GRANT TERMS AND CONDITIONS

GRANT NUMBER: MT-2210-07-NC-07
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Narrative Final Report (Attachment C)
NCPTT 2007 Grants
Title Page

Grant Number: MT-2210-07-NC-07

Project title: Microbial Detoxification of Mercury Contaminated Museum Collections: Effect of Material Composition on Mercury Removal

Institution/Organization: Regents of the Univ. of Colorado, University of Colorado Denver

Principal investigator: Dr. Timberley Roane

Project team:

Principal investigator contact information:
Department of Biology, Campus Box 171
P.O. Box 173364
University of Colorado
Denver, CO 80217-3364

Phone: (303) 556-6592
Fax: (303) 556-4352
Email: Timberley.Roane@ucdenver.edu

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Executive Summary
Arsenic and mercury, the most persistent of museum pesticides, continue to pose health risks. With the enactment of NAGPRA (Native American Graves Protection and Repatriation Act) in 1990, there was concern about exposure of tribal peoples and museum personnel to these pesticides. Under NAGPRA, as tribes seek the return of their artifacts the risk of exposure increases. Linked with illnesses including kidney damage and neurological disorders, the removal and detoxification of arsenic and mercury are imperative. As artifacts are returned to tribes under NAGPRA, artifacts are often put back into cultural practice. Consequently, there is an urgent need for an effective, culturally-sensitive, less or non-destructive method for mitigating metal toxicity on artifacts. The goal of this research funded by the NCPTT program was to develop a microbiological technology for the removal of mercury, in particular, from artifacts. Mercury was chosen as an initial pesticide of study due to our understanding of microbial mercury detoxification. The widespread distribution of mercury-treated artifacts makes this research timely and will provide the methodological basis for the microbial removal of arsenic and other museum-associated pesticides in future studies.

Some microorganisms are known to be metal-resistant and can reduce toxic metal concentrations by turning the metal into a gaseous form that can be safely collected and properly disposed of. Mercury-volatilizing bacteria can be used to reduce the concentration of mercury associated with a particular environment, or in this case, material. Given that many of the traditional methods for mercury-mitigation from museum artifacts are potentially destructive, the overall goal of this research was to determine if mercury-volatilizing bacteria can be used as a less destructive, culturally sensitive approach to metal mitigation on sensitive museum collections, especially those earmarked for repatriation.

With 2004 NCPTT funding, sixteen mercury-resistant bacteria associated with mercury-treated cultural collections at the Arizona State Museum were collected and isolated. Representing common bacterial species resistant to parts per billion to parts per million mercuric chloride (HgCl₂), several isolates converted dissolved and solid HgCl₂ into gaseous mercury. Arthrobacter sp. 2604, collected from a mercury-treated native leather bag, removed up to 20% of the mercury (from a 10 ppm Hg starting concentration) from solid agar (gelatin) and paper within 10 days. Agar, as a porous gelatinous material, required the bacteria to remove mercury from a large solid volume, while the paper provided a complex surface for removal. Mercury removal occurred when 10⁶ cells/mL of Arthrobacter sp. 2604 were surface applied in a dilute nutrient solution enhancing gaseous mercury conversion. A bacterium from a metal-contaminated soil, Cupriavidus metallidurans CH34, removed up to 60% of the mercury associated with agar and paper within 10 days. Initially, used as an experimental control, the experiments discussed here conducted with the 2007 NCPTT funding were conducted with C. metallidurans CH34 only.

Upon the design of a bacterial: mercury treatment chamber (see Figure 1), standardized mercury removal using the mercury-resistant bacterium Cupriavidus metallidurans CH34 was attempted with three material types: paper, hair, and cloth. This work has shown that the direct application of 10⁴ - 10⁶ cells/mL of C. metallidurans CH34, to mercury-treated paper was able to remove up to 28% of the mercury under 25% humidity, 6% of the mercury under 60% humidity, and 0% of the mercury under 76% humidity within 10 days at 28°C (from a starting mercury concentration of 10 ppm). Removal of mercury from treated hair samples was also attempted; however, an inconsistent absorption of mercury by the hair samples made treatment comparisons difficult. Further work with hair, as a representative museum material, has been temporarily suspended to allow for troubleshooting. Experimentation then shifted to removal of mercury from untreated cotton cloth material. From cloth, preliminary data indicated a maximum removal of 11% mercury at 60% humidity and 17% mercury at 25% humidity. We
hope to continue this study to further develop the conditions for optimized removal of mercury from these and other material types, including other hair sources, cloth materials, and, a new material type for this study, wood.

**Introduction**

Museums throughout the U.S. and Canada have mercury- and arsenic-contaminated materials in their collections. Original collectors frequently applied arsenic- and mercury-based salts to items for preservation purposes, protecting them primarily from insect and rodent damage. Treatment with such metal-based salts also reduced microbial activity, thereby further reducing material degradation and deterioration. These materials, some more than a hundred years old, are now housed in museums and are still contaminated. Their contamination is extensive enough to be toxic and pose health risks to those who handle the items. While the practice of using arsenic and mercuric salts ceased in the 1970s, the passing of the Native American Graves Protection and Repatriation Act (NAGPRA) in 1990 has resulted in active pursuit to understanding the extent of the problem, the true risks associated, and remediation efforts to mitigate those risks. NAGPRA requires the return, upon request, of cultural items to their native owners. Tribes across the U.S. actively seek the return of their artifacts, such as headdresses, ceremonial masks, pipes and clothing. Less chemically intensive methods of preservation are now in use, including freezing and treatments with insect pheromones.

Current proposed remediation practices include material washing, scrapping, heating, and cross-linking the metal to the material via UV light exposure. While successful under some circumstances, these approaches are considered too harsh or too aggressive for some materials. Additionally, some native communities are uncomfortable with these practices being used on their artifacts. The research proposed here used a naturally-occurring mercury-removing bacterium as an alternative to current practices.
Some microorganisms, such as bacteria, are known to be metal-resistant and can reduce toxic metal concentrations in a process called volatilization where the metal is converted into a gaseous form that can be collected and properly disposed of. Readily found in the environment and generally non-pathogenic, metal-volatilizing bacteria can be used to mitigate and remediate metal-contaminated soil and water systems.

Bacteria capable of converting various forms of mercury, including mercuric chloride (HgCl$_2$), into volatile, gaseous forms can be found in a variety of environments. Naturally exposed to low background levels of mercury or high anthropogenic sources of mercury, these mercury-resistant bacteria provide a unique opportunity for the removal of mercury from museums. The gaseous forms of mercury can be easily contained and properly handled for disposal. The long term goal of this research was to determine if mercury-volatilizing bacteria can be used as a less destructive, culturally sensitive approach to metal mitigation on sensitive museum collections.

To achieve this goal, C. metallidurans CH34 was used for the removal of mercury. The specific objectives for the proposed work were (1) to use bacteria in the removal of mercury from museum-simulated materials; and (2) to optimize mercury removal from different material types through environmental control. C. metallidurans CH34 was suspended in a dilute nutrient broth solution and applied directly to the surface of paper, hair, and cloth. Bacteria treated materials were incubated at 28°C at three humidities (76%, 60%, and 25%). Following incubation in the specifically-designed treatment chamber for 10 days, total remaining mercury was assessed using a microwave acid digestion technique with quantification by cold vapor atomic absorption spectroscopy (CV-AAS) mercury analyzer. Removal was determined by subtracting the remaining mercury from the starting mercury concentration.

**Materials and Methods**

The overall objective of this research was to assess the ability of the bacterium, *Cupriavidus metallidurans* CH34, to reduce the concentration of mercury associated with treated simulated museum material types.

**Material testing:** Samples of paper, hair, and cloth were dipped in solutions containing different amounts of mercuric chloride (0 and 27 ppm HgCl$_2$; 0 and 10 ppm Hg) and air dried. The dried materials were then subjected to bacterial application and incubation. The paper used was Whatman #2 filter paper. The hair samples included human hair and horse hair. The cloth was untreated cotton (cheese cloth).

**Mercury analysis:** Mercury concentrations associated with the mercury-treated materials were determined using a specially modified microwave acid digestion method (from the previous 2004 NCPTT supported work; modified from EPA Mercury Method 1631-EPA-821-R-01-013 Jan. 2001) using a Floyd, Inc. Remote Microwave System followed by analysis with a cold vapor atomic absorption spectroscopy (CV-AAS) mercury analyzer (Leeman Labs PS200II). Both bacterially-applied materials and negative controls (no bacteria added) were analyzed in this manner. Negative controls were to determine the amount of mercury lost during analysis, treatment and incubation.

**Optimization of mercury removal:** Previous research found that maintaining slightly humid, aerobic conditions during bacterial treatment were important for more efficient removal. Incubation time, temperature, humidity, cell concentration, and application method influence bacterial activity and, therefore, mercury removal. To help optimize mercury removal, humidity and cell concentration were
analyzed. Acrylic environmental treatment chambers (12”Lx12”Wx18”H) equipped with saturated salt solutions provided accurate humidity control (20-80%) and mercury containment (Figure 1). The following saturated salt solutions were used to maintain the appropriate humidity: zinc sulfate heptahydrate (Zn(SO₄)₂·7H₂O) maintained 76% humidity; magnesium nitrate hexahydrate (MgNO₃·6H₂O) maintained 60% humidity; and potassium acetate (CH₃COOK) averaged 25% humidity. During incubation, each chamber was equipped with a Supco temperature/humidity data logger for continuous monitoring of conditions. The chambers were incubated at 28°C for 10 days prior to mercury analysis. Bacterial application involved the use of a nebulizer to aerosolize a bacterial suspension of C. metallidurans CH34.

![Figure 1. Acrylic treatment chamber designed to maintain consistent environmental conditions during the bacterial removal of mercury from treated materials.](image)

Results and Discussion

**Objective 1. To use bacteria in the removal of mercury from museum-simulated materials.**

The treatment chamber designed allowed for control of humidity, through the placement of saturated salt solutions inside the chamber prior to incubation. Through the door to the chamber, treated and untreated materials were placed inside. Once closed, the chamber was sealed against air exchange with the outside environment through a rubber gasket along the edge of the door. The chambers are small enough to allow for placement inside of temperature regulated incubators, if necessary. Finally, consistent temperature and humidity levels were constantly monitored with a temperature/humidity data logger placed inside. Between experiments, chambers were disinfected with repeated rinses with 70% ethanol.

Initial material treatment experiments focused on mercury removal from treated paper samples. The maximum amount of mercury removed from paper by the remediating bacterium, *Cupriavidus metallidurans* CH34, was 28% at 25% humidity (Figure 4a and b). Other types of materials examined included hair and cloth. Unfortunately, experiments with hair samples had to be suspended due to difficulties encountered in experimental set-up. Two types of hair were initially examined: horse and human. Both hair types, however, inconsistently absorbed the mercury solution used to amend the hair, e.g., a 10 ppm Hg solution. Upon consultation with a professional stylist, hair texture can vary from root to end, as can dryness, pH, and porosity. Each of these variables can influence the amount of chemical absorbed from solution. To support our hypothesis, consistent mercury application is required in order to show repeatable mercury removal. The preliminary removal from hair is reported in Figure 2; however, some inconsistencies in the results are of concern and need to be addressed.
Figure 2. Reduction of mercury concentrations associated with human hair by the bacterium, *Cupriavidus metallidurans* CH34, upon a 10 day incubation at 28°C under varying humidities.

Several solutions were attempted to resolve the inconsistent absorption of mercury by the hair samples. Attempts were made to obtain the same hair type, e.g., obtaining high amounts of hair from the same individual. The mercury amendment solution was changed to a gel in an effort to “coat” the hair with mercury. To determine if this was going to be a viable approach, the color indicator, methylene blue, was used. A gelatinous matrix was made by suspending concentrations of methylene blue in alcohol-based hand sanitizer. The hair was then dipped in the matrix, allowed to “soak” for 10 min., and then removed and allowed to air dry. Upon drying and evaporation of the alcohol, the gel dried on the hair surface. The methylene blue provided a visual determination of application evenness and consistency (Figure 3).

Figure 3. Examples of methylene blue treated hair in an attempt to ensure even, consistent application of the gelatinous matrix.

Current work is continuing to assess evenness of application. New experiments will be conducted with a mercury-containing gel matrix, and assessed analytically for consistent mercury concentrations. Once this is established, experiments analyzing bacterial removal of the mercury will resume.
Concurrent with the methylene blue experiments, remediation experiments with cloth were being conducted. Only one cloth type has been examined so far, that is untreated cotton (cheesecloth). Cotton was chosen for its ease of acquisition and ability to standardize (e.g., the purchase of scientific grade cheesecloth). From cloth, *C. metallidurans* CH34 was able to remove up to 17% mercury at 25% humidity (Figure 4a and b). Less removal, 11% and 0% mercury, occurred at 60% and 76% humidity, respectively.

**Objective 2. To optimize mercury removal from different material types through environmental control.**

In the mercury removal studies with *C. metallidurans* CH34, the bacterium was able to remove differing amounts of mercury under different humidity conditions (Figure 4a and b). Humidity control was achieved through the placement of saturated salt solutions within the treatment chambers (Figure 5). Within a contained environment, saturated salt solutions will establish a known, constant humidity. For this work, saturated solutions of zinc sulfate heptahydrate (Zn(SO$_4$)$_2$·7H$_2$O) maintained 76% humidity; magnesium nitrate hexahydrate (MgNO$_3$·6H$_2$O) maintained 60% humidity; and potassium acetate (CH$_3$COOK) averaged 25% humidity. Temperature effects were not investigated in this work (all
experiments were conducted at 28°C; however, the treatment chambers were designed in such a way that they can easily be placed in a temperature controlled incubator for temperature modification.

Figure 5. Use of saturated salt solutions and the resulting humidities established when used inside the acrylic treatment chambers.

The specific method of bacterial application required some experimentation. Several methods were initially examined, including wet application of bacterial suspensions (direct application of a known volume of a bacterial culture), micro-droplet application via sonication and via a nebulizer. Direct application of a bacterial suspension resulted in too much wetting of the material. The ultrasonic applicator method resulted in killing the bacteria during the application process due to the intense sonic vibrations generated. Nebulizer application (through the vertical installation of the nebulizer at the top of the chamber) appeared to be the most effective method of bacterial application, in terms of not wetting the material, achieving an even coat of bacteria, maintaining bacterial viability, and ease of use within the bacterial application chamber.

Through the use of the nebulizer, applied cell concentrations were determined throughout the chamber by placing glass slides in a grid pattern, applying bacteria via the nebulizer, and then collecting and counting the number of bacterial cells using the total microscopic cell count method of Hobbie et al. (1977). Using this approach, bacterial application throughout the chamber was even and consistent, averaging $10^4 - 10^5$ cells/cm$^2$ onto the surface of the material. Future experiments will identify the optimal cell concentration for inoculation of materials for treatment.

Work to date demonstrates a potential for the use of bacteria in the removal of pesticides from sensitive materials. Not only did removal occur, but the degree of removal was directly influenced by the humidity level. For this work, 25% humidity was optimal for both paper and cloth treatment. There is much work that remains, including continuing with the remediation of materials, e.g., hair, other cloth types, wood, etc.; evaluation of material changes upon bacterial removal of mercury; and removal of bacteria upon the conclusion of treatment. We look forward to advancing this work, and further developing an alternative to current treatment methods.
Conclusions
The use of microorganisms in the remediation or restoration of artwork is gaining attention. Cappitelli et al. (2006) propose the use of sulfate-reducing bacteria in the removal of black crusts on stonework. Restoration of frescos using direct application of Pseudomonas stutzeri A29 to dissolve interfering adhesive animal glue was examined by Ranalli et al. (2005). Other scientists are examining the microbial composition of Paleolithic paintings (Schabereiter-Gurtner et al., 2004). Finally, Ramirez et al. (2005) calls for more research on the use of biotechnology for the preservation and restoration of cultural heritage. The research here addresses a novel use for microorganisms in the removal of toxic mercury from cultural and other collections in museums. We look forward to continuing to refine and define the treatment method so as to begin remediating actual museum materials. As more cultural artifacts are earmarked for repatriation, the detoxification of mercury and other associated pesticides is imperative to the health of individuals and to maintaining our cultural heritage.

Acknowledgements
a) We would first and foremost like to thank the National Center for Preservation Technology and Training for supporting this work. The support from NCPPT has led to the development of a potentially new methodology in the restoration of culturally-sensitive museum collections.
b) We would like to acknowledge the participation of the Arizona State Museum in providing access to their collections. As a forerunner in the issue of pesticide mitigation in museums, Nancy Odegaard, Head Conservator, and the Arizona State Museum were instrumental in the success of this research. We look forward to continued collaborations with the Museum.
c) We would like to thank Mr. Jeff Boon of the Shared Analytical Services Laboratory at the University of Colorado Denver for his analytical expertise and support for the project.
d) We would like to thank Dr. Mary Striegel, Environmental and Materials Research Program Director, for her support and enthusiasm for this work.

References


Abstract:
Bacteria capable of detoxifying and, in some cases, sequestering metals are being investigated in the remediation of contaminated environments such as soil and water and, in this project, the removal of mercury from museum type materials. Mercury on such materials poses a unique remediation challenge because it forms non-degradable, persistent chemicals. Because mercury-resistant bacteria have the ability to convert mercury into a gaseous form, they may facilitate mercury removal. In the work presented here, a diverse bacterial community was isolated from mercury-treated items; two of the non-pathogenic bacterial isolates were capable of reducing 10 ppm mercury concentrations. One, *Arthrobacter* sp. 2604, reduced the mercury associated with a gelatin medium by 30% and a paper matrix by 20% within 7 days at 28°C. Another, *Cupriavidus metallidurans* CH34, reduced up to 50% and 60%, respectively. Current work is optimizing the conditions for bacterial mercury removal including the nutritional requirements and appropriate food sources for bacteria during the remediation process.
Presentation given at the annual Eastern Analytical Symposium and Exposition, November 2007

The Use of Bacteria in Mitigating Collection Associated Mercury
L.J. Snelling and T.M. Roane
Department of Biology, University of Colorado Denver

Abstract:
Mitigation technologies are being developed for the remediation of mercury-treated museum collections. While effective pesticides, mercury-based chemicals pose health risks and are toxic to all biological systems. However, bacteria capable of removing mercury from contaminated systems have been identified from a variety of habitats, including contaminated soils and waters. In this study, such mercury-remediating bacteria are being investigated in the removal of mercury from museum type materials. Mercury-treated museum materials pose a unique remediation challenge due to the persistence of the mercury, the complex nature of the materials involved, and the need for preservation of a given artifact. Because mercury-remediating bacteria have the ability to convert mercury into a gaseous form, they may facilitate mercury removal from a variety of substrates. The research proposed here represents a novel, microbiologically-based mercury mitigation method for the remediation of contaminated museum collections. Results to date show a diverse mercury-tolerant bacterial community already present on several mercury-treated museum artifacts. Bacteria isolated from the surfaces of native artifacts were resistant up to mg/L levels of mercury. Two bacterial isolates, *Arthrobacter* sp. 2604 and *Cupriavidus metallidurans* CH34, were resistant up to 50 mg/L and 10 mg/L mercury, respectively. Additionally, within 10 days at 28°C, these isolates were able to remove from 20-60% of the mercury associated with three substrate types: an aqueous solution, a solid gelatin medium, and a paper matrix. Current work is optimizing the conditions for bacterial mercury removal including the method of application of the bacteria to a material surface and the environmental parameters needed during the remediation process.

Presentation given at the Rocky Mountain American Society for Microbiology annual meeting, April 2008

Bacterial Conversion of Mercury in the Remediation of Museum Materials
M.H. Albuthi and T.M. Roane
Department of Biology, University of Colorado Denver

Abstract
The Native American Graves Protection and Repatriation Act (NAGPRA) of 1990 has created a demand for a process to remediate mercury-contaminated cultural collections that is culturally—sensitive and minimally destructive. The objective of this research was to use mercury-resistant bacteria to remove mercury from materials in a long-term effort to mitigate the toxicity associated with some museum collections. Results showed that two isolates were able to successfully convert mercuric chloride into gaseous mercury. *Arthrobacter* sp. 2604, collected from a mercury-treated leather bag, removed up to 20% of the mercury (from a 13.5ppm HgCl₂ starting concentration) from solid agar and paper within 10 days. Additionally, a bacterium from a metal-contaminated soil, *Cupriavidus metallidurans* CH34, removed up to 60% of the mercury associated with agar and paper within 10 days. Optimization of the removal by *C. metallidurans* CH34 under varying environmental conditions, such as humidity, was examined. Saturated salt solutions maintained specific humidities within a closed treatment chamber. After 7 days, zinc sulfate heptahydrate resulted in an average humidity of 76%, magnesium nitrate
hexahydrate averaged 60% humidity and potassium acetate averaged 25% humidity. When *C. metallidurans* CH34 was applied to filter paper treated with 13.5 ppm HgCl₂, incubation at 28°C at each of the humidities resulted in mercury removal. Following incubation, the papers were digested using a modified microwave acid digestion method followed by mercury analysis using cold vapor atomic absorption spectroscopy. Results indicated that within the closed chamber, *C. metallidurans* CH34 was able to remove 73% of the mercury at 76% humidity, 85% of the mercury at 60% humidity, and 79% of the mercury at 25% humidity within a 10 day period. This work will lead to a protocol for the treatment of mercury-impacted museum collections.

**Presentation given at the American Society for Microbiology annual meeting, June 2008**

Optimizing the Bacterial Remediation of Mercury-Treated Museum Collections  
M.H. Albuthi and T.M. Roane  
Department of Biology, University of Colorado Denver

Abstract:  
The Native American Graves Protection and Repatriation Act (NAGPRA) of 1990 has created a demand for a process to remediate mercury-contaminated cultural collections that is culturally-sensitive and minimally destructive. The objective of this research was to use mercury-resistant bacteria to remove mercury from materials in a long-term effort to mitigate the toxicity associated with some museum collections. Results showed that two isolates were able to successfully convert mercuric chloride into gaseous mercury. *Arthrobacter* sp. 2604, collected from a mercury-treated leather bag, removed up to 20% of the mercury (from a 13.5ppm HgCl₂ starting concentration) from solid agar and paper within 10 days. Additionally, a bacterium from a metal-contaminated soil, *Cupriavidus metallidurans* CH34, removed up to 60% of the mercury associated with agar and paper within 10 days. Optimization of the removal by *C. metallidurans* CH34 under varying environmental conditions, such as humidity, was examined. Saturated salt solutions maintained specific humidities within a closed treatment chamber. After 7 days, zinc sulfate heptahydrate resulted in an average humidity of 76%, magnesium nitrate hexahydrate averaged 60% humidity and potassium acetate averaged 25% humidity. When *C. metallidurans* CH34 was applied to filter paper treated with 13.5 ppm HgCl₂, incubation at 28°C at each of the humidities resulted in mercury removal. Following incubation, the papers were digested using a modified microwave acid digestion method followed by mercury analysis using cold vapor atomic absorption spectroscopy. Results indicated that within the closed chamber, *C. metallidurans* CH34 was able to remove 73% of the mercury at 76% humidity, 85% of the mercury at 60% humidity, and 79% of the mercury at 25% humidity within a 10 day period. This work will lead to a protocol for the treatment of mercury-impacted museum collections.

**Presentation given at the American Society for Microbiology annual meeting, May 2009**

Bacterial Remediation of Mercury-Treated Museum Materials  
M.H. Lantz and T.M. Roane  
Department of Biology, University of Colorado Denver

Abstract:  
In 1990 the Native American Graves Protection and Repatriation Act (NAGPRA) was passed requiring that Native American cultural items be returned to their respective tribes. Historically, cultural collections were treated with mercury-based salts as a preservative against biological damage. Currently these mercury-contaminated collections require the application of a culturally-sensitive and
minimally destructive process to remediate the mercury prior to repatriation. The objective of this research is to use mercury-resistant bacteria to remove mercury from various materials used to simulate the materials found in the cultural items. Preliminary results identified two bacterial isolates capable of transforming dissolved and precipitated mercuric chloride into gaseous mercury. Paper and hair samples were amended with 13.5 ppm HgCl₂ and air dried prior to the application of a bacterial aerosol and incubated under specific humidities within a closed chamber. Zinc sulfate heptahydrate was used to maintain an average humidity of 76%, magnesium nitrate hexahydrate averaged 60% humidity and potassium acetate averaged 25% humidity. When Cupriavidus metallidurans CH34 was applied to paper treated with 13.5 ppm HgCl₂, incubation at 28°C at each of the humidities resulted in mercury removal. Results indicated that when compared to negative controls, C. metallidurans CH34 was able to remove 24% of the mercury at 76% humidity, 9% at 60% humidity, and 34% at 25% humidity within a 10 day period. Likewise, when C. metallidurans CH34 was applied to human hair samples treated with 13.5 ppm HgCl₂ and incubated at each of the humidities the isolate was able to remove 40% of the mercury at 76% humidity and 28% of the mercury at 60% humidity. Arthrobacter sp. 2604 removed up to 12% of a 13.5 ppm HgCl₂ starting concentration from treated paper within 10 days while incubated at 60% humidity. The removal of mercury by these isolates demonstrates the potential for bacterially-facilitated mercury removal from cultural collections.
Appendix A
Press releases

University of Colorado at Denver and Health Sciences Center
Downtown Denver Campus

NEWS RELEASE

Contact:
Timberley Roane, Associate Professor: 303-556-6592
Katy Brown, Marketing Coordinator, College of Liberal Arts & Sciences: 303-556-6663

UCDHSC Research Shows Progress in Helping to Safely Restore Native Artifacts

July 6, 2007 – Efforts to conserve artifacts of ancient life have been undertaken by many well-meaning conservationists throughout human history. But some of the methods and materials used by early conservationists have been since determined to cause additional damage and make exposure to such items hazardous to humans.

University of Colorado at Denver and Health Sciences Center (UCDHSC) Associate Professor of Biology Timberley Roane has been working on ways to safely remove harmful chemicals from artifacts under a grant from the National Center for Preservation Technology and Training (NCPTT), an office of the National Park Service.

“Early methods of preserving many native artifacts, such as headdresses, pipes, blankets and ceremonial masks, relied heavily on the use of pesticides,” says Roane. “Two common ingredients in those pesticides were mercury and arsenic. Concentrations of those chemicals now make it risky for humans to come into contact with the artifacts.”

Roane, who is of Lumbee descent, discussed the idea with a Navajo colleague, who works on environmental issues, and came up with the use of bacteria as a possible means to extract mercury from artifacts without damaging them. Roane has found 12 types of bacteria that are able to grow in high concentrations of toxic mercury with two bacteria capable of removing approximately 20-30% and 40-60%, respectively, of the mercury from a surface within 10 days. Due to the presence of mercury, the risk of skin exposure or inhalation makes it dangerous for artifacts to be handled and used in their historic and cultural contexts when items are repatriated. “These bacteria may be the key to helping return artifacts to the people who created them, and to return them without endangering individuals coming in contact with the items,” says Roane.

The NCPTT has awarded Roane a second round of funding, enabling her to continue the work through optimization of the mercury removal process.
Other proposed methods for removing toxic materials include using chemicals or ultraviolet light and heat. But such techniques could damage the items. Roane’s approach is to use bacteria to change the mercury into a gaseous form which then can be disposed of safely. In other work, Roane uses a similar approach to manage environmental cleanup with naturally occurring bacteria.

When Roane began her work with native artifacts, not much was known about contamination levels. But through the renewable grant, she was able to begin her work with the Native American collections at the Arizona State Museum. “It is very important to handle the items with great care because they are considered to be living by the tribes from which they came,” says Roane. “So we believe this research offers hope to ensure their continued legacies.”

The University of Colorado at Denver and Health Sciences Center is Colorado’s premier urban university offering more than 100 degrees and programs in 12 schools and colleges and serving more than 27,000 students in Metro Denver and online.

Article released in the University of Colorado Denver Network Newsletter

Roane’s research a step closer to reuniting artifacts with tribes
January 12, 2009

The Native American repatriation act has reunited many tribes with sacred artifact that had previously been relegated to museum. However, much remains to be done and toxic preservation methods have hindered the process.

That’s where Timberley Roane, associate professor of biology, and her students come in. “It’s a very complex problem for everyone involved; for scientists, for tribes, and for museum curators,” Roane explains. “This is new for everyone.”

Since the Native American Graves Protection and Repatriation Act passed in 1990, many Native American artifacts have been returned to the American Indian tribes to which they belong. However, as an early method of preservation, many of these objects were treated with harmful pesticides such as mercury and arsenic. Museums are working to remove the toxic chemicals before allowing the artifacts to be returned to use.

Roane is using her expertise in environmental microbiology to discover bacterium able to remove the chemicals by reducing mercury ions to an elementary form, which then diffuse away.

Most recently, Roane has narrowed in on two specific bacterium that show positive results. One bacterium, isolated from zinc mine tailings, has proven to remove 60-80 percent of the mercury. She has also identified 12 other potential bacteria that have not yet been isolated for use.

In the second year of research, Roane is currently testing materials such as human and horse hair, and is looking for chemical-free cloths to simulate artifact materials. She is also hoping to receive museum donations of materials and artifacts that are broken or can no longer be used in museums or tribal rituals.
To properly treat the artifacts, Roane must try to understand the complex problems surrounding the contamination. What is the artifact used for and how often is it used? What is the exposure of contamination to humans? How do we quantify the amount of mercury on the object when we don’t know its history? If we do find a solution, how do we make sure it is equally effective on different materials and that it doesn’t degrade any materials in the process?

Although her Native American background wasn’t the initial determining factor in her research — which has always been concentrated in environmental clean-up — it does give her certain sensitivity to the problem. “It allows me to better understand the issues and better communicate with the tribes.”

Once a real solution is developed, the most realistic way of detoxifying these artifacts would be to install some type of control centers where tribal members could participate in the cleanup process. “With control and purity in technique, anyone can do this work,” says Roane.

Roane is working on funding once the second year of research ends. “I’m really excited about where the research is going; we’re making some great progress.”
Appendix B
Interim Report

GRANT TERMS AND CONDITIONS

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Interim Report (Attachment A)
NCPTT 2007 Grants

1. **Institution/Organization:** University of Colorado Denver

2. **Project title:** Microbial Detoxification of Mercury Contaminated Museum Collections: Effect of Material Composition on Mercury Removal

3. **Grant number:** MT-2210-07-NC-07

4. **Summarize requested amendments (if any) to the original Grant Agreement or Work Cost/Budget and provide the approval date(s).**

   None

5. **Briefly describe progress to date for completing the project objectives as outlined in the Grant Agreement. Address each objective and associated task(s).**

   **Research Objective 1.** To use bacteria in the removal of mercury from museum-simulated materials.

   **Work to date:** Bacterial isolates *Arthrobacter* sp. 2604 and *Cupriavidus metallidurans* CH34 (from NCPTT grant MT-2210-04-NC-08, project 1) continue to be investigated for their abilities to remove mercuric salts from simulated museum materials. In project 1, mercury-treated broth, agar and paper were analyzed upon bacterial application. In project 2 (the proposed work here), the ability of the isolates to remove mercury from other material types is being examined. To date, different hair types have been examined, including human hair and horse hair in addition to the re-evaluation of paper. Preliminary results so far indicated the continued ability of both *Arthrobacter* sp. 2604 and *Cupriavidus metallidurans* CH34 to remove mercury from paper and two different hair types within a 10 day incubation period. Both bacterial isolates were able to convert 10 ppm of mercuric chloride to gaseous mercury, successfully reducing the concentration of mercury associated with the materials by up to 80%.

   In order to address Objectives 1 and 2 (below) a treatment chamber was designed in order to provide containment and the ability to control environmental conditions during bacterial treatment. The chamber (pictured below) consists of an acrylic 12 in. by 12 in. by 12 in. clear box equipped with a gasket sealed door for material placement and manipulation. Within the chamber environmental parameters, such as temperature and humidity can be controlled. An additional bacterial application chamber has been designed. This chamber is equipped with the necessary components to allow for consistent application of bacteria to the surface of the material to be treated.
Acrylic treatment chamber designed to maintain consistent environmental conditions during the bacterial removal of mercury from treated materials.

The specific method of bacterial application required some experimentation. Several methods were initially examined, including wet application of bacterial suspensions (direct application of a known volume of a bacterial culture), micro-droplet application via sonication and via a nebulizer. Direct application of a bacterial suspension resulted in too much wetting of the material. The ultrasonic applicator method resulted in killing the bacteria during the application process due to the intense sonic vibrations generated. Nebulizer application appeared to be the most effective method of bacterial application, in terms of not wetting the material, achieving an even coat of bacteria, maintaining bacterial viability, and ease of use within the bacterial application chamber.

Research Objective 2. To optimize mercury removal from different material types through environmental control.

Work to date: In mercury removal studies with *Cupriavidus metallidurans* CH34 where environmental parameters were tightly controlled, *C. metallidurans* CH34 was able to remove up to 75% of the mercury under 76% humidity, 85% of the mercury under 60% humidity, and 79% of the mercury under 25% humidity within 10 days at 28°C. Humidity control was achieved through the placement of saturated salt solutions within the treatment chambers. Within a contained environment, saturated salt solutions will establish known, constant humidity. For this project, saturated solutions of zinc sulfate heptahydrate maintained 76% humidity; magnesium nitrate hexahydrate maintained 60% humidity; and potassium acetate averaged 25% humidity. Temperature control will be achieved by placing the chambers inside of temperature-controlled incubators. The incubators are in place and we are ready to proceed to temperature optimization of the mercury removal.

6. What difficulties have you encountered to date in completing the grant work?

The use of mini-humidifiers was initially proposed for use in controlling humidity for the project. However, upon further investigation of the humidifiers, they were determined to be too bulky and high maintenance for use in this work. A simpler alternative was sought. Consultation with the Laboratory Manager in the Shared Analytical Services Laboratory at the University of Colorado Denver revealed the
potential use of saturated salt solutions to control humidity, an established method in analytical chemistry. This approach turned out to be very successful and is currently being used. However, establishing the saturated salts protocol took longer than expected and delayed the start of the mercury work.

Another challenge has been encountered in obtaining non-chemically treated wool. Commercially available wool (to represent a cloth material) is treated with a variety of preservatives and dyes. The contact information for local sheep herders has been obtained in hopes of being able to purchase untreated wool.

7. What changes in objectives or budget or products are anticipated?

Pending continuation of funding until December 31, 2008, I do not anticipate any objective changes. Pending a June 30, 2008, end date, I do not expect to be able to complete all of the material testing and environmental optimization necessary to fully address the project objectives. No change in products is anticipated.

8. Will you be able to complete work under this grant as scheduled? If not, why?

As a result of a longer than expected method development phase (development of the treatment chambers, bacterial application method, and humidity control), the proposed objectives will not be fully realized by June 30, 2008. However, pending continuation of the funding until December 31, 2008, I do anticipate successful completion of the project.

9. What products (if any) have been produced to date?

A press release titled “UCDHS Research Shows Progress in Helping to Safely Restore Native Artifacts” was released on July 6, 2007.

One peer-reviewed proceedings paper is currently in press on this project. Titled “Bacterial Removal of Mercury from Museum Materials: A New Remediation Technology?” (in the Proceedings of the Smithsonian Institution: Mitigation of Pesticides on Museum Collections April 23-24, Washington, D.C.). Authored by T.M. Roane and L.J. Snelling (graduate student), this paper was initially presented as part of an invited symposium at the Smithsonian Institution on materials conservation.

A regional poster presentation on the project was given at the Rocky Mountain American Society for Microbiology conference in Denver, CO, in April 2008. The poster was titled “Bacterial conversion of mercury in the remediation of museum materials” by M.H. Albuthi (graduate student) and T.M. Roane.

10. What products (if any) are currently underway?

Our abstract titled “Optimizing the Bacterial Remediation of Mercury-Treated Museum Collections” for this project was recently accepted for presentation at the American Society for Microbiology national conference to be held June 1-5, 2008, in Boston, MA. This national forum will allow us to present our research findings to scientists, regulators, politicians, and industry representatives.
A popular press article titled “Roane’s research a step closer to reuniting artifacts with tribes” is being published in the University of Colorado Network publication available online and sent out to faculty, staff, students and alumni. The article is being published by reporters Janae Reed and Catherine Beuten.