of the wall, and crossed through the cemetery delineating large sections; narrower 10' wide paths further subdivided the grounds. "These drives and paths are covered with coarse broken stone, and, being but little used, are very uncomfortable to drive or walk over." The major interior paths had brick gutters and were lined with dense rows of the same types of trees. In addition, there were eight painted artillery guns, "planted vertically, as monuments" throughout the cemetery. In August 1871, it was reported that more than $142,287 had been spent developing and maintaining the cemetery to date. The next year Jefferson Barracks was categorized as a "First Class" cemetery, an Army designation based on "the extent and importance" of the facilities, which also determined the superintendent's salary of $75 per month. In 1875, the first enlargement of the cemetery took place.

During the early 1880s cast-metal tablets containing verse, "The Gettysburg Address" the War Department's General Orders No. 80, and text of the 1867 Act to establish and protect national cemeteries.

As space within the enclosure walls became limited, an expansion that would more than double the size of the cemetery was underway by the early 1890s. The original entrance with its "double iron gates hung on handsome piers of rough dressed limestone" and the old administration building/lodge were located on the north side of the existing cemetery. The landscape in some areas of Jefferson Barracks National Cemetery was one of the most contentious. Behind this building there were:

> …two deep depressions in the ground, similar to the "sink-holes" in limestone formations, each having in its bottom a small pond; one has been enlarged and surrounded by a stone wall, making a miniature lake; the other is in its natural state. The ponds have subterranean communications with each other and with the Mississippi, and are affected by the rise and fall of water in that river, but are never dry.

The superintendent's personal domain included a grape arbor, privy and cistern, as well as evergreen trees and shaped planting beds of flowers and vegetables. By 1893 the approach to the entrance was established via a gravel road flanked by deciduous trees and "plank fences." Already there were a fountain, two sheds, two stables, a two-room cottage for seasonal laborers, and a rectangular rostrum (1872) located on the expanded property.

In 1922 an Executive Order assigned 170 acres of military reservation to the Veterans Bureau (now Department of Veterans Affairs). In July 1936, the War Department formally named Jefferson Barracks National Cemetery as a component of Jefferson Barracks, along with similar designations of military reservations at instillations including those named in honor of persons, target ranges and national cemeteries.

From April 1936 through the early 1940s, Depression-era government make-work programs brought improvements to the cemetery. Works Progress Administration (WPA) laborers were responsible for building 23,000' of hard-surfaced roads and walks, 46,000' concrete curbs, nearly 16,000' of "asphalt macadam" roads, and resurfacing of the same. They also removed some of the original stone wall and constructed nearly 4,600' of "common ashler (sic) stone wall, as well as miscellaneous grading. In 1946 a new stone boundary wall and entrance gate were erected. The WPA renovated the 1872 brick rostrum that measured 23'x 38' in 1941.

Gradually the importance of the post lessened and Jefferson Barracks was deactivated in 1946. Expansion of the cemetery, however, was granted by 1947 legislation authorizing the Secretary of War to "utilize and expand existing facilities" at Jefferson Barracks "when practicable, through the use of federally owned lands under the jurisdiction of the War Department" that were no longer needed for military purposes.

World War II casualties introduced a new focus to the cemetery as the central repository for group interments resulting from national disasters, when individual remains cannot be identified.

Among the more than 560 group burials—meaning two or more veterans in a common grave—are 123 victims of a 1944 Japanese massacre of POWs in the Philippines, and the remains of 41 unidentified marines who perished in a South Vietnam helicopter crash in 1968.

Jefferson Barracks National Cemetery was listed on the National Register of Historic Places in 1998.[3]

San Francisco National Cemetery

When Spain colonized what would become California, this area was selected as the site for a fort, or presidio, to defend San Francisco Bay. About 40 families traveled here from northern Mexico in 1776 and built the first settlement, a small quadrangle, only a few hundred feet west of what is now Funston Avenue. Mexico controlled the Presidio following 1821, but the fort became increasingly less important to the Mexican government. In 1835, most soldiers and their families moved north to Sonoma, leaving it nearly abandoned. During the Mexican War, U.S. troops occupied and repaired the damage to the fort.

The mid-century discovery of gold in California led to the sudden growth and importance of San Francisco, and prompted the U.S. government to establish a military reservation here. By executive order, President Millard Fillmore established the Presidio for military use in November 1850. During the 1850s and 1960s, Presidio-based soldiers fought Native Americans in California, Oregon, Washington and Nevada. The outbreak of the Civil War in 1861 re-emphasized the importance of California's riches and the military significance of San Francisco's harbor to the Union. This led, in 1862, to the first major construction and expansion program at the Presidio since the United States acquired it.

The Indian Wars of the 1870s and 1880s resulted in additional expansion of the Presidio, including large-scale tree planting and a post beautification program. By the following decade the Presidio had shed its frontier outpost appearance and was elevated to a major military installation and base for American expansion into the Pacific.

In 1890, with the creation of Sequoia, General Grant and Yosemite national parks in the Sierra Nevada mountains of California, the protection of these scenic and natural resources was assigned to the U.S. cavalry stationed at the Presidio. Soldiers patrolled these parks during summer months until the start of World War I in 1914. The Spanish American War in 1898 and subsequent Philippine-American War, from 1899 to 1902, increased the role of the Presidio. Thousands of troops camped in tent cities while awaiting shipment to the Philippines. Returning sick and wounded soldiers were treated in the Army's first permanent hospital, later renamed Letterman Army General Hospital. In 1914, troops under the command of Gen. John Pershing departed the Presidio for the Mexican border in pursuit of Pancho Villa and his men. When World War I began, Pershing became commander of the American Expeditionary Forces in Europe.

When the United States entered World War II after the Japanese attack on Pearl Harbor, Presidio soldiers dug foxholes along the nearby beaches. Fourth Army Commander Gen. John L. DeWitt conducted the interment of thousands of Japanese and Japanese-Americans on the West Coast while U.S. soldiers of Japanese descent were trained to read and speak Japanese at the first Military Intelligence Service language school organized at Crissy Field. During the 1950s, the Presidio served as the headquarters for the Nike missile defense program and headquarters for the famed Sixth U.S. Army. The Presidio of San Francisco, encompassing more than 350 buildings with historic value, was designated a National Historic Landmark in 1962. In 1989, the Presidio closed as a military entity and was transferred to the National Park Service in October 1994.

On Dec. 12, 1884, the War Department designated nine acres, including the site of the old post cemetery, as San Francisco National Cemetery. It was the first national cemetery established on the West Coast and, as such, marks the growth and development of a system of national cemeteries extending beyond the battlefields of the Civil War. Initial interments included the remains of the dead from the former post cemetery as well as individuals removed from cemeteries at abandoned forts and camps elsewhere along the Pacific coast and western frontier. In 1934, all unknown remains in the cemetery were disinterred and reinterred in one plot. Many soldiers and sailors who

[3] http://www.cem.va.gov/nchp/jeffersonbarracks.htm

died overseas serving in the Philippines, China and other areas of the Pacific Theater are interred in San Francisco National Cemetery.

The cemetery is enclosed with a stone wall and slopes down a hill that today frames a view of the Golden Gate Bridge. Its original ornamental cast-iron entrance gates are present but have been unused since the entrance was relocated. Tall eucalyptus trees further enclose the cemetery. The lodge and rostrum date to the 1920s and reflect the Spanish Revival styling introduced to several western cemeteries.

Two unusual interments at San Francisco National Cemetery are "Major" Pauline Cushman and Miss Sarah A. Bowman. Cushman's headstone bears the inscription "Pauline C. Fryer, Union Spy," but her real name was Harriet Wood. Born in the 1830s, she became a performer in Thomas Placide's show Varieties and took the name Pauline Cushman. She married theater musician Charles Dickinson in 1853, but after her husband died of illness related to his service for Union forces, she returned to the stage. During spring 1863, while performing in Louisville, Ky., she was asked by the provost marshal to gather information regarding local Confederate activity. From there she was sent to Nashville, where she had some success conveying information about troop strength and movements. In Nashville, she was also captured and nearly hanged as a spy. She returned to the stage in 1864, to lecture and sell her autobiography. Entertainer P.T. Barnum promoted her as the "Spy of the Cumberland" and through Barnum's practiced boostership she quickly gained fleeting fame. After spending the 1870s working the redwood logging camps, she remarried and moved to the Arizona Territory. By 1893 she was divorced, destitute and desperate; she applied for her first husband's military pension and returned to San Francisco, where she died from an overdose of narcotics allegedly taken to soothe her rheumatism. Members of the Grand Army of the Republic and Women's Relief Corps conducted a magnificent funeral for the former spy. "Major" Cushman's remains reside in Officer's Circle.

Also buried at San Francisco National Cemetery is Sarah Bowman, also known as "Great Western," a formidable woman over 6 feet tall with red hair and a fondness for wearing pistols. Married to a soldier, she traveled with Zachary Taylor's troops in the Mexican War helping to care for the wounded, for which she earned a government pension. After her husband's death she had a variety of male companions and ran an infamous tavern and brothel in El Paso, Texas. Bowman left El Paso when she married her last husband. The two ended up at Fort Yuma, where she operated a boarding house until her death from a spider bite in 1866. She was given a full military funeral and was buried in the Fort Yuma Cemetery. Several years later her body was exhumed and reburied at San Francisco National Cemetery.

San Francisco National Cemetery was listed as a National Historic Landmark as part of the Presidio in 1962.[4]

Santa Fe National Cemetery

Santa Fe National Cemetery is located within the city limits of Santa Fe, N.M., approximately one mile northwest of the main plaza.

Thirteen years before the Pilgrims settled in Plymouth Colony, the Spanish had established a small settlement in Santa Fe, N.M. Santa Fe would soon become the seat of power for the Spanish Empire north of the Rio Grande and the oldest capital city in North America. Santa Fe is the site of both the oldest public building in America, the Palace of the Governors, and the nation's oldest community celebration, the Santa Fe Fiesta, established in 1712 to commemorate the Spanish reconquest of New Mexico in summer 1692. Conquistador Don Pedro de Peralta and his men laid out the plan for Santa Fe at the base of the Sangre de Cristo Mountains on the site of the ancient Pueblo ruin of Kaupoge, or "place of shell beads near the water."

When Mexico gained its independence from Spain, Santa Fe became the capital of the province of New Mexico. With the Spanish defeat came an end to the policy of a closed empire; American trappers and traders journeyed into the region along the 1,000 mile Santa Fe trail beginning in Arrow Rock, Mo. For a brief period in 1837, northern New Mexico farmers rebelled against Mexican rule, killing the provincial governor in what has been called the Chimayó

[4] http://www.cem.va.gov/nchp/sanfrancisco.htm

Rebellion, and occupying the capital. The insurrectionists were soon defeated and peace returned to Santa Fe for almost a decade.

In 1846, at the outset of the Mexican-American War, President James K. Polk asked General Stephen Watts Kearny to muster an army and march 1,000 miles into the Southwest to claim that region for the United States and organize territorial governments along the way. Kearny, faced with a Mexican administration weakened by years of occupation and political turmoil, was able to take Santa Fe without firing a shot. In quick succession, he won over the local leadership, assured a peaceful transition to a new civilian government and implemented a new legal code for the territory before continuing on to Arizona and California.

While there was little armed conflict in the territory of New Mexico during the Civil War, there were some engagements in the area of Santa Fe. Confederate General Henry H. Sibley raised and equipped a column to secure the secessionist claims in the New Mexico and Arizona region. Undermanned, often commanded by secessionist sympathizers and largely abandoned, the U.S. installations in the region were initially unable to defend themselves. News of the Confederate advance into New Mexico quickly raised volunteers from the Colorado Territory who took up the march. In addition, a large "California column" was raised to help defend the city of Santa Fe.

Toward the end of March 1862, Union Major John M. Chivington encountered a Confederate force southeast of the city, where the Santa Fe Trail crossed the mountains. Several days of skirmishes culminated in a battle at Glorieta Pass. Although the Confederates held their own, several hundred Union soldiers moved to the far end of the canyon and attacked the unprotected supply train. After bayoneting the pack animals and burning the wagons, the Union forces left Sibley's men little choice but to make the long trek back to Texas. The campaign not only ended Southern ambitions in the Southwest but it also forced the Confederate abandonment of Fort Bliss outside El Paso, Texas.

At the close of the Civil War, the federal government established a cemetery for the reinterment of Union soldiers who died during the brief military activity in the area. The ground initially chosen was located just west of Santa Fe and is currently part of Santa Fe National Cemetery. The Roman Catholic Diocese of Santa Fe, who owned the property, donated the land to the United States in 1870. Santa Fe's initial designation as a national cemetery was short lived. In July 1876, the War Department decided that, to save expenses, its status should be downgraded to that of a post cemetery. The superintendent was transferred to Mound City National Cemetery, Ill., and the quartermaster was transferred to Fort Macy, a local post in Santa Fe. Nine years later, however, it was re-established as a national cemetery.[5]

Enumeration of Microorganisms

Bacteria and fungi were enumerated by plating samples on solid media. Plates were incubated at room temperature for two days and colonies were counted. Bacteria were plated on Difco Nutrient Agar (Becton-Dickinson, Sparks, MD) and fungi were plated on malt extract agar (6.4 g/L maltose, 1.4 g/L dextrose, 1.2 g/L glycerol, 0.4 g/L peptone, 7.5 g/L agar, 4875 U penicillin G, 3250 U bacitracin). Photosynthetic microorganisms (algae) were analyzed using a hemocytometer. The numbers of algae in at least 10 fields of view were counted at 40X magnification.

RESULTS

Fungi and bacteria were enumerated by plating on solid media and counting colonies after incubation. Numbers of bacteria and fungi in samples were variable (Fig. 1-5). Numbers of fungi were generally lower than bacteria. No consistent differences were found between marble types (i.e., Georgia and Colorado) or sunny and shaded areas of tombstones. Algae were not found in any samples.

[5] http://www.cem.va.gov/nchp/santafe.htm

The number of bacteria in samples from Alexandria National Cemetery were highest in sample K162-A (Fig. 1A) while numbers of fungi were greatest in sample C416-A (Fig. 1B). Bacteria and/or fungi were present in most samples. In contrast, bacteria and fungi were detected in few samples from Bath National Cemetery (Fig. 2A and 2B). Three samples contained relatively large numbers of bacteria (A21D, D175) and fungi (A21C). Bacterial numbers in samples from Jefferson Barracks National Cemetery were highly variable (Fig. 3A). Bacteria were not detected in many samples while the highest numbers were (>17,000/cm^2) were found in sample 32-2934A. Numbers of fungi were lower than bacteria, but fungi were detected in all samples except 72-1269 from Jefferson Barracks National Cemetery (Fig. 3B). Like Alexandria National Cemetery, bacteria and/or fungi were found in almost all samples. Bacteria were not detected in many samples from San Francisco National Cemetery, but the greatest number was found in sample WS1033B (Fig. 4B). Fungi were found more frequently in samples than were bacteria, and the greatest number of fungi were in samples WS1033B and WS1035A. Samples WS1033B is interesting in that this is one case in which high numbers of bacteria and fungi were found in the same sample. Numbers of bacteria and fungi were much greater in samples from Santa Fe National Cemetery than any of the other sites (note the difference in scales). Bacteria were found in most samples and numbers were highest in sample U313 (Fig. 5A). Fungi were also detected in most samples and numbers were highest in sample H526-H (Fig. 5B).

CONCLUSIONS

- Bacteria and/or fungi were found in most samples.
- Numbers of bacteria were generally greater than numbers of fungi.
- Algae were not detected in the samples.
- Our analysis of microbial growth showed wide variability in the size of the microbial community.
- Numbers of bacteria and fungi were low in most samples.
- These data will provide a useful baseline for further tests of biocide effectiveness and cleaning strategies.

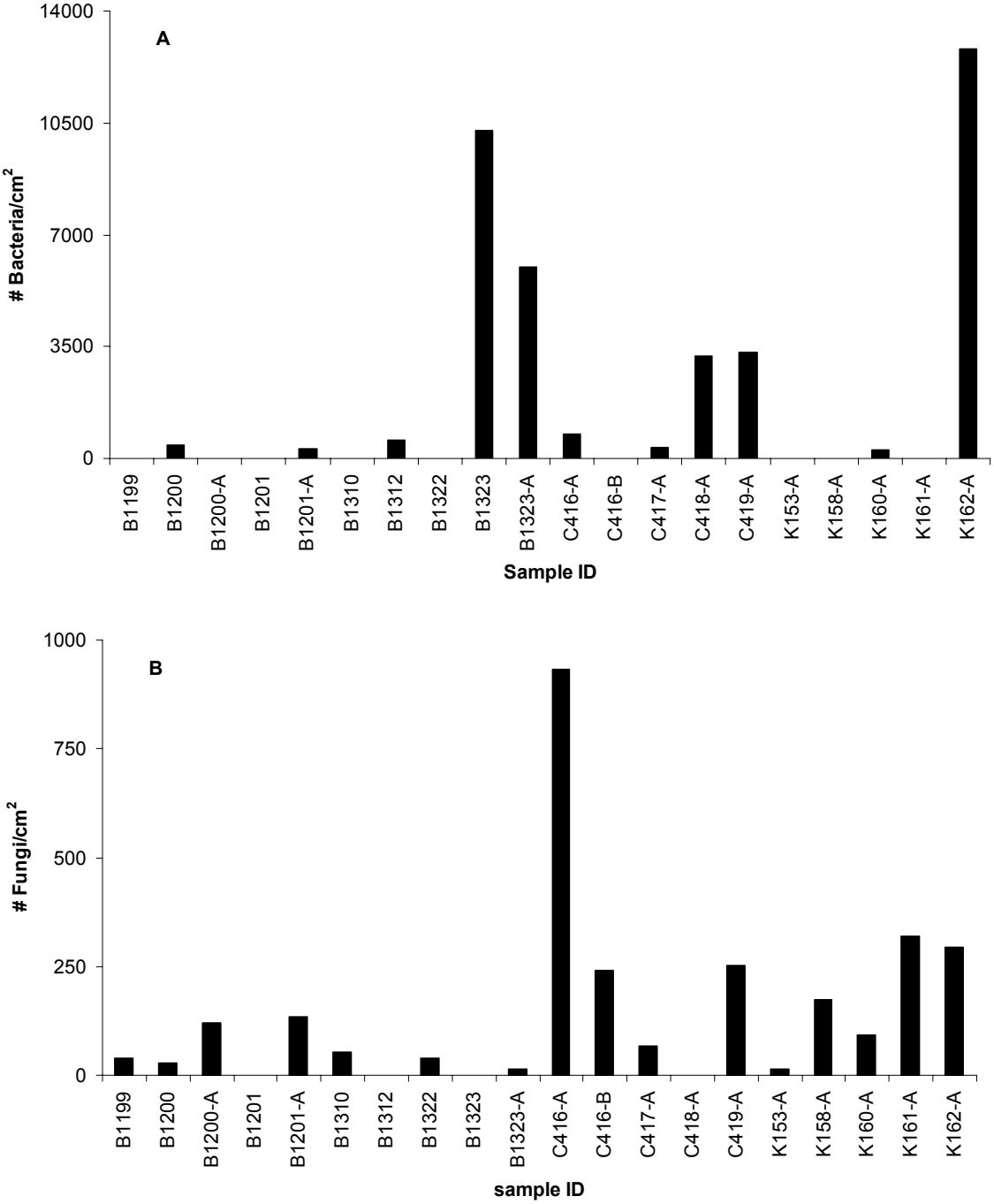


Figure 1. Numbers of bacteria (A) and fungi (B) in samples collected from Alexandria National Cemetery.

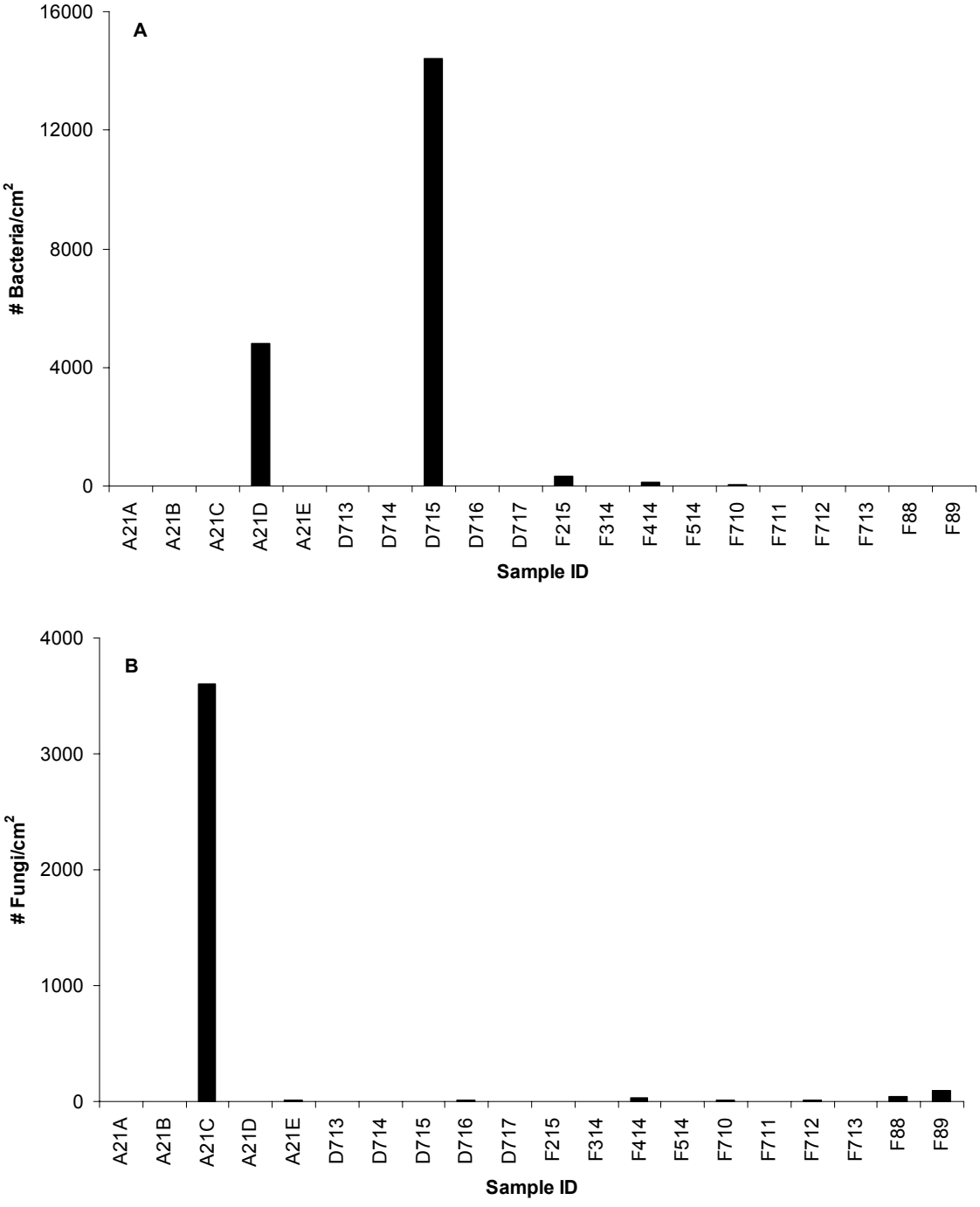


Figure 2. Numbers of bacteria (A) and fungi (B) in samples collected from Bath National Cemetery.

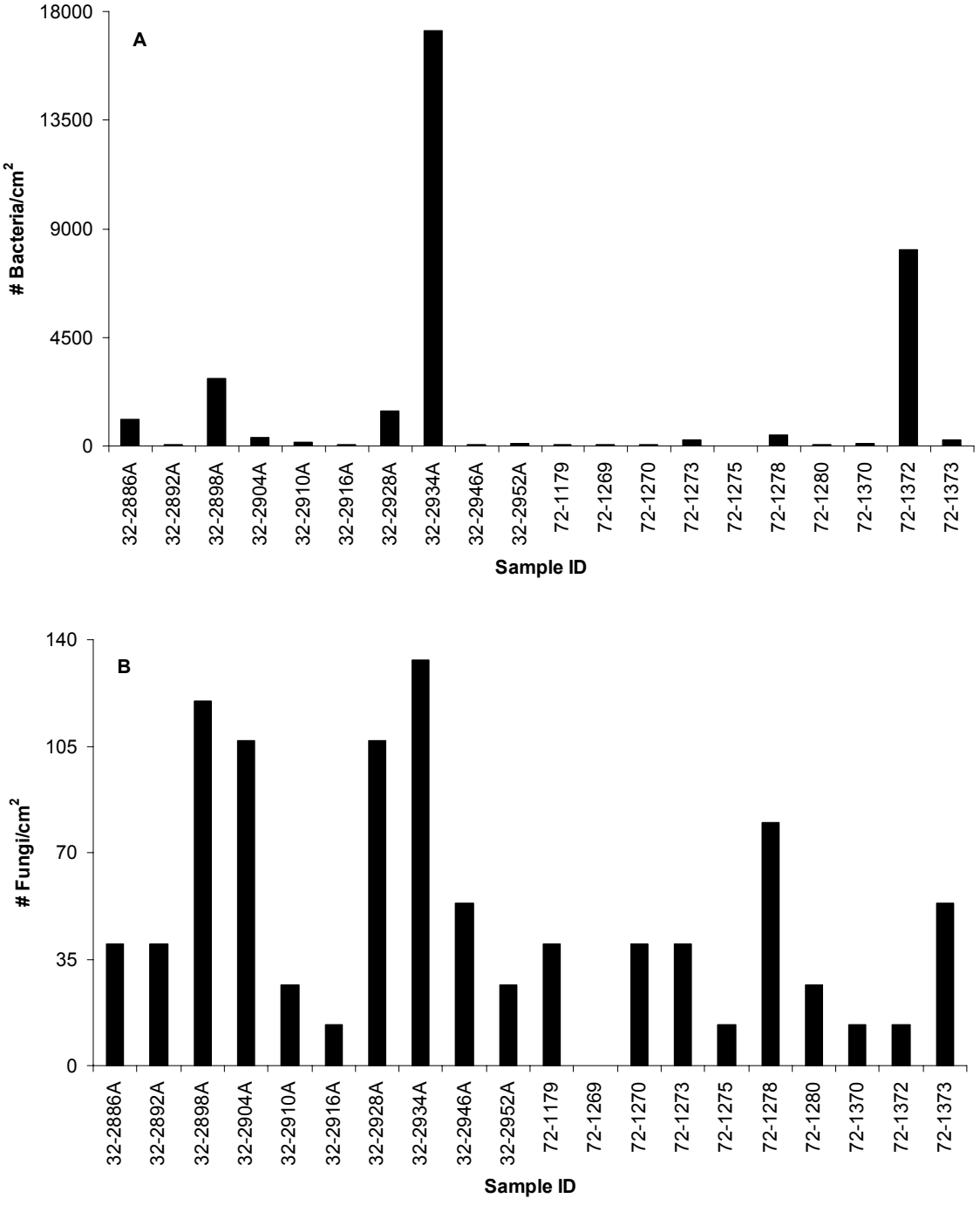


Figure 3. Numbers of bacteria (A) and fungi (B) in samples collected from Jefferson Barracks National Cemetery.

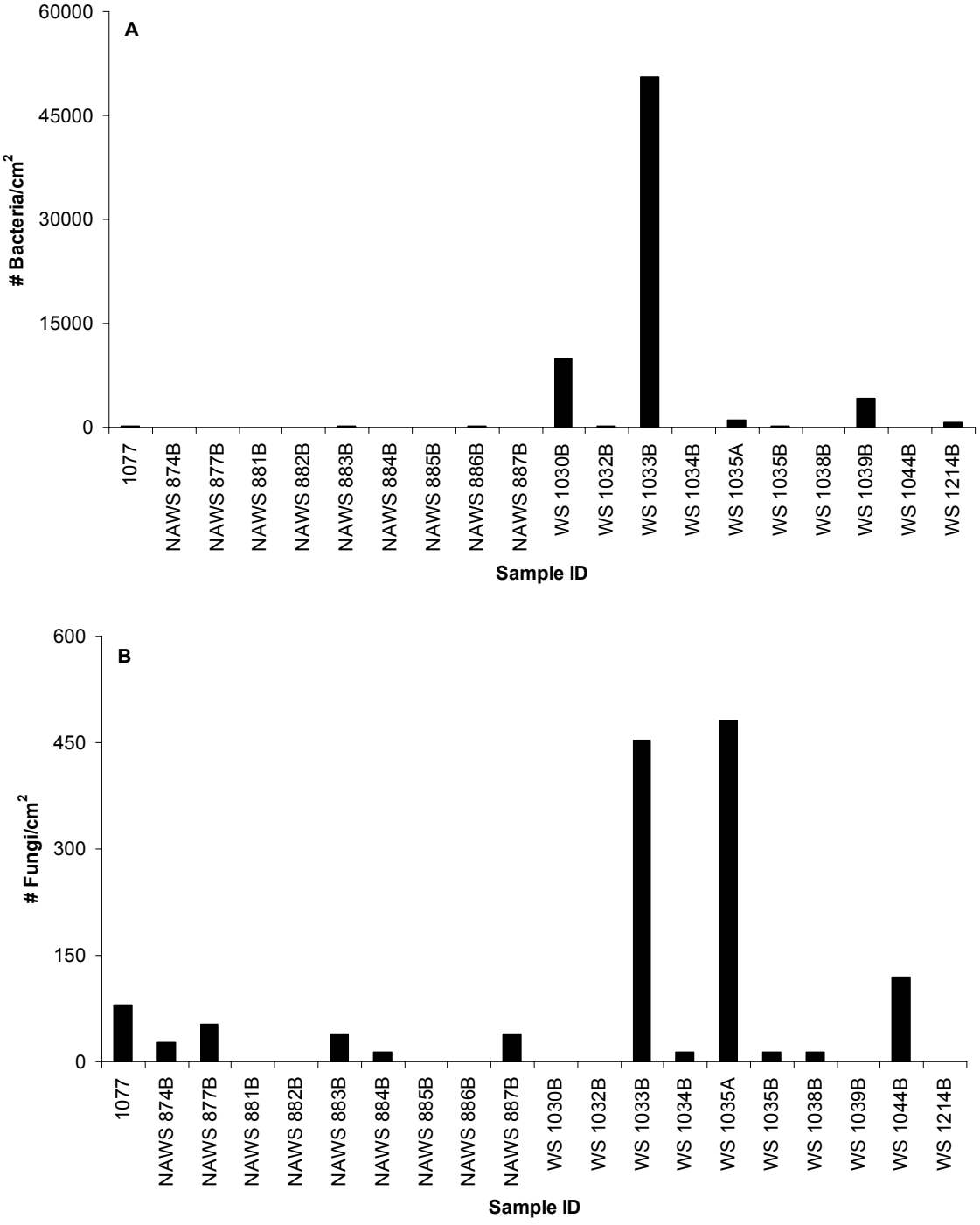


Figure 4. Numbers of bacteria (A) and fungi (B) in samples collected from San Francisco National Cemetery.

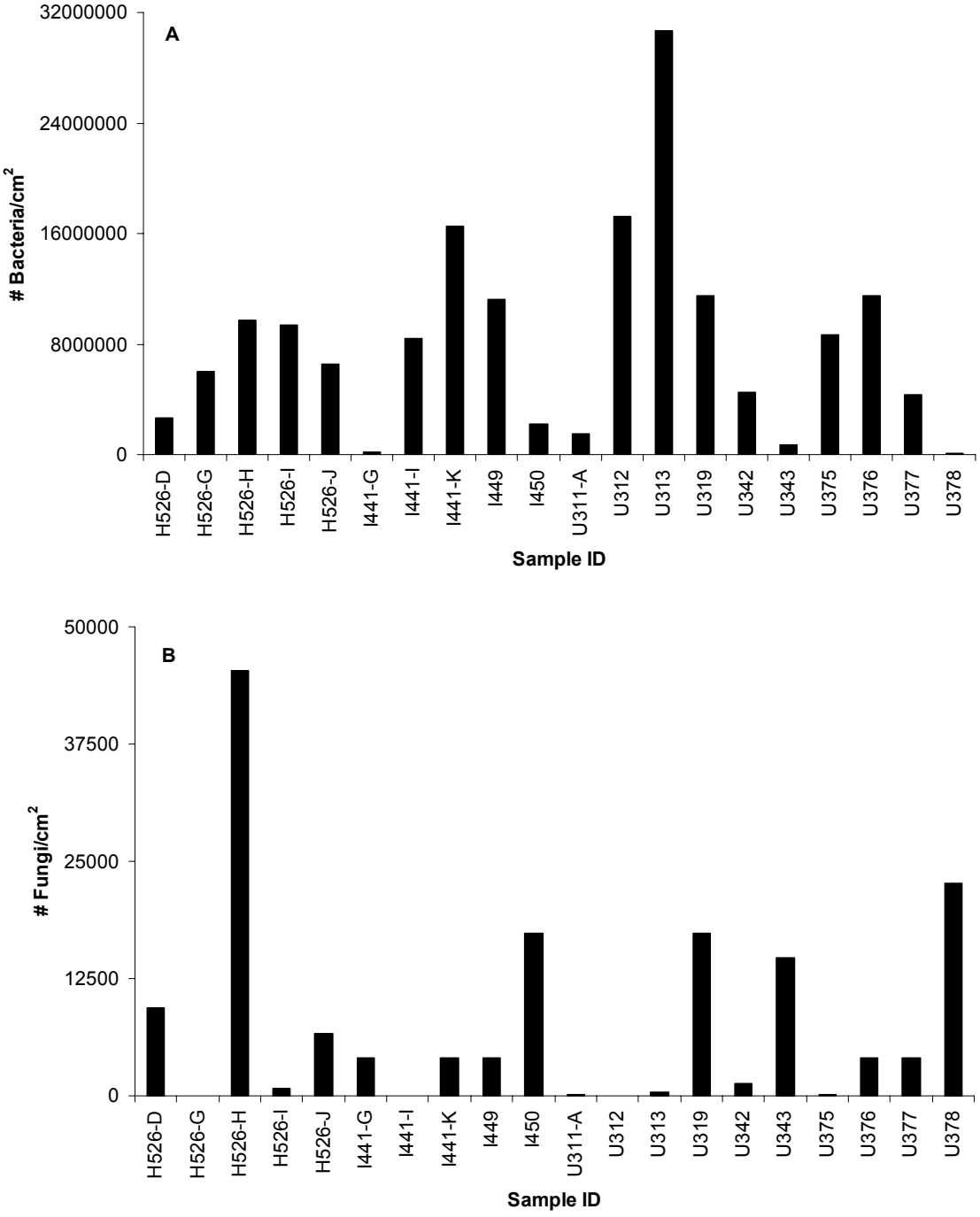


Figure 5. Numbers of bacteria (A) and fungi (B) in samples from Santa Fe National Cemetery.

APPENDIX I

Raw data and calculated numbers for bacteria and fungi.

Date Collected	Cemetery location	Stone ID	Bacteria #	Bacteria/cm^2	Fungi #	Fungi/cm^2
10/4/2005	Alexandria National	B1199	0	0	3	40
10/4/2005	Alexandria National	B1200	31	413	2	27
10/4/2005	Alexandria National	B1200-A	0	0	9	120
10/4/2005	Alexandria National	B1201	0	0	0	0
10/4/2005	Alexandria National	B1201-A	22	293	1	133
10/4/2005	Alexandria National	B1310	0	0	4	53
10/4/2005	Alexandria National	B1312	44	587	0	0
10/4/2005	Alexandria National	B1322	0	0	3	40
10/4/2005	Alexandria National	B1323	77	10267	0	0
10/4/2005	Alexandria National	B1323-A	45	6000	1	13
10/4/2005	Alexandria National	C416-A	58	773	7	933
10/4/2005	Alexandria National	C416-B	0	0	18	240
10/4/2005	Alexandria National	C417-A	25	333	5	67
10/4/2005	Alexandria National	C418-A	24	3200	0	0
10/4/2005	Alexandria National	C419-A	25	3333	19	253
10/4/2005	Alexandria National	K153-A	0	0	1	13
10/4/2005	Alexandria National	K158-A	0	0	13	173
10/4/2005	Alexandria National	K160-A	19	253	7	93
10/4/2005	Alexandria National	K161-A	0	0	24	320
10/4/2005	Alexandria National	K162-A	96	12800	22	293
10/6/2005	Bath National	A21A	0	0	0	0
10/6/2005	Bath National	A21B	0	0	0	0
10/6/2005	Bath National	A21C	0	0	27	3600
10/6/2005	Bath National	A21D	36	4800	0	0
10/6/2005	Bath National	A21E	0	0	1	13
10/6/2005	Bath National	D713	0	0	0	0
10/6/2005	Bath National	D714	0	0	0	0
10/6/2005	Bath National	D715	108	14400	0	0
10/6/2005	Bath National	D716	0	0	1	13
10/6/2005	Bath National	D717	0	0	0	0
10/6/2005	Bath National	F215	24	320	0	0
10/6/2005	Bath National	F314	0	0	0	0
10/6/2005	Bath National	F414	9	120	2	27
10/6/2005	Bath National	F514	0	0	0	0
10/6/2005	Bath National	F710	2	27	1	13
10/6/2005	Bath National	F711	0	0	0	0
10/6/2005	Bath National	F712	0	0	1	13
10/6/2005	Bath National	F713	0	0	0	0
10/6/2005	Bath National	F88	0	0	3	40
10/6/2005	Bath National	F89	0	0	7	93
10/19/2005	Jefferson Barricks National	32-2886A	84	1120	3	40
10/19/2005	Jefferson Barricks National	32-2892A	4	53	3	40
10/19/2005	Jefferson Barricks National	32-2898A	21	2800	9	120
10/19/2005	Jefferson Barricks National	32-2904A	25	333	8	107
10/19/2005	Jefferson Barricks National	32-2910A	10	133	2	27
10/19/2005	Jefferson Barricks National	32-2916A	4	53	1	13

Date Collected	Cemetery location	Stone ID	Bacteria #	Bacteria/cm^2	Fungi #	Fungi/cm^2
10/19/2005	Jefferson Barricks National	32-2928A	11	1467	8	107
10/19/2005	Jefferson Barricks National	32-2934A	129	17200	1	133
10/19/2005	Jefferson Barricks National	32-2946A	4	53	4	53
10/19/2005	Jefferson Barricks National	32-2952A	8	107	2	27
10/19/2005	Jefferson Barricks National	72-1179	5	67	3	40
10/19/2005	Jefferson Barricks National	72-1269	3	40	0	
10/19/2005	Jefferson Barricks National	72-1270	4	53	3	40
10/19/2005	Jefferson Barricks National	72-1273	20	267	3	40
10/19/2005	Jefferson Barricks National	72-1275	0	0	1	13
10/19/2005	Jefferson Barricks National	72-1278	34	453	6	80
10/19/2005	Jefferson Barricks National	72-1280	3	40	2	27
10/19/2005	Jefferson Barricks National	72-1370	8	107	1	13
10/19/2005	Jefferson Barricks National	72-1372	61	8133	1	13
10/19/2005	Jefferson Barricks National	72-1373	18	240	4	53
10/19/2005	San Francisco National	1077	19	253	6	80
10/19/2005	San Francisco National	NAWS 874B	5	67	2	27
10/19/2005	San Francisco National	NAWS 877B	4	53	4	53
10/19/2005	San Francisco National	NAWS 881B	5	67	0	0
10/19/2005	San Francisco National	NAWS 882B	0	0	0	0
10/19/2005	San Francisco National	NAWS 883B	10	133	3	40
10/19/2005	San Francisco National	NAWS 884B	1	13	1	13
10/19/2005	San Francisco National	NAWS 885B	5	67	0	0
10/19/2005	San Francisco National	NAWS 886B	14	187	0	0
10/19/2005	San Francisco National	NAWS 887B	5	67	3	40
10/19/2005	San Francisco National	WS 1030B	75	10000	0	0
10/19/2005	San Francisco National	WS 1032B	14	187	0	0
10/19/2005	San Francisco National	WS 1033B	38	50667	34	453
10/19/2005	San Francisco National	WS 1034B	0	0	1	13
10/19/2005	San Francisco National	WS 1035A	8	1067	36	480
10/19/2005	San Francisco National	WS 1035B	16	213	1	13
10/19/2005	San Francisco National	WS 1038B	2	27	1	13
10/19/2005	San Francisco National	WS 1039B	31	4133	0	0
10/19/2005	San Francisco National	WS 1044B	1	13	9	120
10/19/2005	San Francisco National	WS 1214B	49	653	0	0
11/14/2005	Santa Fe National	H526-D	197	2626667	7	9333
11/14/2005	Santa Fe National	H526-G	45	6000000	4	53
11/14/2005	Santa Fe National	H526-H	73	9733333	34	45333
11/14/2005	Santa Fe National	H526-I	70	9333333	6	800
11/14/2005	Santa Fe National	H526-J	49	6533333	5	6667
11/14/2005	Santa Fe National	I441-G	11	146667	3	4000
11/14/2005	Santa Fe National	I441-I	63	8400000	0	0
11/14/2005	Santa Fe National	I441-K	124	16533333	3	4000
11/14/2005	Santa Fe National	I449	84	11200000	3	4000
11/14/2005	Santa Fe National	I450	168	2240000	13	17333
11/14/2005	Santa Fe National	U311-A	11	1466667	1	133
11/14/2005	Santa Fe National	U312	129	17200000	3	40
11/14/2005	Santa Fe National	U313	23	30666667	27	360
11/14/2005	Santa Fe National	U319	86	11466667	13	17333
11/14/2005	Santa Fe National	U342	34	4533333	1	1333

Date Collected	Cemetery location	Stone ID	Bacteria #	Bacteria/cm^2	Fungi #	Fungi/cm^2
11/14/2005	Santa Fe National	U343	54	720000	11	14667
11/14/2005	Santa Fe National	U375	65	8666667	1	133
11/14/2005	Santa Fe National	U376	86	11466667	3	4000
11/14/2005	Santa Fe National	U377	328	4373333	3	4000
11/14/2005	Santa Fe National	U378	7	93333	17	22667

Appendix E.
Analysis of Microorganisms on Headstones in VA Cemeteries, Second Report: June 2006

Analysis of Microorganisms on Headstones in VA Cemeteries
Second Report: June 2006

Ralph Mitchell, Kristen Bearce and Christopher McNamara

Laboratory of Applied Microbiology
Division of Engineering and Applied Sciences
Harvard University

OBJECTIVES

The objective of this project is to test cleaning agents for use in cleaning headstones within national cemeteries overseen by the National Cemetery Administration. The purpose of the current work was to analyze of numbers of microorganisms in samples collected from tombstones in five Veterans Administration cemeteries six months after cleaning.

RESULTS FROM PREVIOUS SAMPLE COLLECTION

Bacteria and/or fungi were found in most samples collected in October and November 2005 (see Appendix 1). Numbers of bacteria were generally greater than numbers of fungi and algae were not detected in the samples. Our analysis of microbial growth showed wide variability in the size of the microbial community. However, numbers of bacteria and fungi were low in most samples.

METHODS

Sample Collection and Study Sites

Samples were collected during April and May 2006 by Jason Church from five cemeteries: 1) Alexandria National Cemetery, Alexandria, VA, 2) Bath National Cemetery, Steuben County, NY, 3) Jefferson Barracks National Cemetery, St. Luois, MO, 4) San Francisco National Cemetery, San Francisco, CA, and 5) Santa Fe National Cemetery, Santa Fe, NM. Within each cemetery, samples were collected from 20 locations. A three cm^2 area of the tombstones were sampled for microorganisms using BBL Culture Swabs (Becton-Dickinson, Sparks, MD). Sample locations were cleaned in October and November 2005 using five different agents: Daybreak, 5914 (NCH Corporation, Irving, TX), Marble and Granite Cleaner Concentrate (World Environmental Group, Inc., Ocala, FL), Photo-Flo 200 (Eastman Kodak Company, Rochester, NY), $H_2Orange_2$ Grout Safe (EnvirOx LLC, Danville, IL), and D/2 Architectural Antimicrobial (Sunshine Makers, Inc., Huntington Harbour, CA). Samples were stored un-refrigerated for a number of days prior to shipment to Harvard University. Samples were shipped overnight to Harvard University.

Enumeration of Microorganisms

Samples collected from the headstones were enumerated (Table 1). Bacteria and fungi were enumerated by plating samples on solid media. Plates were incubated at room temperature for two days and colonies were counted. Bacteria were plated on Difco Nutrient Agar (Becton-Dickinson, Sparks, MD) and fungi were plated on malt extract agar (6.4 g/L maltose, 1.4 g/L dextrose, 1.2 g/L glycerol, 0.4 g/L peptone, 7.5 g/L agar, 4875 U penicillin G, 3250 U bacitracin). When present, photosynthetic microorganisms (algae) were analyzed using a hemocytometer. The numbers of algae in at least 10 fields of view were counted at 40X magnification.

RESULTS

No algae were detected in samples from any of the five cemeteries sampled. Green coloration in some samples was due to the presence of fungi. Fungi and bacteria were enumerated by plating on solid media and counting colonies after incubation. Numbers of bacteria and fungi in samples were variable.

Large numbers of bacteria were found in samples from Alexandria National Cemetery (Fig. 1A). The largest number of bacteria were found in the sample from the sun-exposed location cleaned with Photoflow. The smallest number of bacteria was found in the shaded location sample location cleaned with Marble/Granite cleaner. Numbers of fungi from Alexandria National Cemetery were much lower than numbers of bacteria (Fig. 1B). The smallest numbers of fungi were found in samples cleaned with Daybreak while the largest number of fungi was found in the shaded location cleaned with D2.

Numbers of bacteria from Bath National Cemetery were very high in all samples, and were greatest in the shaded sample cleaned with Photoflow (Fig. 2A). The lowest number of bacteria was found in the sun-exposed sample cleaned with Photoflow. Numbers of fungi in samples from Bath National cemetery were much lower than number of bacteria, but were greater than the numbers of fungi found in Alexandria (Fig. 2B). The greatest numbers of fungi were found in the locations cleaned with D2. Fungi were below the detection limit in the shaded sample cleaned with Daybreak.

Numbers of bacteria in samples from Jefferson National Cemetery were greatest in sun-exposed samples cleaned with H_2 Orange and Photoflow (Fig. 3A). The lowest numbers of bacteria were found in samples cleaned with D2, Daybreak, and Marble/Granite Cleaner. Numbers of fungi were generally higher in shaded locations (fig. 3B). The lowest numbers of fungi were found in samples cleaned with Marble/Granite cleaner.

The lowest number of bacteria at San Francisco National Cemetery were found in sun-exposed locations cleaned with Daybreak (Fig. 4A). Large numbers of bacteria were found in all other samples from this cemetery. Numbers of fungi were extremely variable. The lowest numbers of fungi were found in locations treated with H_2 Orange (Fig. 4B). The highest numbers of fungi were observed in samples cleaned with D2 and Photoflow.

Numbers of bacteria in samples from Santa Fe National Cemetery were generally higher in shaded locations than in sun-exposed areas (Fig. 5A). The lowest numbers of bacteria were found in samples from sun-exposed locations cleaned with Daybreak and Marble/Granite cleaner. Numbers of fungi were quite low, with the exception of the shaded location cleaned with Marble/Granite cleaner (Fig. 5B). Fungi were below detection limits in the sun-exposed location cleaned with D2 and the shaded location cleaned with Photoflow.

CONCLUSIONS

- Large numbers of bacteria and fungi were found in all samples.
- The large numbers of microorganisms enumerated is inconsistent with visual observations made by Jason Church, in which locations cleaned with D2 and Daybreak appeared to be free from microbial growth.
- Inconsistencies between visual observations and microbial counts may be due to growth of microorganisms in the swabs after sampling.
- The swabs used in this study contain Amies medium to prolong survival of the microorganisms during transport. Because swabs were stored for long periods of time without refrigeration before shipment to Harvard, growth of microorganisms may have occurred.
- We recommend that future samples be shipped to Harvard on ice immediately after collection.

Table 1. Samples enumerated in this study.

Cemetery	Stone Identifier	Environment	Marble Type
Alexandria	C417A	Shady	Colorado
Alexandria	B1312	Sunny	Colorado
Bath	D713	Shady	Colorado
Bath	D714	Shady	Colorado
Bath	D715	Shady	Colorado
Bath	F710	Sunny	Colorado
Bath	F711	Sunny	Colorado
Bath	F712	Sunny	Colorado
Jefferson	32-2886A	Shady	Colorado
Jefferson	32-2904A	Shady	Colorado
Jefferson	32-2928A	Shady	Colorado
Jefferson	72-1269	Sunny	Colorado
Jefferson	72-1270	Sunny	Colorado
Jefferson	72-1370	Sunny	Colorado
San Francisco	NAWS 886 B	Shady	Colorado
San Francisco	WS 1032 B	Sunny	Colorado
Santa Fe	U311-A	Shady	Colorado
Santa Fe	H 526 D	Sunny	Colorado

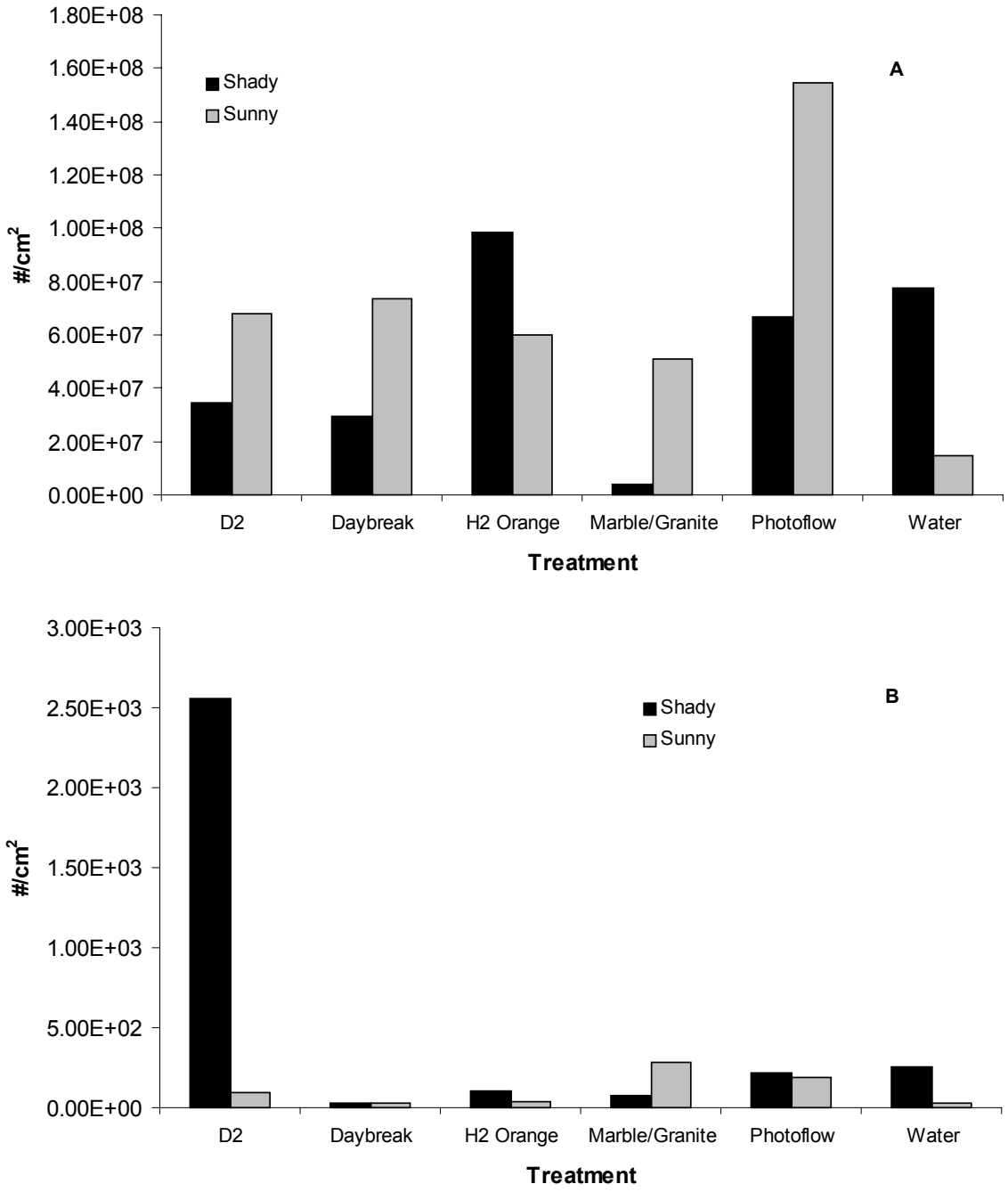


Figure 1. Numbers of bacteria (A) and fungi (B) in samples from Alexandria National Cemetery.

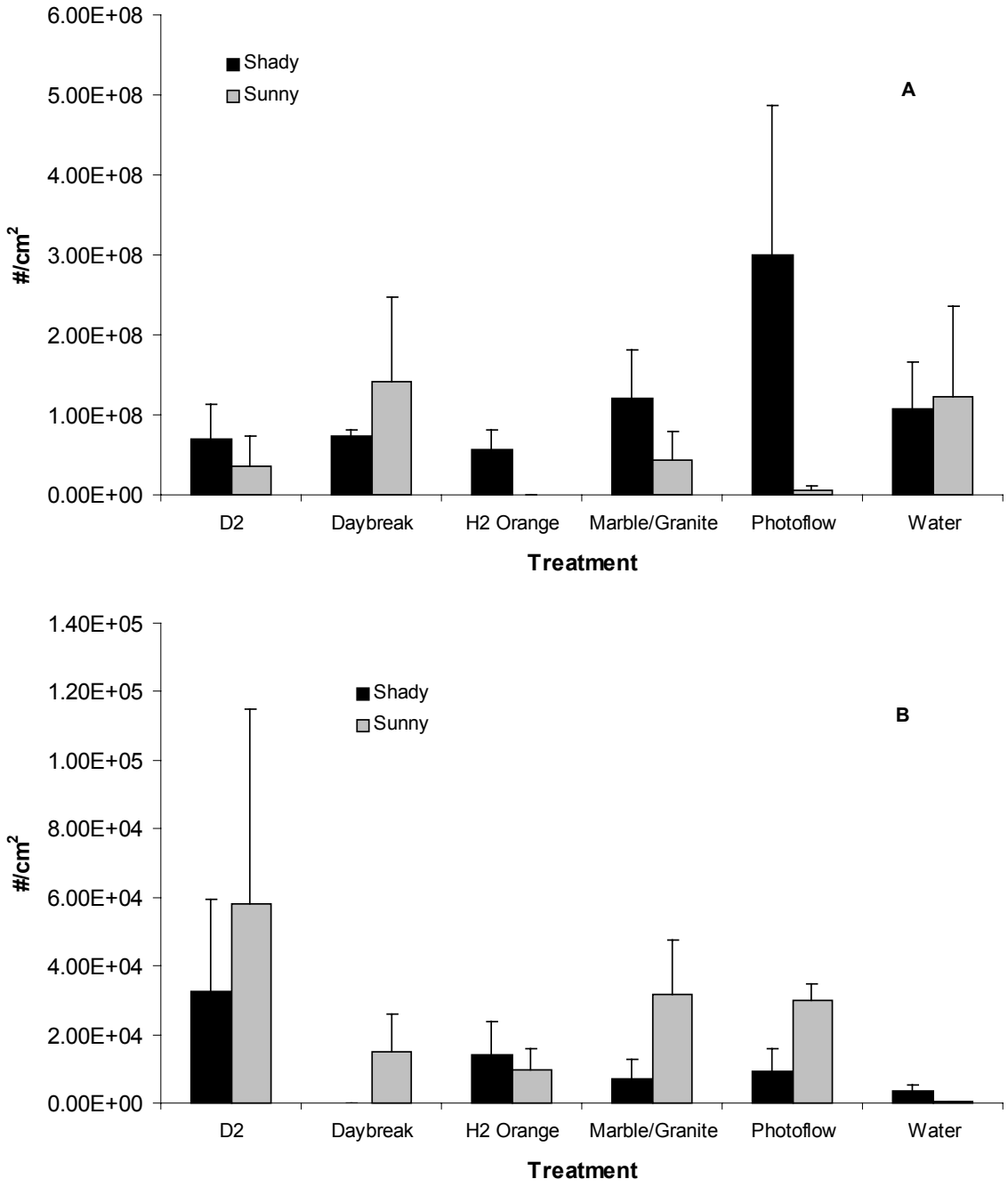


Figure 2. Numbers of bacteria (A) and fungi (B) in samples from Bath National Cemetery.

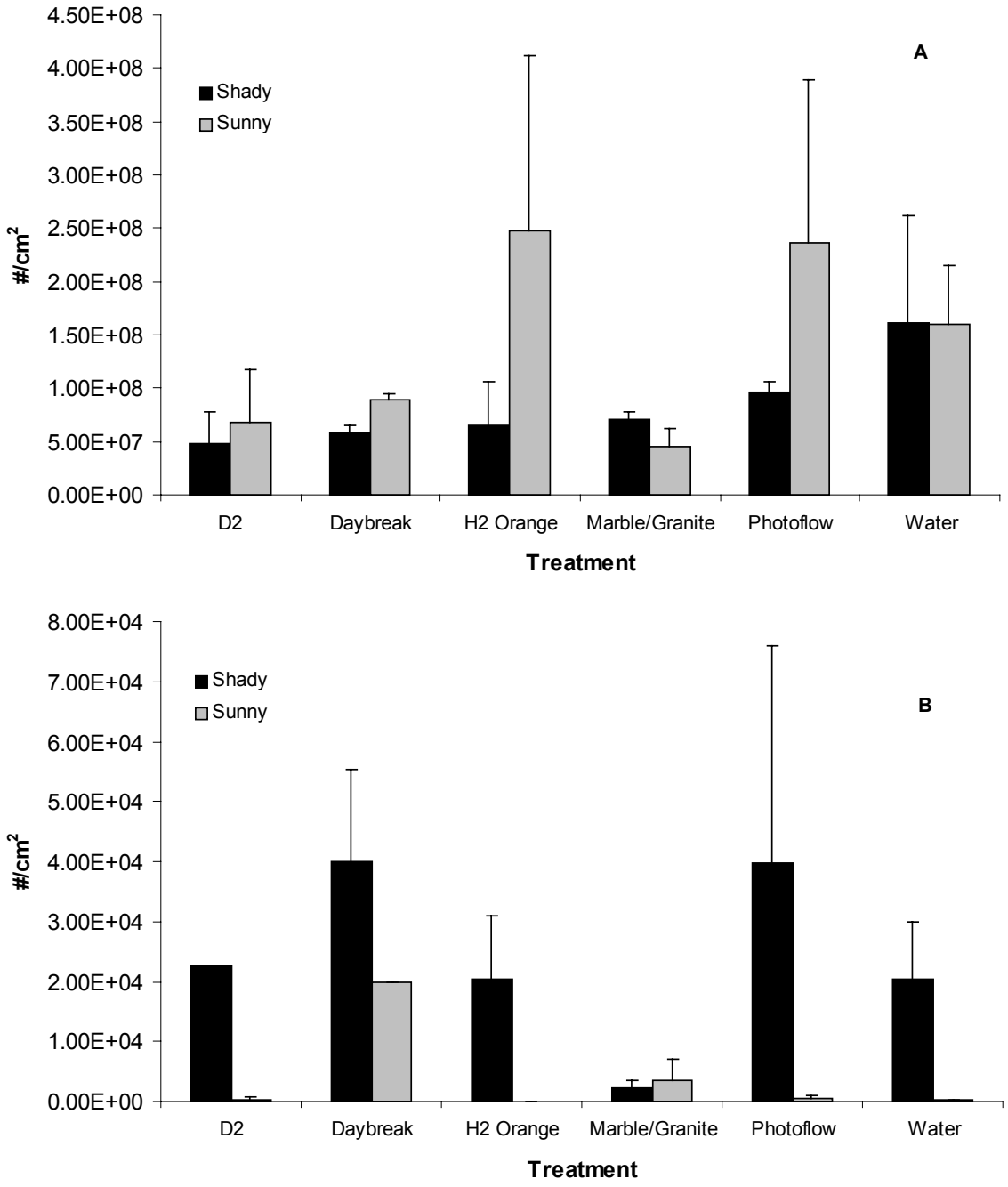


Figure 3. Numbers of bacteria (A) and fungi (B) in samples from Jefferson Barracks National Cemetery.

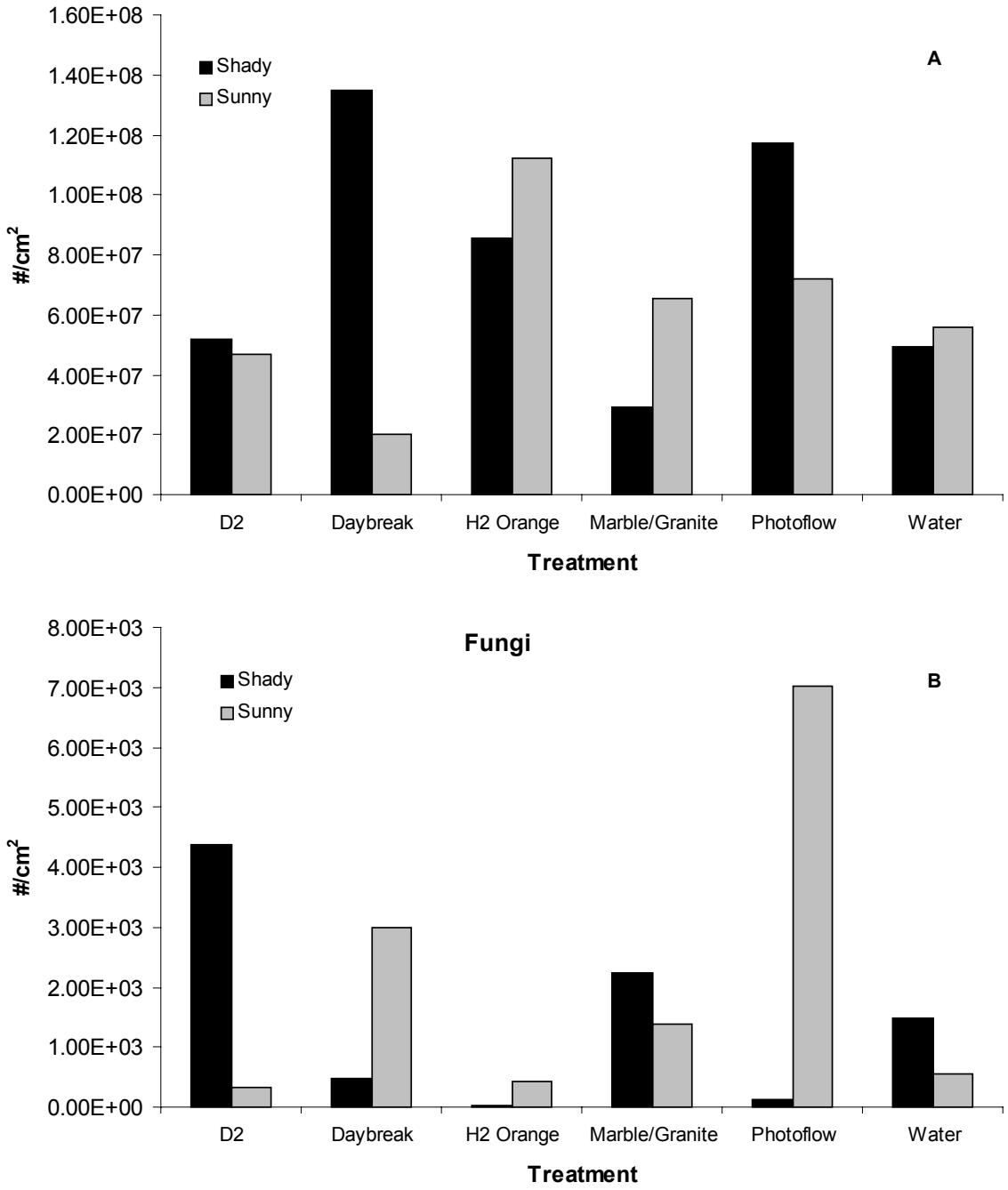


Figure 4. Numbers of bacteria (A) and fungi (B) in samples from San Francisco National Cemetery.

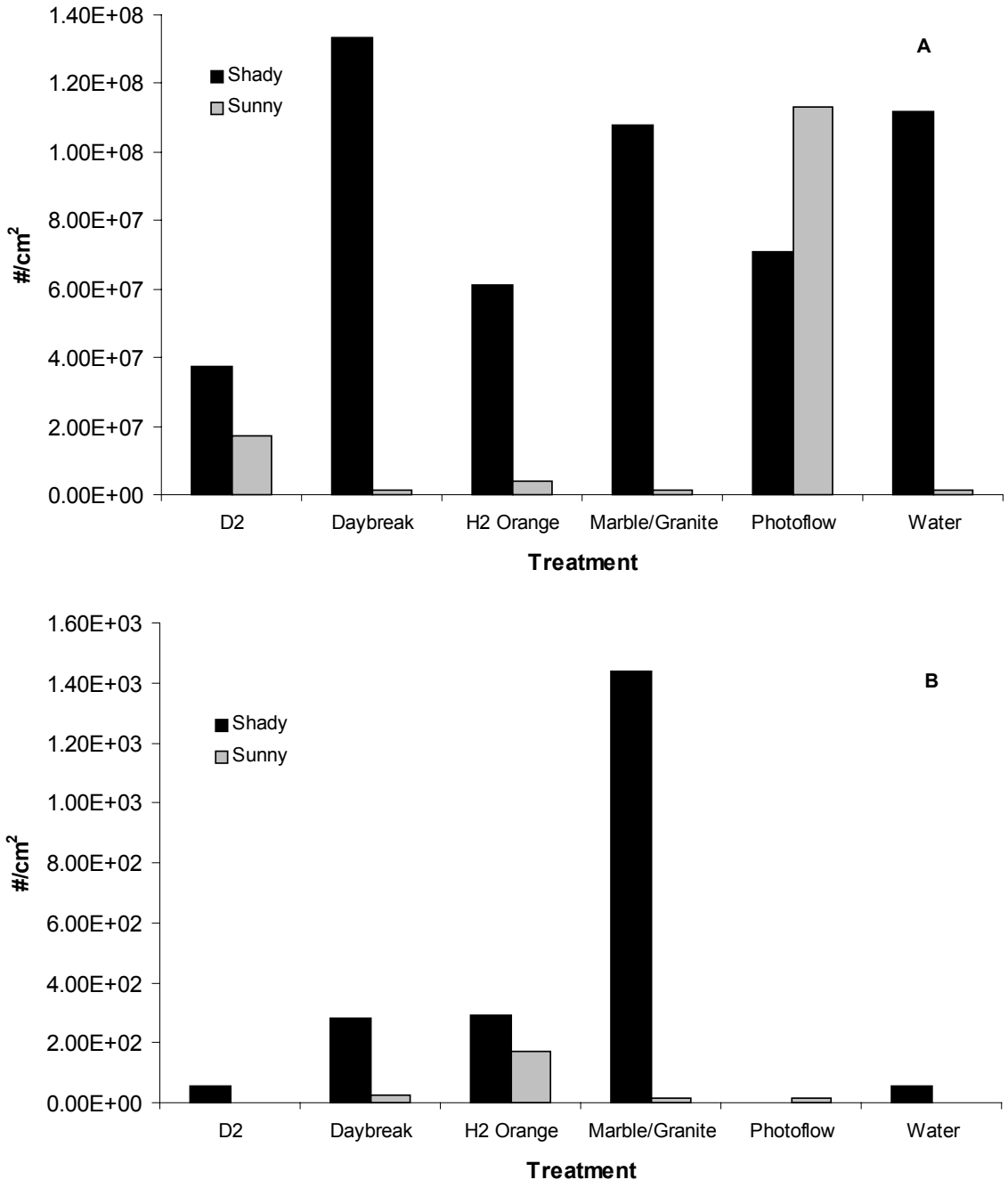


Figure 5. Numbers of bacteria (A) and fungi (B) in samples from Santa Fe National Cemetery.

APPENDIX 1. Results of Initial Samples Collected in October – November 2005

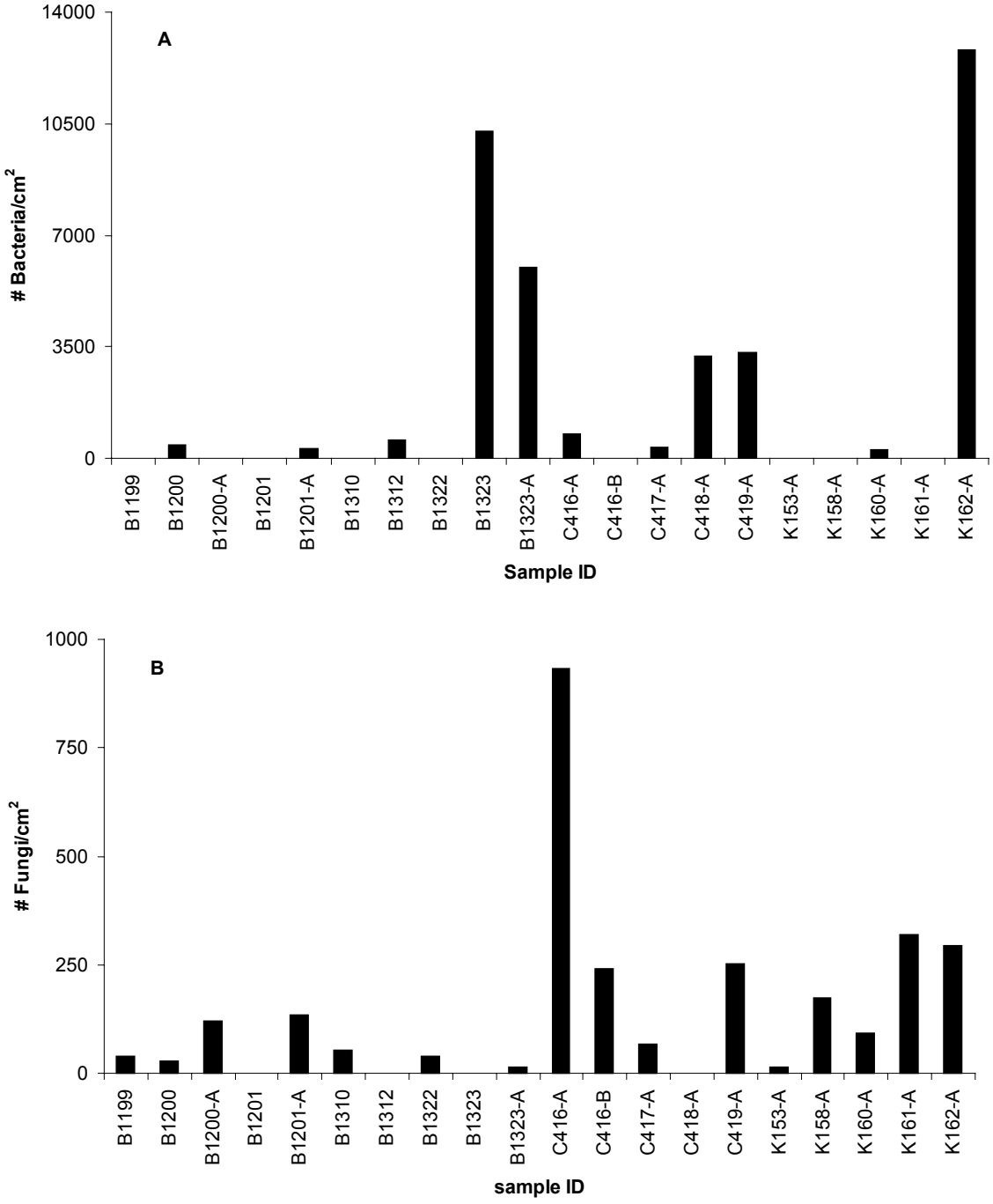


Figure 1-1. Numbers of bacteria (A) and fungi (B) in samples collected from Alexandria National Cemetery.

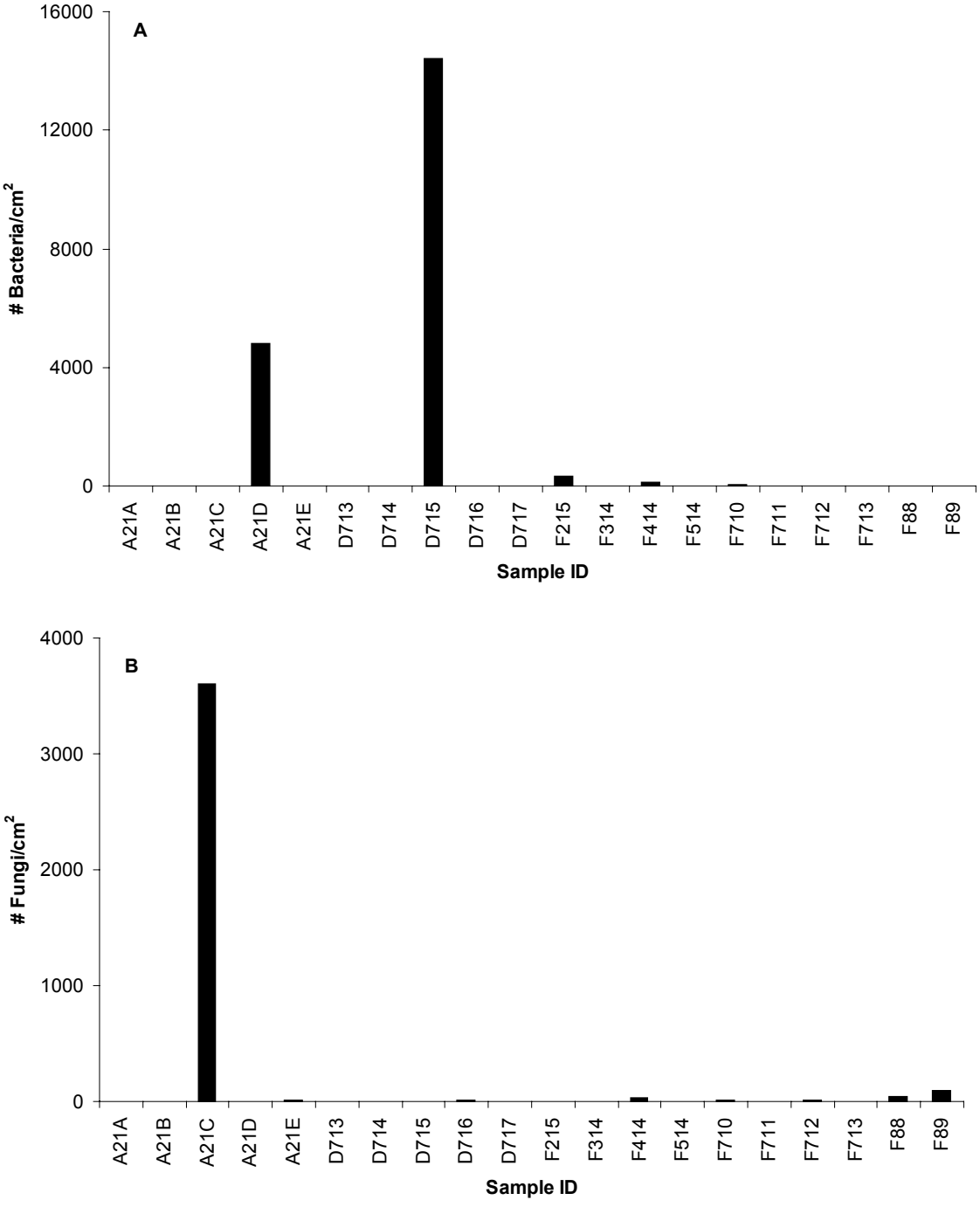


Figure 1-2. Numbers of bacteria (A) and fungi (B) in samples collected from Bath National Cemetery.

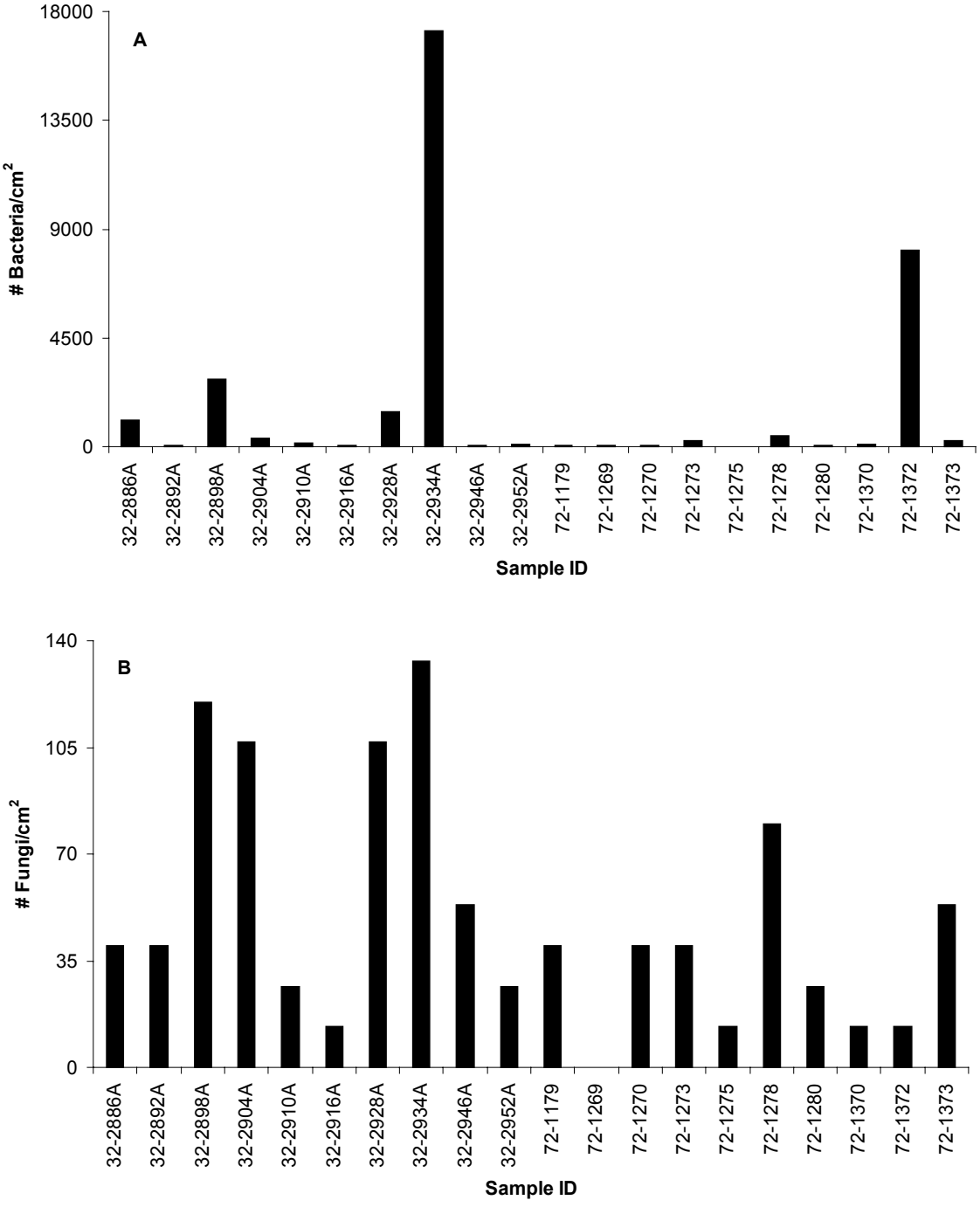


Figure 1-3. Numbers of bacteria (A) and fungi (B) in samples collected from Jefferson Barracks National Cemetery.

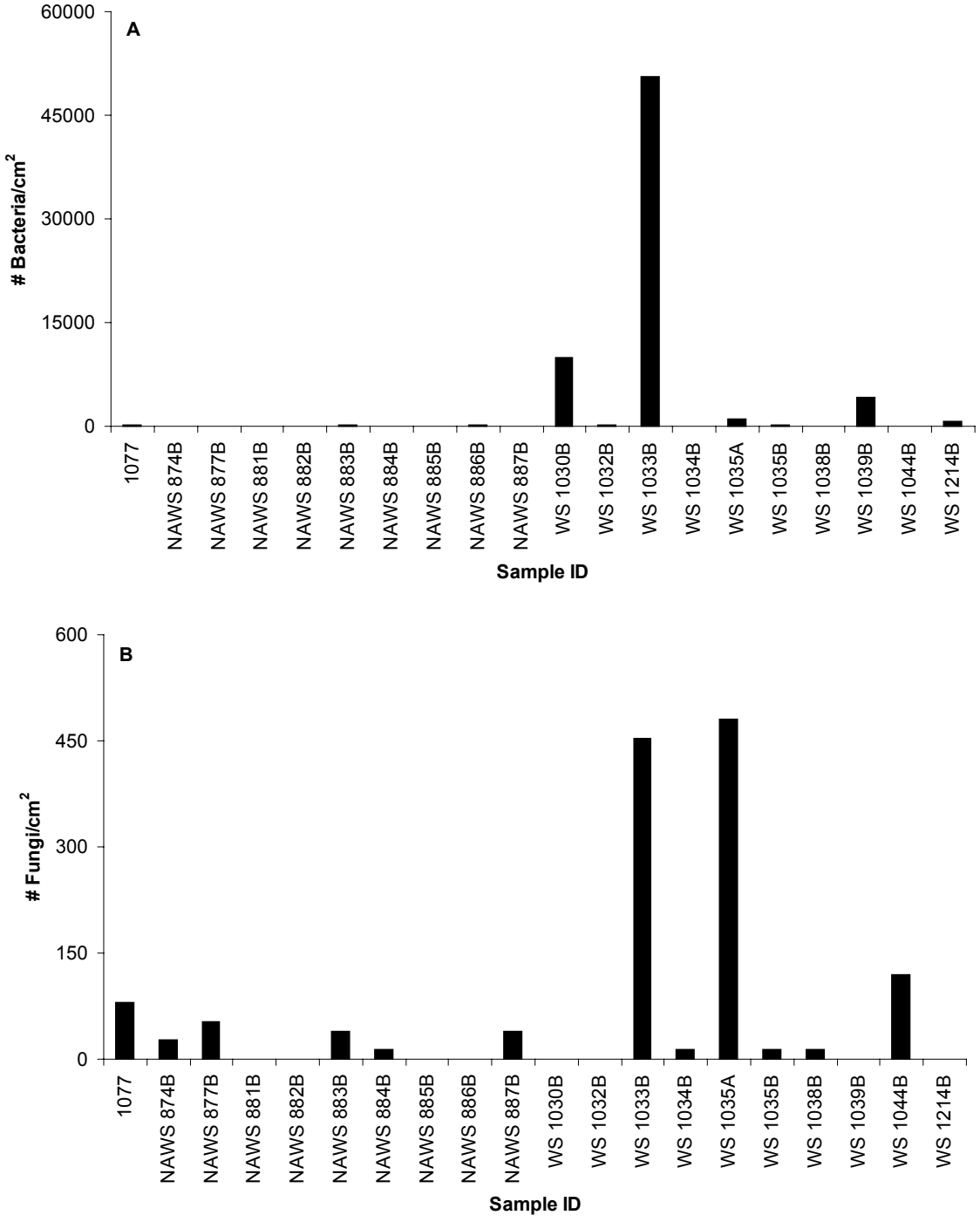


Figure 1-4. Numbers of bacteria (A) and fungi (B) in samples from San Francisco National Cemetery.

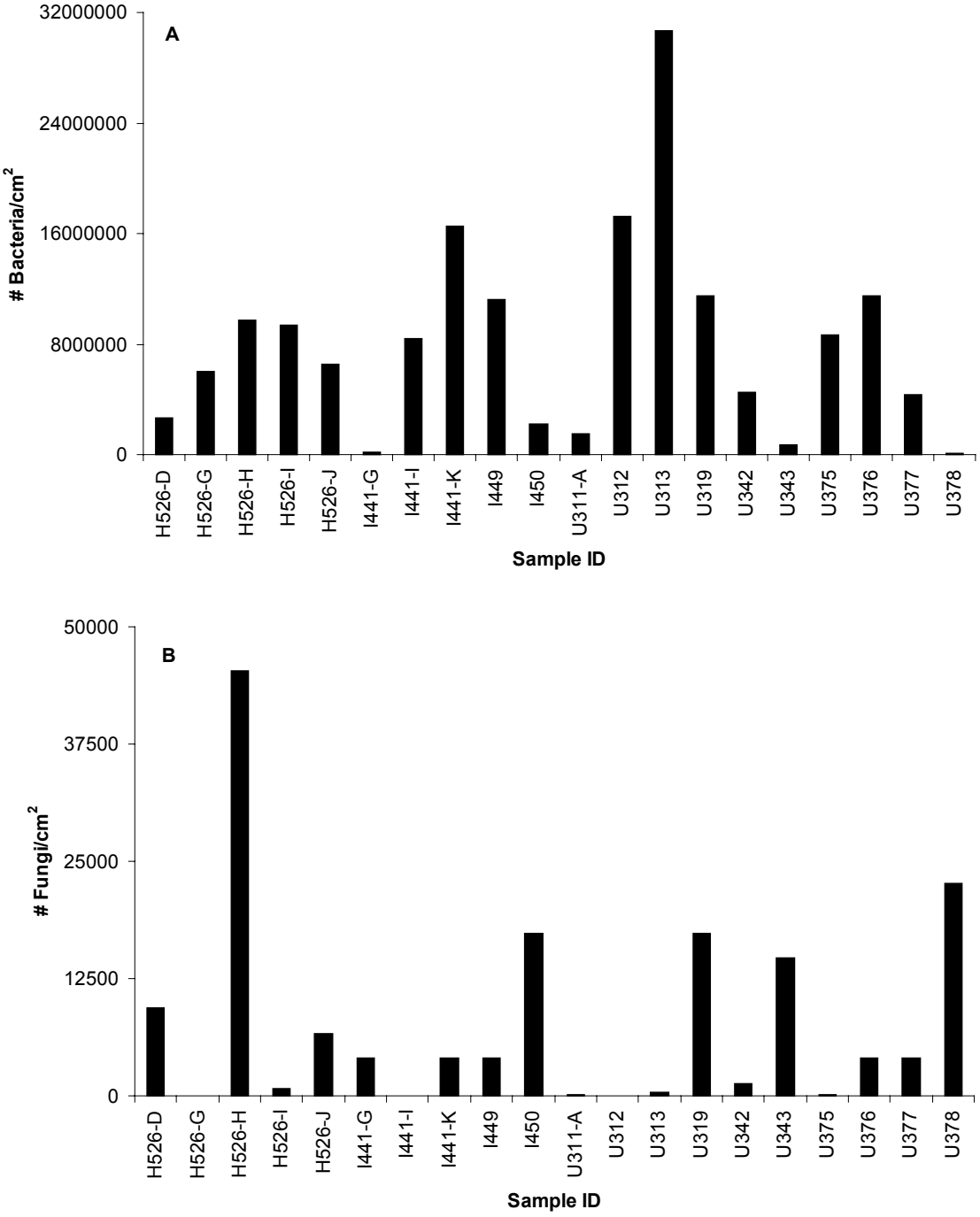
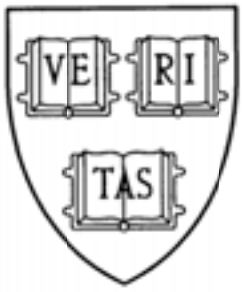


Figure 1-5. Numbers of bacteria (A) and fungi (B) in samples from Santa Fe National Cemetery.

Appendix F.
Analysis of Microorganisms on Headstones in VA Cemeteries, Third Report: February 2007

Analysis of Microorganisms on Headstones in VA Cemeteries
Third Report: February 2007



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SUMMARY

- Few differences were observed between locations cleaned in 2005 and 2006, suggesting that the cleaning agents' protection against bacterial and fungal re-growth is short, probably less than one year.
- No algae or photosynthetic bacteria were observed in the samples.
- The absence of algae or photosynthetic bacteria is significant. These organisms typically provide the most visual evidence of growth on headstones. Their absence, even from the stones treated with water, suggests it is still too early to determine the effectiveness of the biocides.
- Numbers of bacteria were generally greater than numbers of fungi.
- No consistent differences in numbers of bacteria or fungi were found among the cleaning agents.
- The most consistent differences were observed between sunny and shaded locations- abundance of bacteria and fungi were frequently greater in shady locations. This is most likely due to drier conditions and more intense UV irradiation in sunny locations.

OBJECTIVES

The objective of this project is to test cleaning agents for use in cleaning headstones within national cemeteries overseen by the National Cemetery Administration. The purpose of the current work was to analyze of numbers of microorganisms in samples collected from tombstones in five Veterans Administration cemeteries one year after cleaning.

RESULTS FROM PREVIOUS SAMPLE COLLECTION

Fall 2005 samples

Bacteria and/or fungi were found in most samples collected in October and November 2005 (see Appendix 1). Numbers of bacteria were generally greater than numbers of fungi. Algae were not detected in the samples. Our analysis of microbial growth showed wide variability in the size of the microbial community. However, numbers of bacteria and fungi were low in most samples.

Spring 2006 Samples

Large numbers of bacteria and fungi were found in all samples (see Appendix 2). The large numbers of microorganisms enumerated were inconsistent with visual observations made by Jason Church, in which locations cleaned with D2 and Daybreak appeared to be free from microbial growth. Inconsistencies between visual observations and microbial counts may have been due to growth of microorganisms in the swabs after sampling.

METHODS

Sample Collection and Study Sites

Samples were collected during November and December 2006 by Jason Church from five cemeteries: 1) Alexandria National Cemetery, Pineville, LA 2) Bath National Cemetery, Steuben County, NY, 3) Jefferson Barracks National Cemetery, St. Louis, MO, 4) San Francisco National Cemetery, San Francisco, CA, and 5) Santa Fe National Cemetery, Santa Fe, NM. A three cm^2 area of the tombstones were sampled for microorganisms using BBL Culture Swabs (Becton-Dickinson, Sparks, MD). Sample locations were cleaned in either 2005 or 2006 using five different agents: Daybreak, 5914 (NCH Corporation, Irving, TX), Marble and Granite Cleaner Concentrate (World Environmental Group, Inc., Ocala, FL), Photo-Flo 200 (Eastman Kodak Company, Rochester, NY), $H_2Orange_2$ Grout Safe (EnvirOx LLC, Danville, IL), and D/2 Architectural Antimicrobial (Sunshine Makers, Inc., Huntington Harbour, CA). In this round of samples, locations cleaned with D/2, Daybreak, Marble and Granite Cleaner, and water were sampled. Samples were shipped overnight to Harvard University.

Enumeration of Microorganisms

Samples collected from the headstones were enumerated (Table 1). Bacteria and fungi were enumerated by plating samples on solid media. Plates were incubated at room temperature for two days and colonies were counted. Bacteria were plated on Difco Nutrient Agar (Becton-Dickinson, Sparks, MD) and fungi were plated on malt extract agar (6.4 g/L maltose, 1.4 g/L dextrose, 1.2 g/L glycerol, 0.4 g/L peptone, 7.5 g/L agar, 4875 U penicillin G, 3250 U bacitracin). When present, photosynthetic microorganisms (algae) were analyzed using a hemocytometer. The numbers of algae in at least 10 fields of view were counted at 40X magnification.

RESULTS

No algae were detected in samples from any of the five cemeteries sampled. Green coloration in some samples was due to the presence of fungi. Fungi and bacteria were enumerated by plating on solid media and counting colonies after incubation. Numbers of bacteria and fungi in samples were variable.

Large numbers of bacteria were found in samples from Alexandria National Cemetery (Fig. 1A). The largest numbers of bacteria were found in samples cleaned with D/2 (Shady 2006 and Sunny 2005). The smallest number of bacteria was found in the shaded location sample location cleaned with Marble/Granite cleaner. Numbers of fungi from Alexandria National Cemetery were more variable than numbers of bacteria, but frequently were as abundant as the bacteria (Fig. 1B). No fungi were observed in sunny locations cleaned in 2005.

Bacteria in samples from Bath National Cemetery were high in the shady locations and generally low or not found in sunny locations (Fig. 2A). There did not appear to be any differences between cleaning agents. As was found in Alexandria, numbers of fungi were much more variable than bacteria at Bath National Cemetery (Fig. 2B). For all cleaning agents except Daybreak, numbers of fungi were greater shady locations than sunny locations.

Numbers of bacteria in samples from Jefferson Barracks National Cemetery were variable and there were no consistent differences between cleaning agents (Fig. 3A). The lowest numbers of bacteria were found in sunny locations cleaned with Daybreak. Numbers of fungi were generally higher in shaded locations (Fig. 3B). Sunny locations cleaned in 2006 had the lowest numbers of fungi.

The numbers of bacteria were generally very high in samples from San Francisco National Cemetery (Fig. 4A). Again, there were no consistent differences between cleaning agents. Numbers of fungi were variable, but much lower than numbers of bacteria (Fig. 4B). No fungi were observed in sunny locations cleaned with Daybreak.

Numbers of bacteria in samples from Santa Fe National Cemetery were generally high (Fig. 5A). In most cases numbers were greater in samples from shaded locations than sunny locations. The lowest numbers of bacteria were found in samples from sun-exposed locations cleaned with Daybreak in 2006. Numbers of fungi were fairly consistent, with most samples having about 10,000 colony forming units/cm^2 (Fig. 5B). Like the bacteria, the lowest number of fungi was found in the locations cleaned with Daybreak in 2006.

Table 1. Samples enumerated in this study.

Cemetery	Stone Identifier	Environment	Year Cleaned	Marble Type
Alexandria	C 417-A	Shady	2005	Colorado
Alexandria	C 419	Shady	2006	Colorado
Alexandria	B 1312	Sunny	2005	Colorado
Alexandria	B 1202	Sunny	2006	Colorado
Bath	D 7 13	Shady	2005	Colorado
Bath	B1 11	Shady	2006	Colorado
Bath	F 7 12	Sunny	2005	Colorado
Bath	F 8 12	Sunny	2006	Colorado
Jefferson	32 2904-A	Shady	2005	Colorado
Jefferson	3187	Shady	2006	Colorado
Jefferson	72 1370	Sunny	2005	Colorado
Jefferson	72 1268	Sunny	2006	Colorado
San Francisco	NAWS 886B	Shady	2005	Colorado
San Francisco	1075	Shady	2006	Colorado
San Francisco	WS 1032B	Sunny	2005	Colorado
San Francisco	WS 1038B	Sunny	2006	Colorado
Santa Fe	U 311-A	Shady	2005	Colorado
Santa Fe	U 280	Shady	2006	Colorado
Santa Fe	H 526 D	Sunny	2005	Colorado
Santa Fe	H 530	Sunny	2006	Colorado

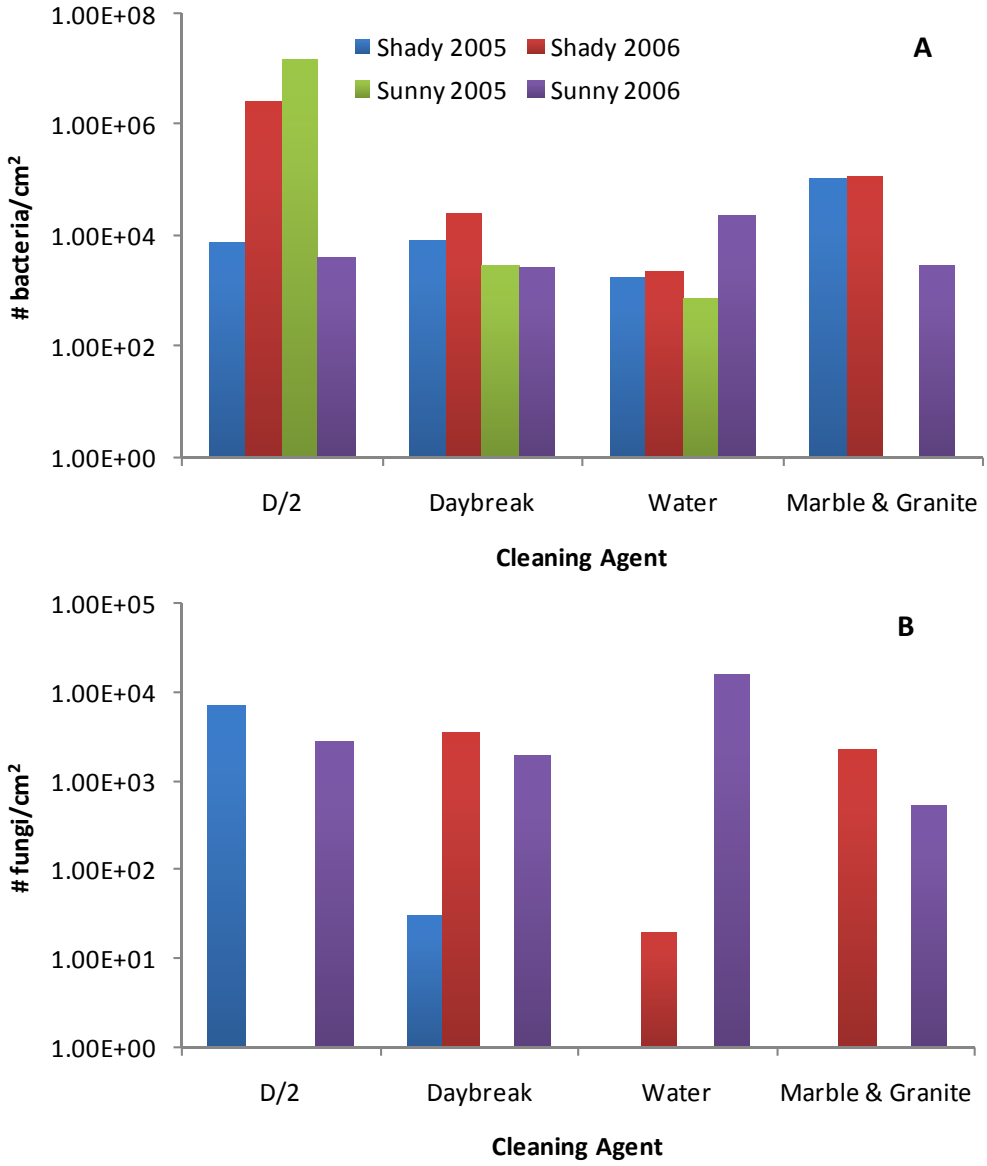

Figure 1. Numbers of bacteria (A) and fungi (B) in Alexandria National Cemetery samples from locations cleaned in 2005 and 2006.

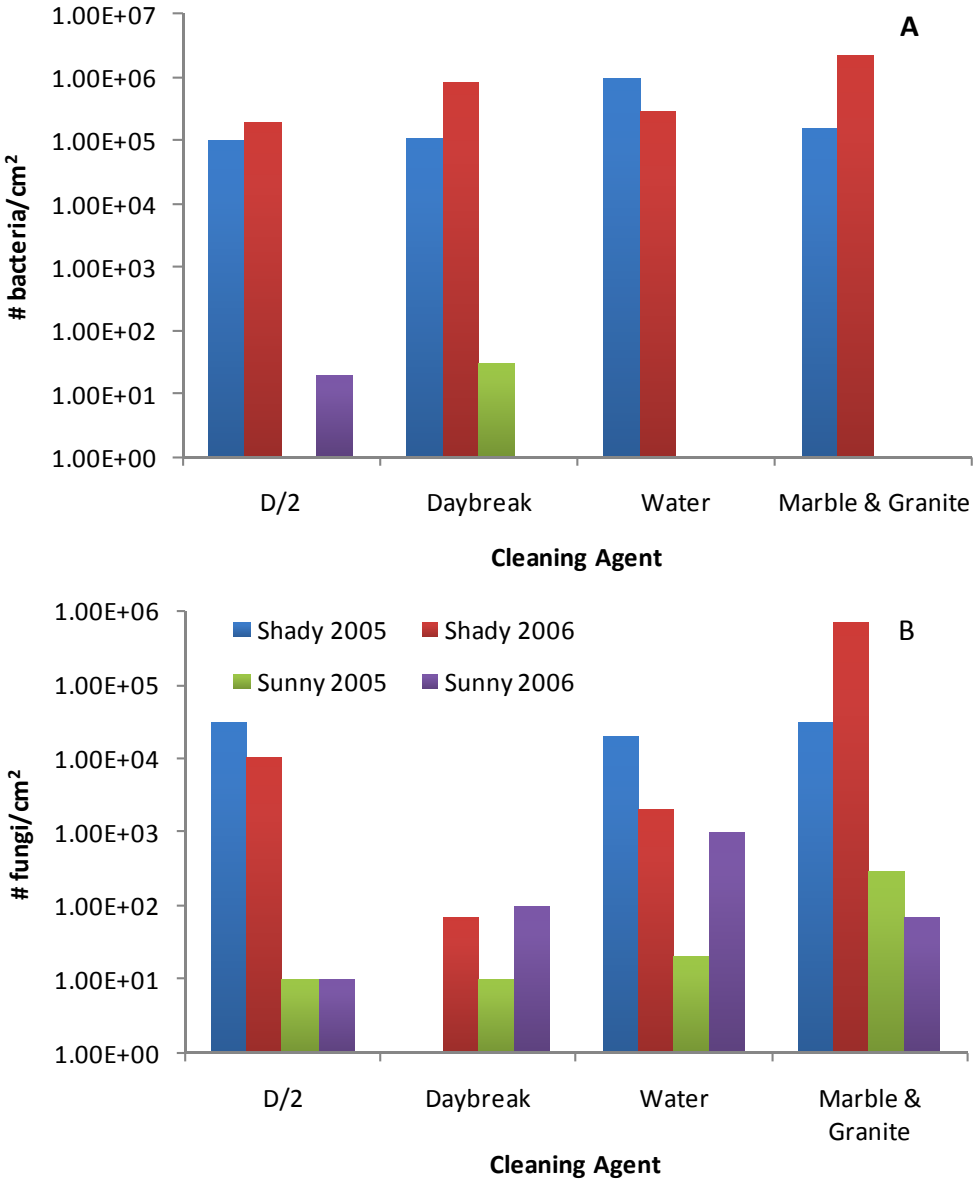

Figure 2. Numbers of bacteria (A) and fungi (B) in Bath National Cemetery samples from locations cleaned in 2005 and 2006.

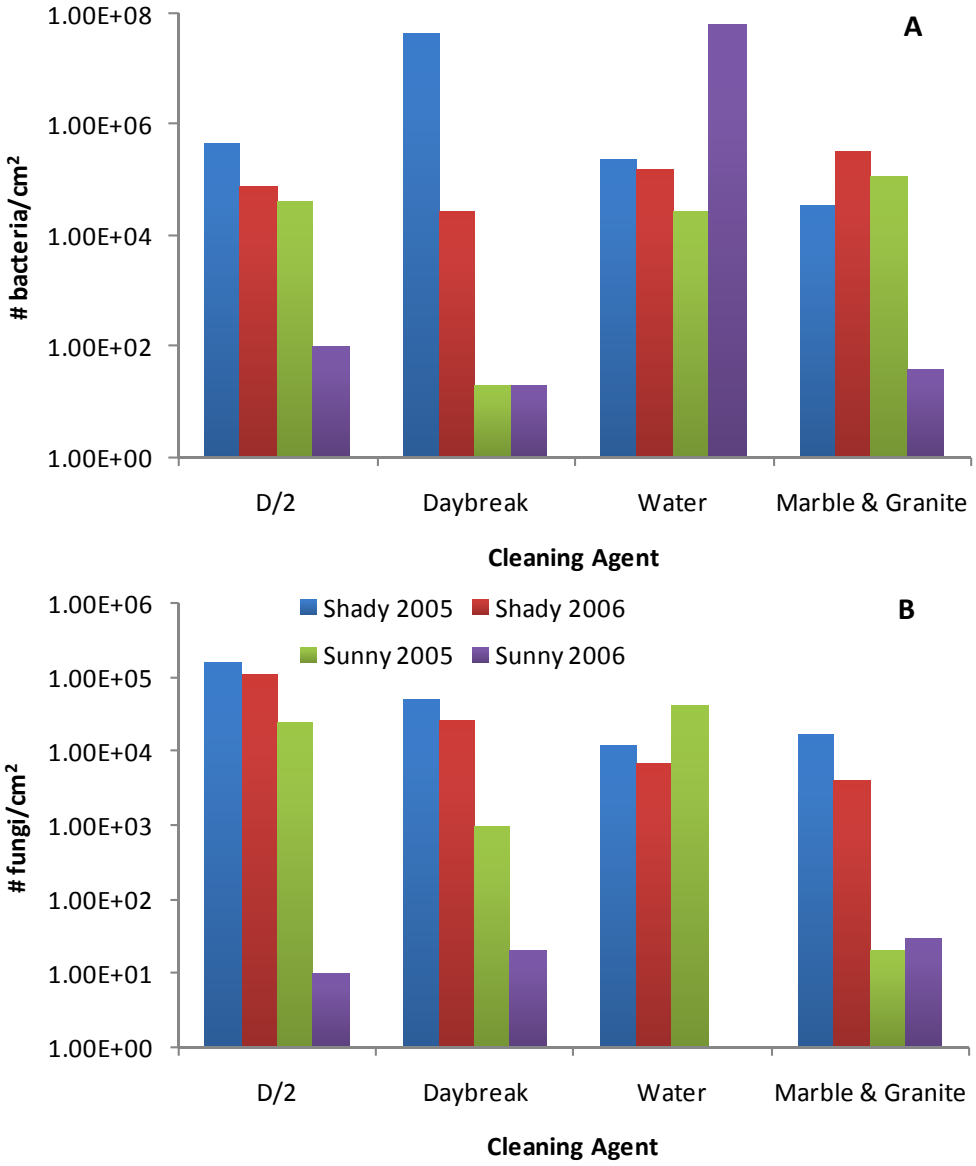

Figure 3. Numbers of bacteria (A) and fungi (B) in Jefferson Barracks National Cemetery samples from locations cleaned in 2005 and 2006.

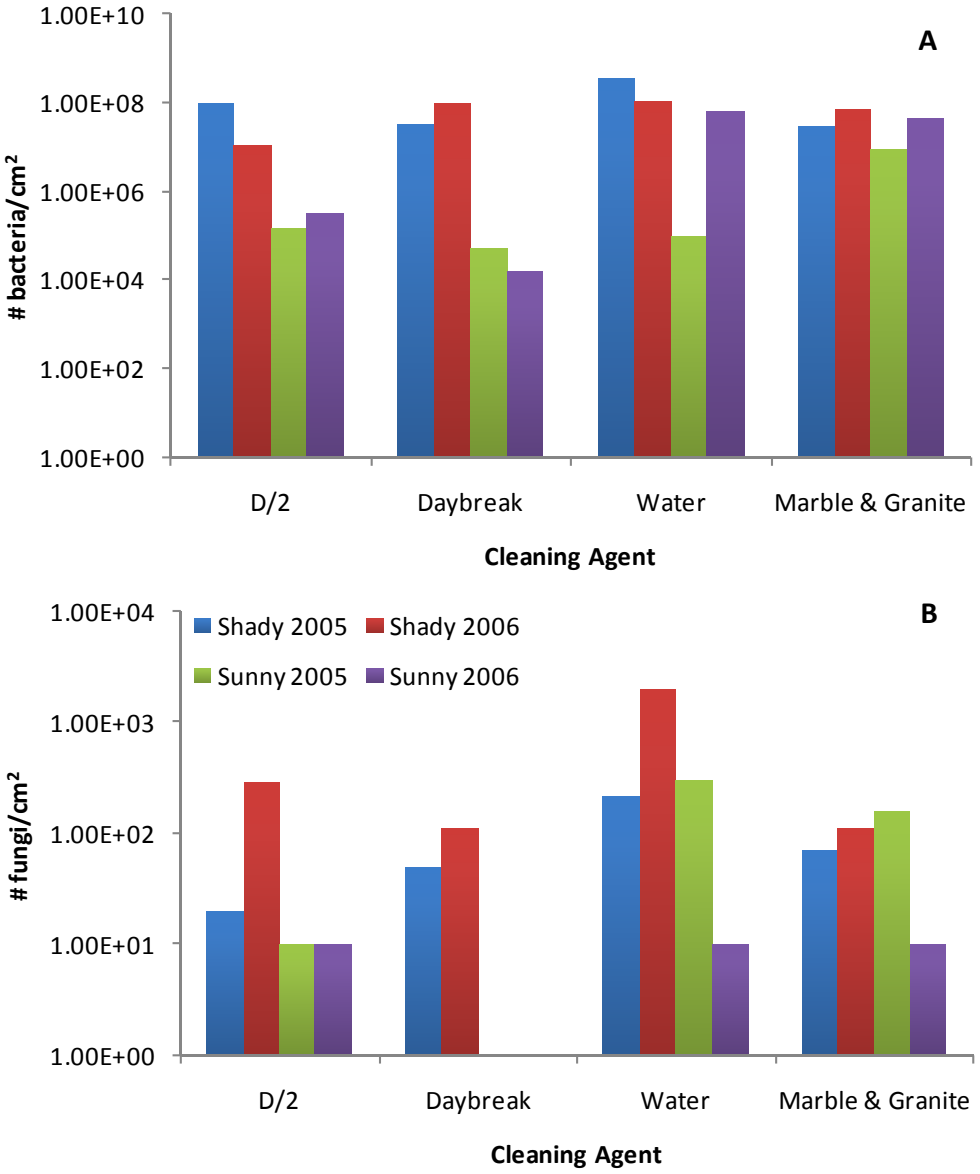

Figure 4. Numbers of bacteria (A) and fungi (B) in San Francisco National Cemetery samples from locations cleaned in 2005 and 2006.

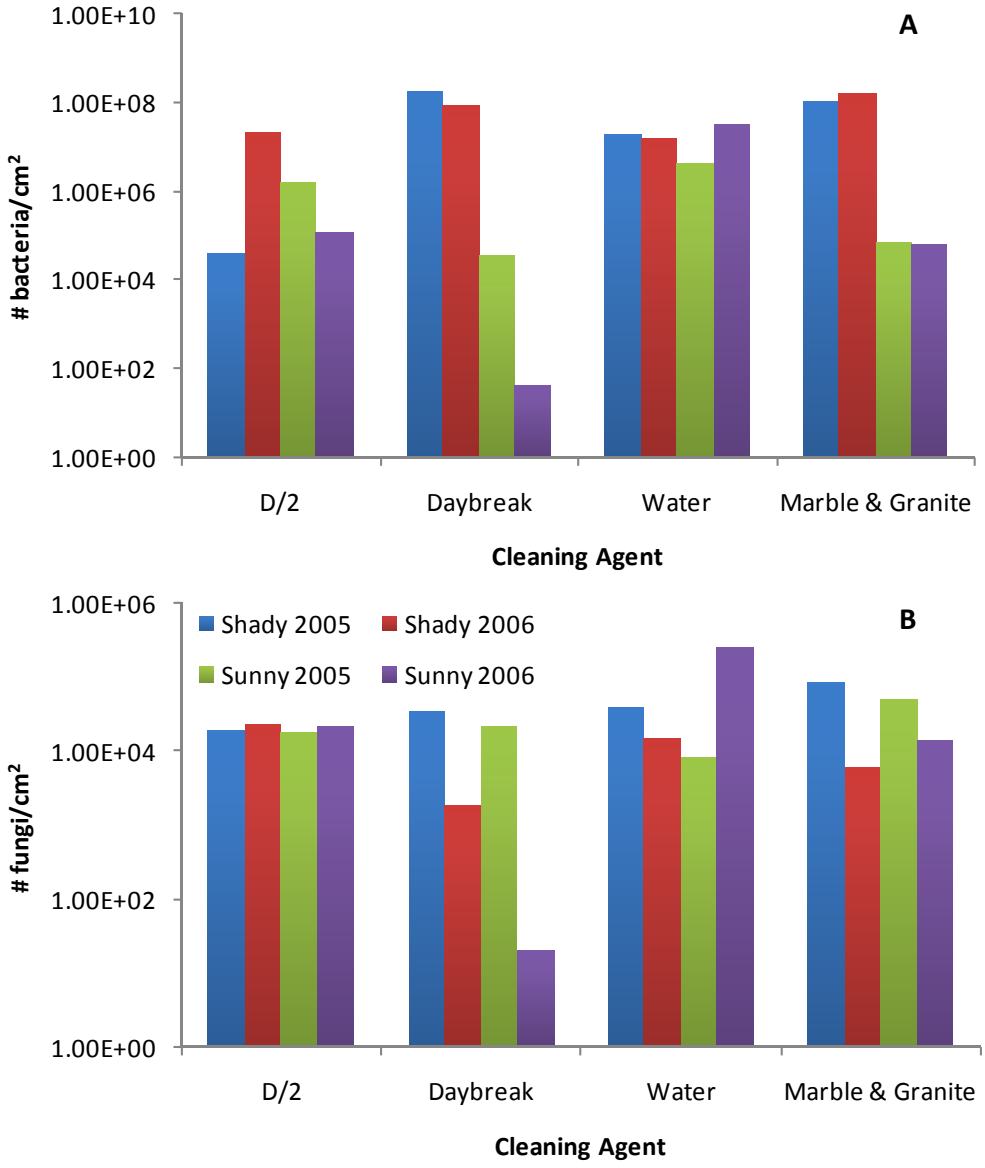

Figure 5. Numbers of bacteria (A) and fungi (B) in Santa Fe National Cemetery samples from locations cleaned in 2005 and 2006.

APPENDIX 1

Results of Initial Samples Collected in October – November 2005

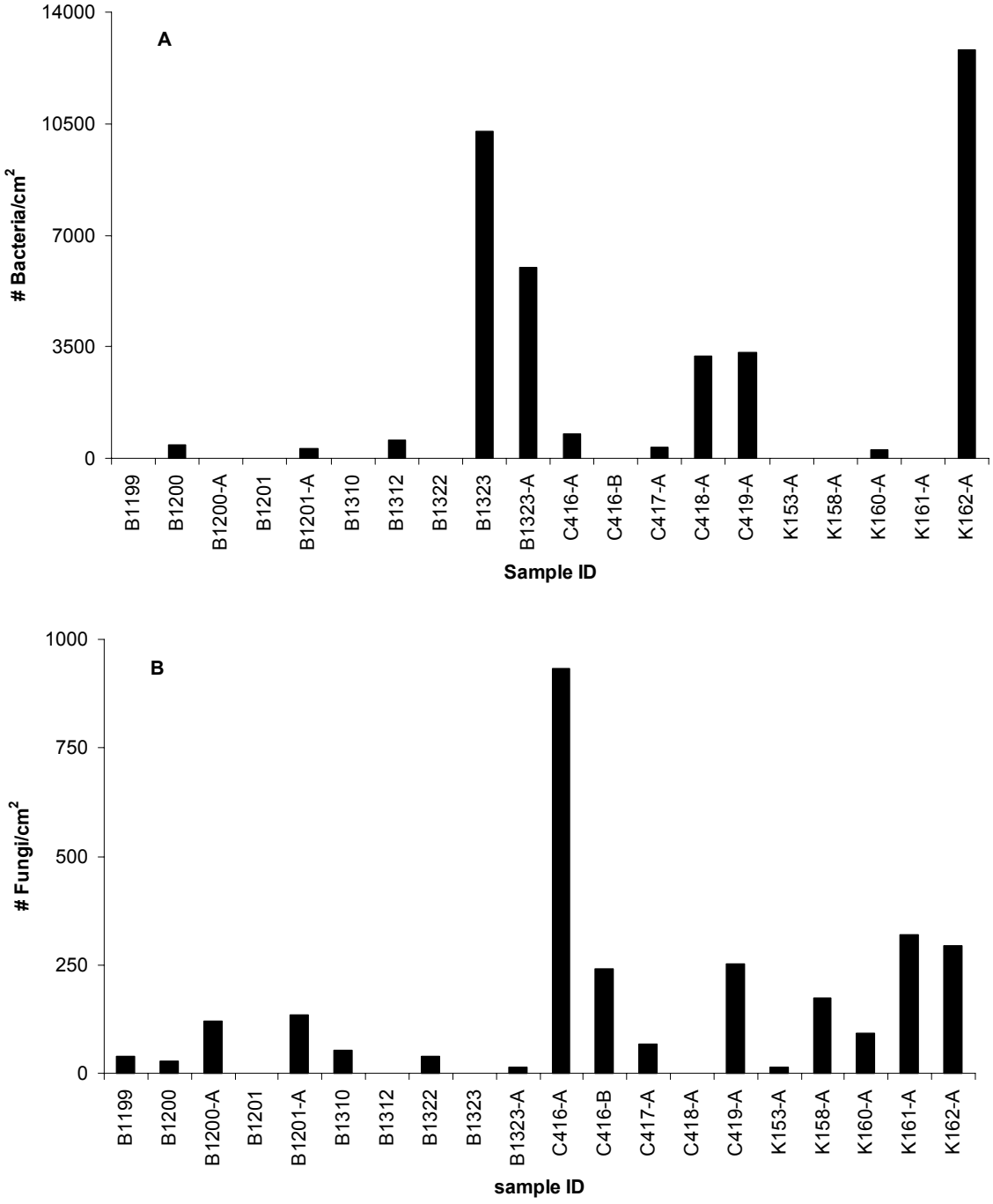

Figure 1-1. Numbers of bacteria (A) and fungi (B) in samples collected from Alexandria National Cemetery.

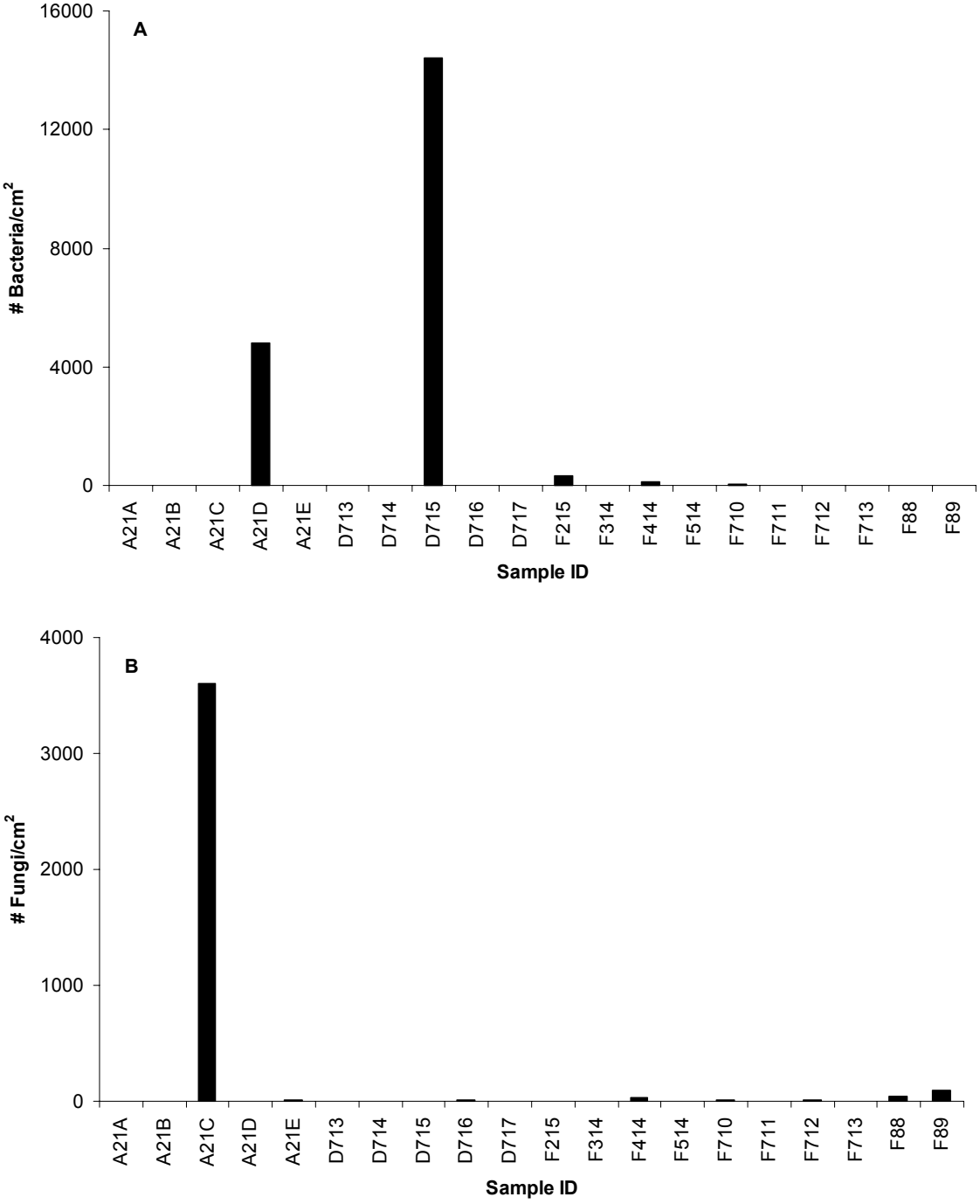

Figure 1-2. Numbers of bacteria (A) and fungi (B) in samples collected from Bath National Cemetery.

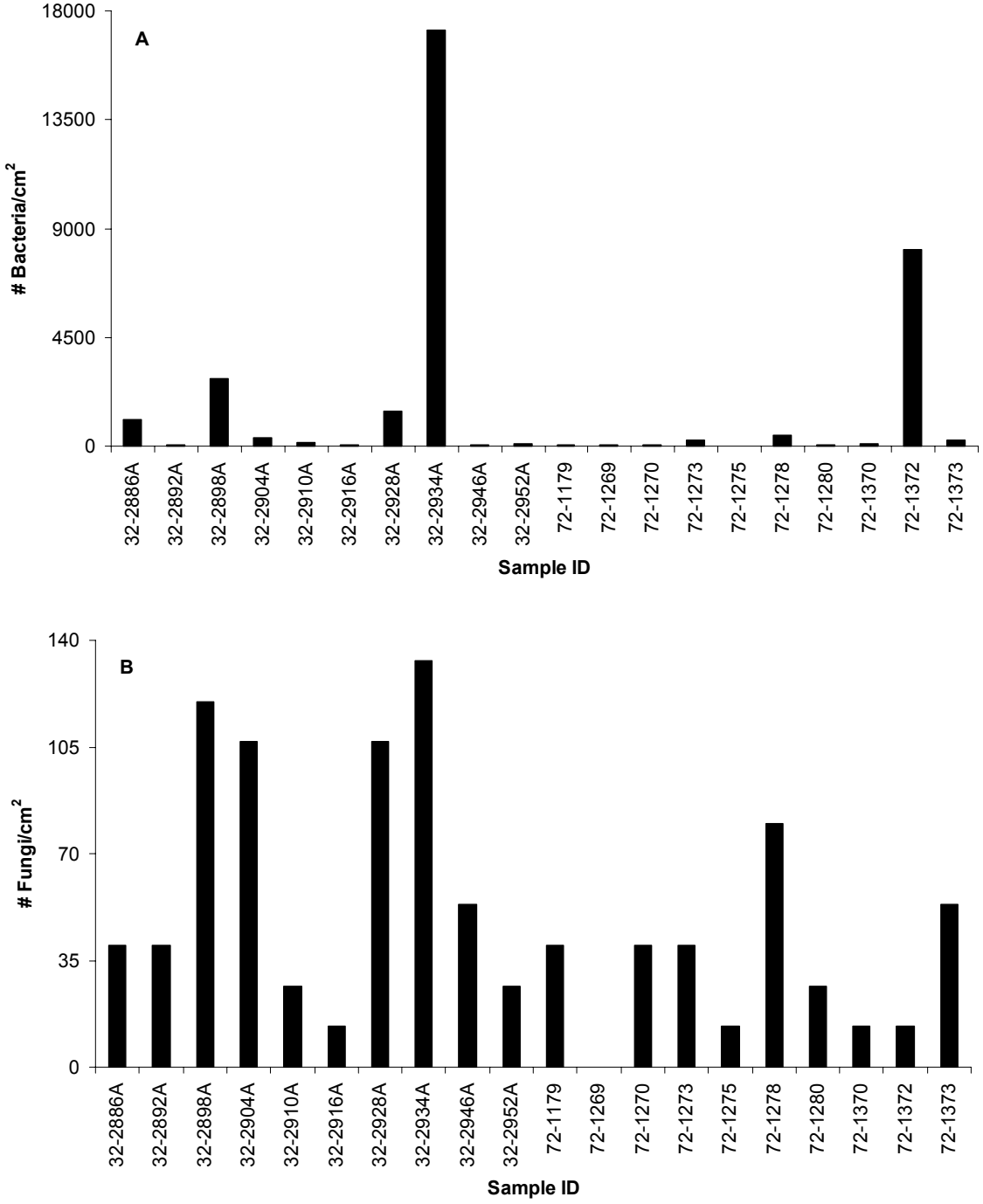

Figure 1-3. Numbers of bacteria (A) and fungi (B) in samples collected from Jefferson Barracks National Cemetery.

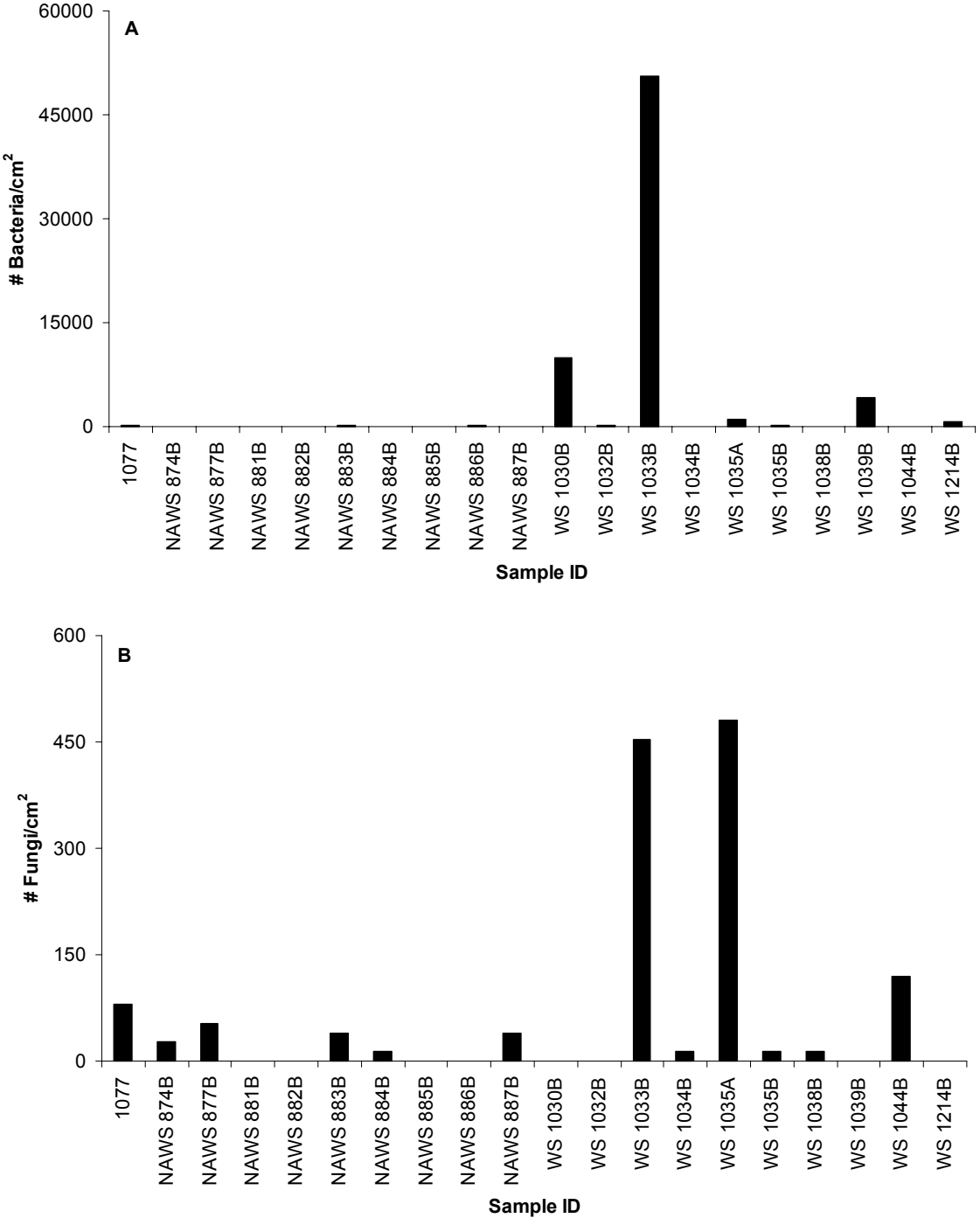

Figure 1-4. Numbers of bacteria (A) and fungi (B) in samples from San Francisco National Cemetery.

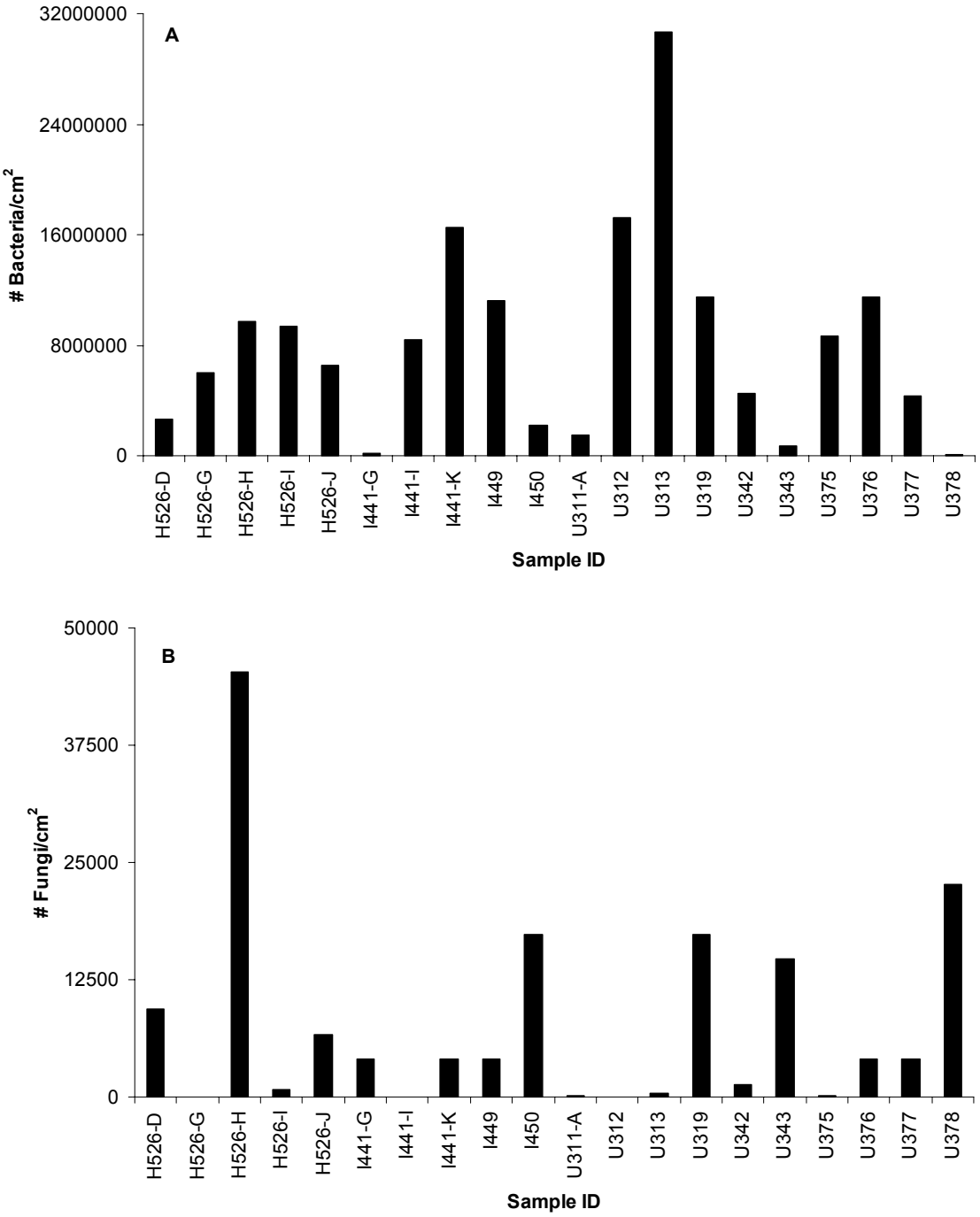

Figure 1-5. Numbers of bacteria (A) and fungi (B) in samples from Santa Fe National Cemetery.

APPENDIX 2

Results of samples collected in April-May 2006

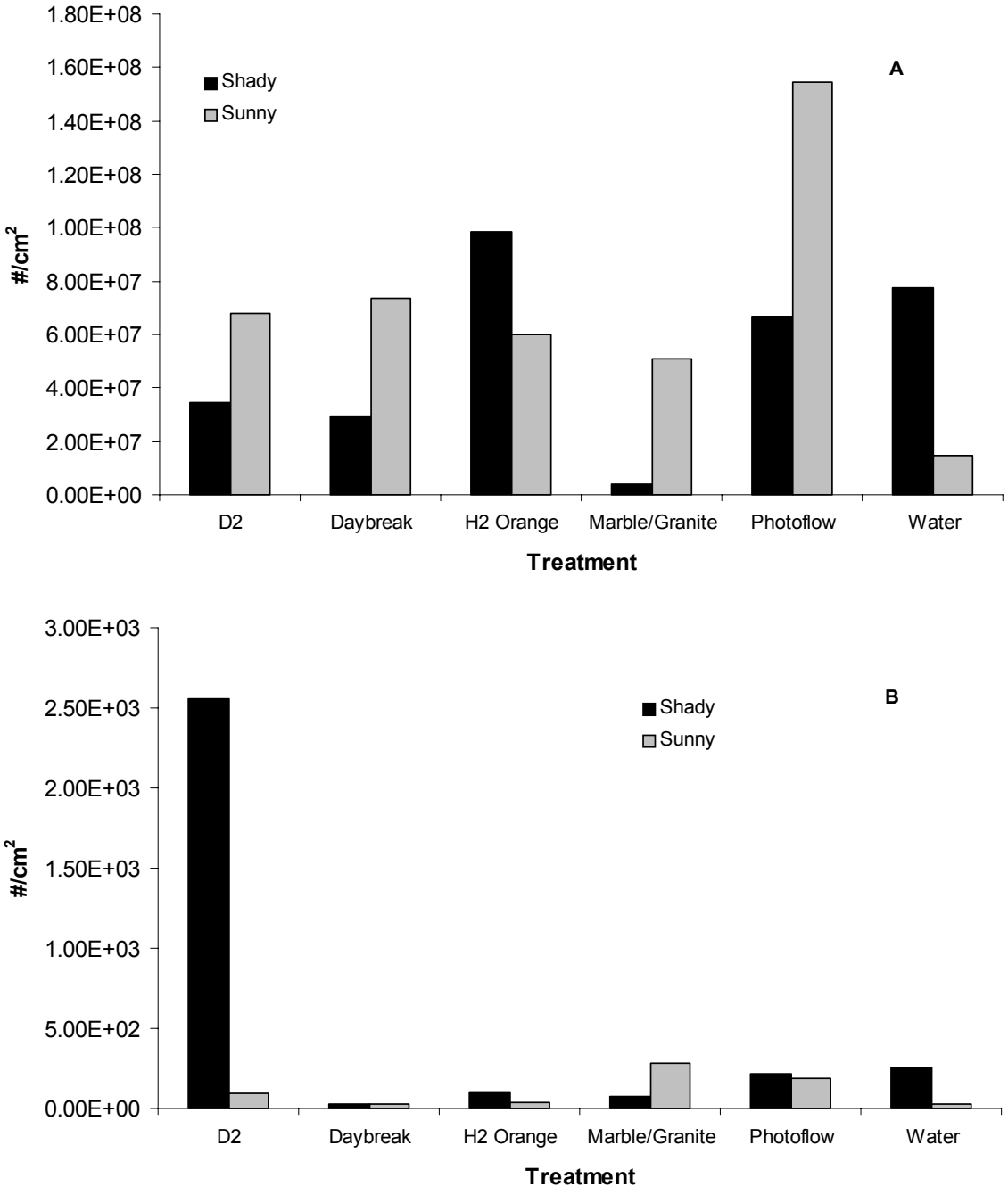

Figure 2-1. Numbers of bacteria (A) and fungi (B) in samples from Alexandria National Cemetery.

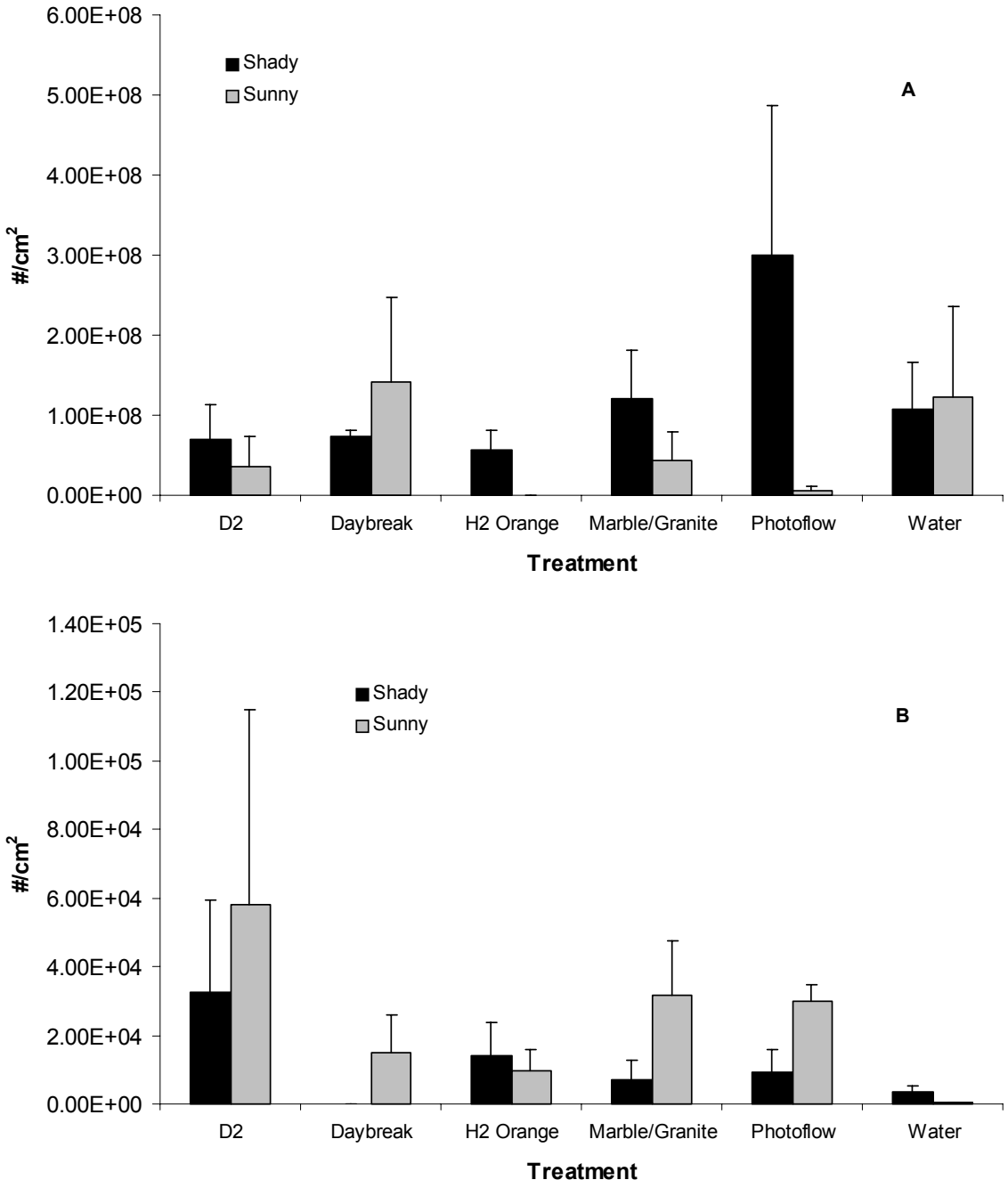

Figure 2-2. Numbers of bacteria (A) and fungi (B) in samples from Bath National Cemetery.

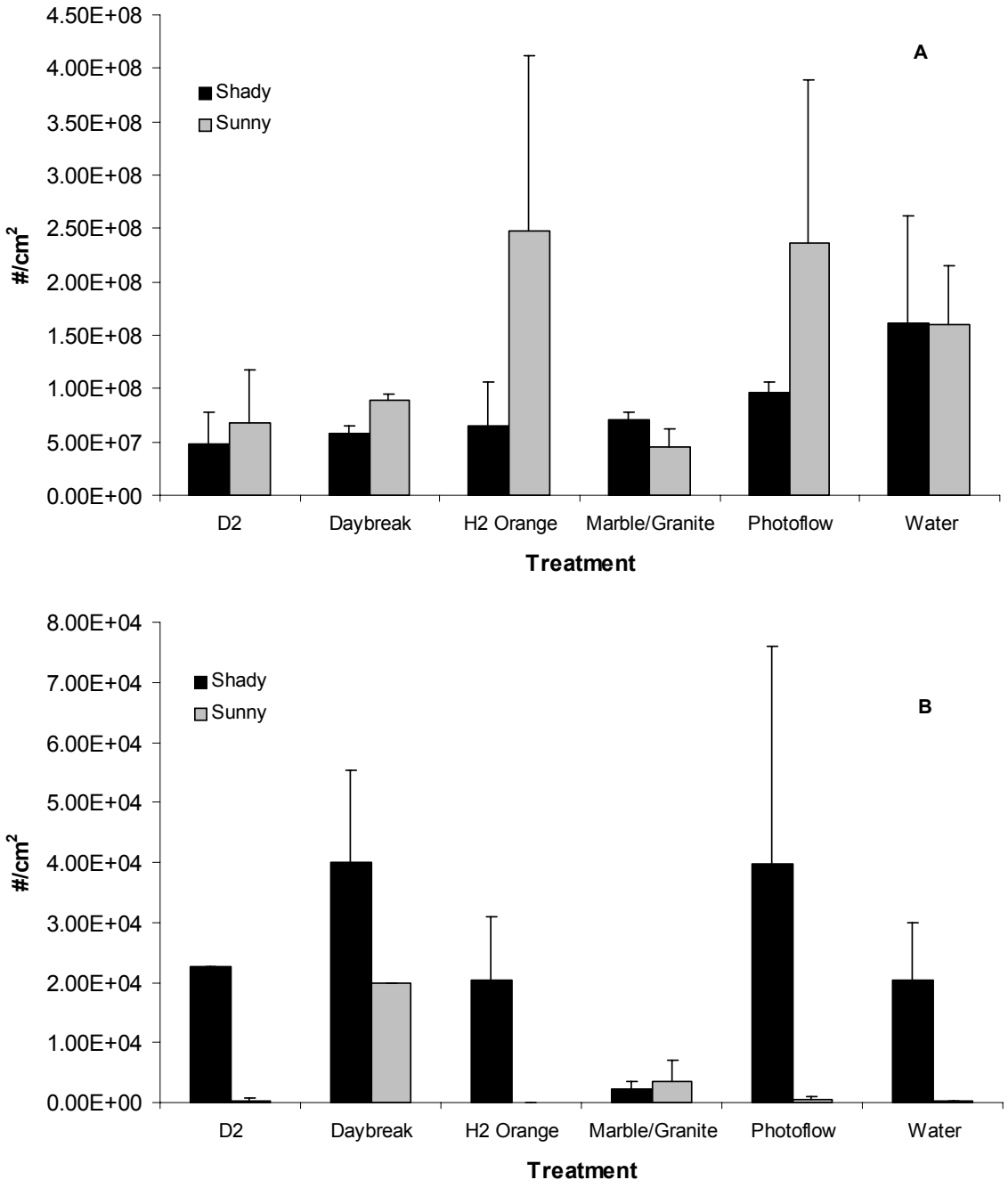

Figure 2-3. Numbers of bacteria (A) and fungi (B) in samples from Jefferson Barracks National Cemetery.

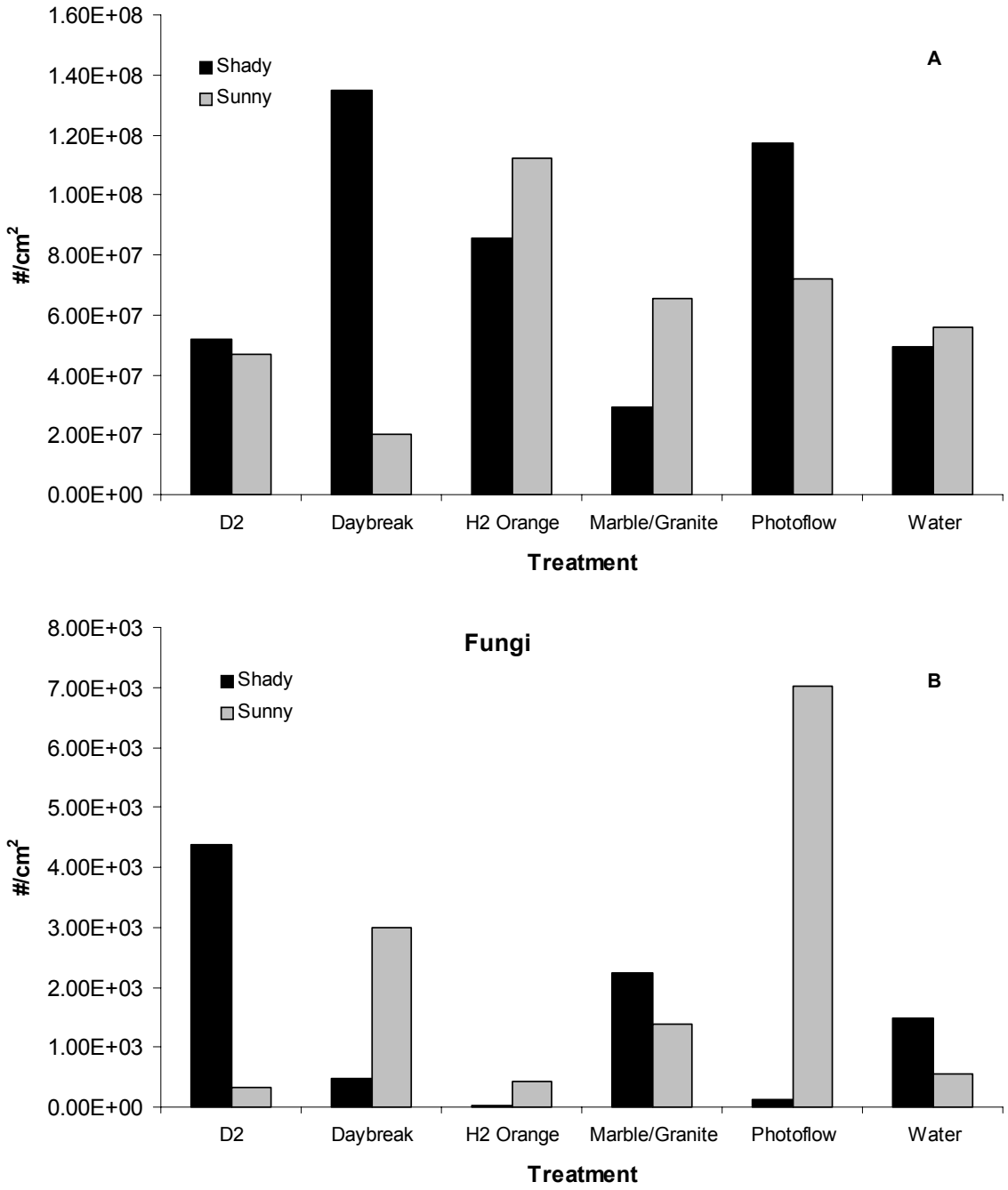

Figure 2-4. Numbers of bacteria (A) and fungi (B) in samples from San Francisco National Cemetery.

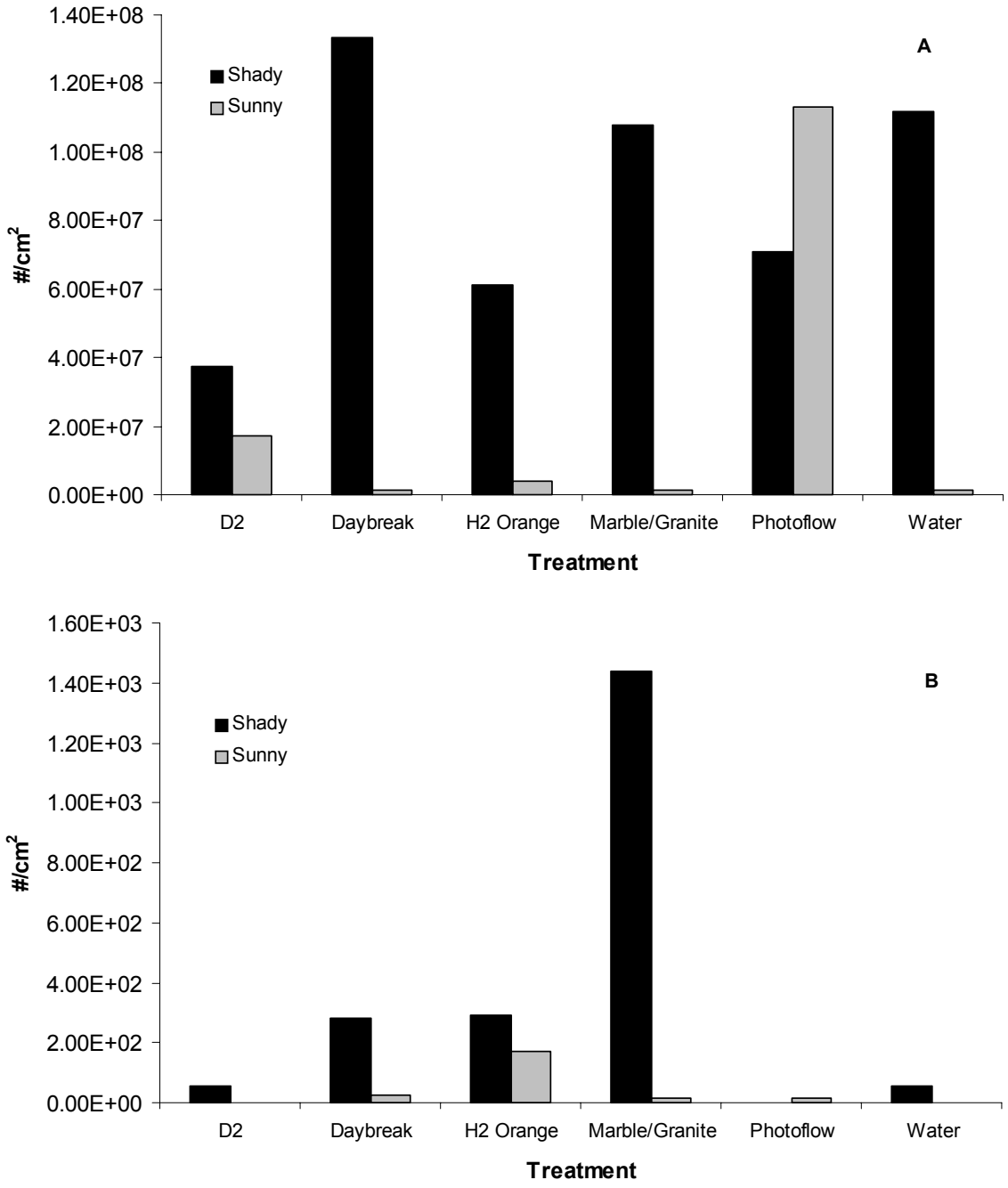

Figure 2-5. Numbers of bacteria (A) and fungi (B) in samples from Santa Fe National Cemetery.

Appendix G.
Biological Performance
Based on June 2006 Report

Appendix, Biological Performance Based on Sun or Shade

	BShady	FShady	BShady	FShady	BShady	FShady	BShady	FShady	BShady	FShady	**Shade Overall**	Bshade	Fshade
D2	4	1	4	1	5	3	4	1	4	6	**3.3**	4.2	2.4
Daybreak	5	6	4	6	4	1	1	5	1	5	**3.8**	3	4.6
H2Orange	1	6	5	3	4	3	3	6	3	5	**3.9**	3.2	4.6
Marble/Granite	6	6	3	4	4	6	5	3	2	1	**4**	4	4
Photo-flo	3	5	1	4	3	1	2	6	3	6	**3.4**	2.4	4.4
Water	2	5	3	5	1	3	4	4	2	6	**3.5**	2.4	4.6

	BSunny	FSunny	BSunny	FSunny	BSunny	FSunny	BSunny	FSunny	BSunny	FSunny	**Sun Overall**	Bsun	Fsun
D2	3	4	4	1	4	6	3	6	5	6	**4.2**	3.8	4.6
Daybreak	3	6	1	4	3	1	5	3	6	4	**3.6**	3.6	3.6
H2Orange	3	5	6	5	1	6	1	6	6	1	**4**	3.4	4.6
Marble/Granite	4	1	4	3	5	5	2	4	6	5	**3.9**	4.2	3.6
Photo-flo	1	2	6	3	1	6	2	1	1	5	**2.8**	2.2	3.4
Water	6	6	2	6	2	6	3	5	6	6	**4.8**	3.8	5.8

	TotGrowth	TotGrowth	TotGrowth	TotGrowth	TotGrowth	TotGrowth	TotGrowth	TotGrowth	TotGrowth	TotGrowth	**TotOverall**	Boverall	Foverall
D2	3.5	2.5	4	1	4.5	4.5	3.5	3.5	4.5	6	**3.75**	4	3.5
Daybreak	4	6	2.5	5	3.5	1	3	4	3.5	4.5	**3.7**	3.3	4.1
H2Orange	2	5.5	5.5	4	2.5	4.5	2	6	4.5	3	**3.95**	3.3	4.6
Marble/Granite	5	3.5	3.5	3.5	4.5	5.5	3.5	3.5	4	3	**3.95**	4.1	3.8
Photo-flo	2	3.5	3.5	3.5	2	3.5	2	3.5	2	5.5	**3.1**	2.3	3.9
Water	4	5.5	2.5	5.5	1.5	4.5	3.5	4.5	4	6	**4.15**	3.1	5.2

Rankings from 1-6, ties allowable (lower numbers indicates worse performance)

Appendix H.
Biological Performance
Based on February 2007 Report

Appendix H, Biological Performance Based on Sun or Shade based on Biological Analyses February 2007

	ANC		BNC		JBNC		SFNC		SFeNC				
	BShady	FShady	BShady	FShady	BShady	FShady	BShady	FShady	BShady	FShady	**Shade Overall**	Bshade	Fshade
D2	1	4	4	2	3	1	4	2	3	1	**2.5**	3	2
Daybreak	4	1	2	4	4	2	1	3	1	4	**2.6**	2.4	2.8
Marble/Granite	3	3	3	3	2	3	2	1	4	2	**2.6**	2.8	2.4
Water	2	2	1	1	1	4	3	4	2	3	**2.3**	1.8	2.8
	BSunny	FSunny	BSunny	FSunny	BSunny	FSunny	BSunny	FSunny	BSunny	FSunny	**Sun Overall**	Bsun	Fsun
D2	3	2	1	4	2	3	3	3	3	2	**2.6**	2.4	2.8
Daybreak	4	3	4	2	4	2	4	4	4	4	**3.5**	4	3
Marble/Granite	2	1	4	1	1	4	1	3	1	1	**1.9**	1.8	2
Water	1	4	4	3	3	1	2	3	2	3	**2.6**	2.4	2.8
	TotGrowth	TotGrowth	TotGrowth	TotGrowth	TotGrowth	TotGrowth	TotGrowth	TotGrowth	TotGrowth	TotGrowth	**TotOverall**	Boverall	Foverall
D2	2	3	2.5	3	2.5	2	3.5	2.5	3	1.5	**2.55**	2.7	2.4
Daybreak	4	2	3	3	4	2	2.5	3.5	2.5	4	**3.05**	3.2	2.9
Marble/Granite	2.5	2	3.5	2	1.5	3.5	1.5	2	2.5	1.5	**2.25**	2.3	2.2
Water	1.5	3	2.5	2	2	2.5	2.5	3.5	2	3	**2.45**	2.1	2.8

Rankings from 1-4, ties allowable (lower numbers indicates worse performance)

Appendix I.
Cost Estimates, Four Options for Continuing the Study Two Years

Budget Option A, Four Cemeteries, Two Annual Field Trips

Salaries & Benefits	Quantity	Unit	Rate	Cost
Jason Church	360	hr	$ 29.05	$ 10,458.00
Mary Striegel	80	hr	$ 59.50	$ 4,760.00
Travel				
R/t Airfare	2	trips	$ 1,200.00	$ 2,400.00
Per Deim	2	trips	$ 1,583.00	$ 3,166.00
Mitchell Lab				
Biological analysis	128	samples	$ 250.00	$ 32,000.00
				$ 52,784.00

Budget Option B, Jefferson Barracks & Alexandria, Two Field Trips

Salaries & Benefits	Quantity	Unit	Rate	Cost
Jason Church	280	hr	$ 29.05	$ 8,134.00
Mary Striegel	80	hr	$ 59.50	$ 4,760.00
Travel				
R/t Airfare	2	trips	$ 800.00	$ 1,600.00
Per Deim	2	trips	$ 545.00	$ 1,090.00
Mitchell Lab				
Biological analysis	64	samples	$ 250.00	$ 16,000.00
				$ 31,584.00

Budget Option C, Alexandria, Four Field Trips, includes Bio-Activity

Salaries & Benefits	Quantity	Unit	Rate	Cost
Jason Church	120	hr	$ 29.05	$ 3,486.00
Mary Striegel	40	hr	$ 59.50	$ 2,380.00
Travel				
R/t Car Fare	4	trips	$ 50.00	$ 200.00
Per Deim	0	day	$ -	$ -
Mitchell Lab				
Biological analysis	32	samples	$ 250.00	$ 8,000.00
				$ 14,066.00

Budget Option D, Alexandria, Four Field Trips, Appearance Only					
Salaries & Benefits	Quantity	Unit	Rate		**Cost**
Jason Church	120	hr	$ 29.05	$	3,486.00
Mary Striegel	40	hr	$ 59.50	$	2,380.00
Travel					
R/t Care Fare	4	trips	$ 50.00	$	200.00
Per Deim		day	$ -	$	-
Mitchell Lab					
Biological analysis		samples		$	-
				$	6,066.00