# Water Resources Research Center
# Annual Technical Report
# FY 2005

## Introduction

Fiscal Year March 2005 - February 2006 Program Report Federal Grant Number 01-HQ-GR-0113.

Prepared by the Arizona Water Resources Research Center, the University of Arizona, Tucson, Arizona 85721.

## Research Program

# Forward and Inverse Transient Analytic Element Models of Groundwater Flow

## Basic Information

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| **Title:** | Forward and Inverse Transient Analytic Element Models of Groundwater Flow |
| **Project Number:** | 2004AZ68G |
| **Start Date:** | 9/1/2004 |
| **End Date:** | 8/31/2007 |
| **Funding Source:** | 104G |
| **Congressional District:** | 7th |
| **Research Category:** | Ground-water Flow and Transport |
| **Focus Category:** | Groundwater, Hydrology, Models |
| **Descriptors:** | Ground-water, Hydrology, Models |
| **Principal Investigators:** | Shlomo P. Neuman |

## Publication


1. Kuhlman, Kristopher L. and Shlomo P. Neuman. Recent advances in Laplace transform analytic element method (LT-AEM) theory and application to transient groundwater flow, In Computation Methods in Water Resources, Volume XVI, 2006.

**USGS-National Institutes for Water Resources Grant Program**
**Award 200AZ68G**
**Forward and Inverse Transient Analytic Element Models of Groundwater Flow**
**Shlomo P. Neuman PI**
**Progress Report**

Mr. Kris Kuhlman, a doctoral student in the department of Hydrology and Water Resources, has been working as Research Associate on this project. Much of the work described below has been accomplished by him under the supervision of the PI in consultation with our USGS co-PI, Dr. Paul A. Hsieh.

The project focuses on extending and implementing the Laplace Transform Analytic Element Method (LT-AEM) to transient groundwater flow by utilizing an analytic element approach in Laplace space and inverting the results numerically back into the time domain. Kris is presently completing the implementation of LT-AEM to point and circular elements. Part of this work entailed converting his earlier MATLAB code into standard Fortran95. This enhanced considerably the computational efficiency and flexibility as well as portability of his code. Currently, Kris has circular elements implemented in a way that allows imposing nearly any boundary condition (variable in space or time) on a circle (Type I, II, III or a matching condition between the inside and outside of the circle.) Using a specified boundary condition on the circumference of the circle, Kris can now simulate finite circular domains. This is an important breakthrough considering that the analytic element method has been traditionally limited to infinite domains.

Other innovations of equal importance include the use of Laplace space convolution (multiplication) to represent any function of time as a convolution of an instantaneous source and a function of time. This approach is similar to the use of transfer functions in systems analysis; a unit response of the system is convolved with an input function to obtain a general response. It allows us to focus solely on deriving elements with instantaneous behavior in time (which are often mathematically much simpler than the same function for other time behaviors), and still achieve very general time behavior, leveraging the properties of the Laplace transform.

Kris has derived and implemented an area flux source over a circular element, which can be used to implement non-zero initial conditions using the LT-AEM.

The above advances are detailed in a paper by Kuhlman and Neuman (2006, attached) scheduled to appear in the Proceedings of the XVI-th International Conference on Computational Methods in Water Resources (CMWR XVI). The paper will be presented orally by Kris in Copenhagen, Denmark, this coming June.

Kris has completed the mathematical development of elliptical elements in Laplace space which he is currently implementing on the computer. The elliptical elements are associated with radial and angular modified Mathieu functions as eigenfunctions, which are analogous to the modified Bessel functions and trigonometric functions that form the eigenfunctions of circular elements.

Kris is also attempting to improve the computational efficiency of his code through the implementation of more efficient iterative solvers, least squares solution techniques which utilize the orthogonality of the eigenfunctions, parallel solution for the coefficients in Laplace space using the message passing interface (MPI), and Taylor series approximations of flow elements at large distances (similar in principle but not in detail to the superblock approach of Strack).

Kris has developed much of the mathematics needed to extend the LT-AEM to three-dimensional problems using spherical, prolate spheroidal and oblate spheroidal elements. Many of the innovations described earlier in the context of circular elements generalize directly to other elements which utilize separable geometries (elliptical, ellipsoidal, etc.).

Kris has developed an adaptive particle tracking program for solute transport, which utilizes the flow velocities calculated directly using the LT-AEM, without a need to employ finite differences of computed heads.

Kris has now completed all courses required for his PhD study, enabling him to devote more time to research under this project in the future.

**References**

Kuhlman, Kristopher L. and Shlomo P. Neuman. “Recent advances in Laplace transform analytic element method (LT-AEM) theory and application to transient groundwater flow”, In *Computation Methods in Water Resources,* Volume XVI, 2006.

# Pharmaceutically Active Compounds: Fate in Sludges and Biosolids Derived from Wastewater Treatment

## Basic Information

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| **Title:** | Pharmaceutically Active Compounds: Fate in Sludges and Biosolids Derived from Wastewater Treatment |
| **Project Number:** | 2004AZ70G |
| **Start Date:** | 9/1/2004 |
| **End Date:** | 8/31/2007 |
| **Funding Source:** | 104G |
| **Congressional District:** | Arizona #7 |
| **Research Category:** | Water Quality |
| **Focus Category:** | Groundwater, Non Point Pollution, Toxic Substances |
| **Descriptors:** | Wastewater, Pharmaceuticals, Sludge |
| **Principal Investigators:** | David Matson Quanrud, Robert Arnold, Jon D Chorover, Gail Cordy, Wendell Ela |

## Publication

**Project Title:** Pharmaceutically Active Compounds: Fate in Sludges and Biosolids Derived from Wastewater Treatment

**Progress Report, May 1, 2006**
(Project Start Date: 09-01-04; project end date: 08-31-07)

**Principal Investigators:**
David Quanrud (UA)
Robert Arnold (UA)
Wendell Ela (UA)
Jon Chorover (UA)
Edward Furlong (USGS)
Gail Cordy (USGS)

**Summary of Project Activities**

The two main objectives of this project are to (1) establish reliable measurements for endocrine disrupting compounds (EDCs) as well as several pharmaceutically active compounds (PhACs) in samples derived from sludges/biosolids from selected wastewater treatment plants utilizing different sludge digestion processes and (2) examine the fate of biosolid-associated EDCs and PhACs in soils receiving land application of finished biosolids.

During the second year of the project, the following tasks were performed to satisfy project objectives:

1. A preliminary comparison of sample extraction techniques (microwave assisted extraction, MAE; accelerated solvent extraction, ASE) on solid samples obtained from the Ina Road Wastewater Pollution Control Facility indicated that ASE provided better recoveries of target analytes than did MAE. Sample extracts were analyzed for 21 PhACs and 61 wastewater compounds using liquid chromatography-mass spectroscopy (LC-MS) and gas chromatography-mass spectroscopy (GC-MS) at Edward Furlong's USGS laboratory in Denver, Colorado. A second round of extraction comparison measurements is underway using biosolid samples spiked with known amounts of several target organic wastewater compounds.

2. Liquid and sludge/biosolid samples were obtained from participating wastewater treatment plants. Extraction and analysis of samples is ongoing. Sampling points included raw and digested sludges, dewatered sludges, finished biosolids, as well as liquid phase samples of plant influent and effluent to support mass balance calculations.

3. As part of the knowledge transfer objective of the project, two PhD students (Otakuye Conroy, Sondra Teske) from the environmental engineering program at the University of Arizona (UA) traveled to Edward Furlong's USGS laboratory in April 2006 for a one-week visit to analyze samples using LC-MS and GC-MS analytical techniques for PhACs and EDCs. This was the second trip by students from UA to Dr. Furlong's laboratory.

4. Three 1-m long stainless steel soil columns were set up at UA to simulate application of biosolids to agricultural soils. The columns were packed with biosolid/soil mixtures obtained from field test plots at the University of Arizona's Marana Agricultural Center near Tucson. The field plots have received annual applications of biosolids over a twenty-year period. The laboratory columns were operated over a nine-month period and were irrigated to simulate irrigation practices in the field. A master's student in the environmental engineering program at UA conducted this work. Leachate and soil samples were obtained periodically from the columns and sent to Dr. Furlong's laboratory for analysis of PhACs and EDCs. The analytical measurements at Dr. Furlong's laboratory were conducted in part by the two visiting graduate students from UA in April 2006. The analytical measurement work is ongoing.

**Planned Activities:**

During the next year of the project, planned activities include:

1. Continued collection and analysis of samples from wastewater treatment plants, including sludge digestion processes.

2. Compilation of analytical results to support conclusions on efficacy of wastewater treatment unit processes and land application practices for attenuation of solid-bound PhACs and EDCs.

# Preliminary Evaluation of Perchlorate Contamination of Ground Water In The Lower Colorado River Region

## Basic Information

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|---|---|
| **Title:** | Preliminary Evaluation of Perchlorate Contamination of Ground Water In The Lower Colorado River Region |
| **Project Number:** | 2005AZ79B |
| **Start Date:** | 3/1/2005 |
| **End Date:** | 2/28/2006 |
| **Funding Source:** | 104B |
| **Congressional District:** | 7 |
| **Research Category:** | Water Quality |
| **Focus Category:** | Water Quality, Toxic Substances, Groundwater |
| **Descriptors:** | Perchlorate, Groundwater, Colorado River |
| **Principal Investigators:** | Charles Sanchez |

## Publication

## A. Problem and Research Objectives

Perchlorate is a chemical linked with thyroid dysfunction. Perchlorate competitively inhibits uptake of iodine by the sodium-iodide symporter (NIS) of the thyroid (Greer et al., 2002). Particularly vulnerable to perchlorate exposure are pregnant women, fetuses, newborns, and individuals suffering from hyperthyroidism (Clark, 2000; NAS 2005). Perchlorate salts are used extensively by the ordinance and rocket propulsion industries as oxidants. It has been established that contamination from these industries have been contributing to the presence of perchlorate in the lower Colorado River (Hogue, 2003). Perchlorate concentrations in the Colorado River below Lake Mead have ranged from 5 to 9 μg/L (California Department of Health Services, 2000). It has been estimated that approximately 20 million people are exposed to perchlorate through drinking water drawn from the Colorado River (Hogue, 2003).

There is also a concern that years of irrigation with perchlorate contaminated water may have contaminated the groundwater of the region. In the lower Colorado River region, over 300,000 ha of land is irrigated using Colorado River water. In the greater Yuma region of southwestern Arizona, Colorado River water is extensively used year round for irrigation.

Figure 1 shows perchlorate concentrations at Willow Beach from 1999 and concentrations at Imperial Diversion Dam since 2003. Perchlorate concentrations have ranged from 1 ug/L to 9 ug/L. More recently, they have declined due to remediation of the perchlorate plumes in the Las Vegas Wash near Henderson.

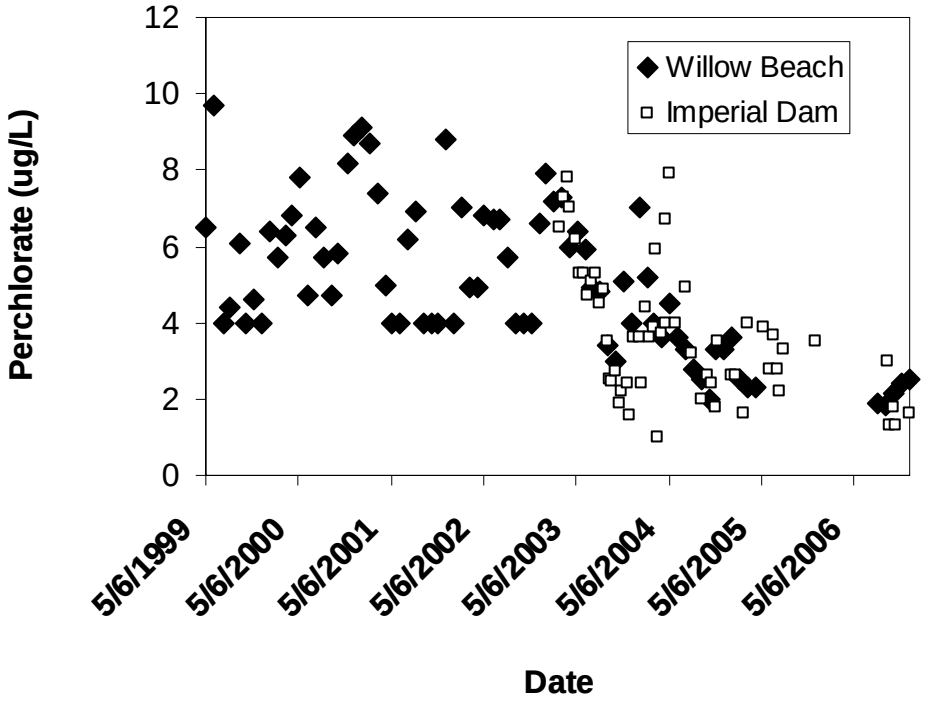
As stated earlier, years of irrigation with perchlorate contaminated Colorado River water may have contaminated the regional aquifer with perchlorate. Thus, rural communities around the greater Yuma region using groundwater as their source of potable water may be exposed to perchlorate by drinking contaminated groundwater. The objective of this survey was to determine the extent of the perchlorate contamination in ground water of lower Yuma Valley area of Arizona.

## B. Methodology

*Sampling program*

The sampling program was initially sub divided into three stages. In the first stage, all the drainage wells associated with the irrigated fields of the Yuma region were sampled. The logic behind this was that the analysis of perchlorate in the water from the active drainage pump discharges would give a direct indication of the effect of irrigation using Colorado River water on the aquifer with respect to perchlorate contamination. After completion of the first stage of sampling, the sampling program was expanded to include the public water system (PWS) wells serving the greater Yuma region. Needless to say

**Figure 1.** **Perchlorate concentrations in the Colorado River at Willow Beach and Imperial Diversion Dam. (The data collected at Willow Beach is from the Nevada Department of Environmental Protection and the data collected at Imperial Diversion Dam were determined in our laboratory).**




this was done to ascertain if the population served by the PWS of the greater Yuma region is being exposed to perchlorate. To improve the geographical distribution of well locations for the survey, the sampling program was expanded to cover rural households and commercial entities having individual wells to serve as the source of potable water. For this part of the survey, the greater Yuma region was sub divided into 6.0 $km^2$ block grids and at least one rural well was sampled per 6.0 $km^2$ block. Expanding the survey to rural households also expanded the scope of the survey to ascertain if the rural households are being exposed to perchlorate via their potable water.

Based on the results of the first three stages of the sampling program, a fourth sampling stage was added, where shallow water depth observation wells (<20.0 ft in depth) of the US Bureau of Reclamation were sampled for perchlorate. All four stages of sampling were conducted between April 2005 and September 2005.

*Sampling procedure*

After a period of flushing to ensure well stabilization indicated by constant conductivity reading for a period of 15 minutes, representative grab water samples were collected in 250 mL containers filled to within an mL of full capacity at each well sampled. Conductivity, pH, and temperature was also reported at each well sampled. At each well sampled, GPS coordinates were also noted, and the well depth was also noted based on prior available data or simply by asking the owner of the well. For domestic wells, simultaneous to sampling, an impromptu survey was also conducted to determine if the water was being used for cooking, drinking, or only for other household uses such as washing, gardening, and other domestic usage. If the water was not being used for cooking and drinking, the participants of the survey were asked as to the reason they were not using the well water as their source of cooking and drinking water. The collected samples were stored at $4^{o}$C for analysis.

*Perchlorate analysis*

These water samples were analyzed for perchlorate using a modification of EPA Method 314.0. Samples for perchlorate analysis were filtered through a 0.2-micron Gelman ion membrane syringe filter to remove particulate matter that may compromise the ion chromatograph column, followed by Dionix "On Guard II Ba", "On Guard II Ag", and "On Guard II H" syringe filters, to remove interfering anions such as chlorides, sulfates, and bicarbonates, and again through a 0.2-micron Gelman ion membrane syringe filter to remove particulate media carried over from the On Guard filters. Perchlorate analysis was performed using an ion chromatograph (Dionex 2500, Dionex Corporation, 527 Lakeside Drive, Building 5, Sunnyvale, California 94086). The Dionex 2500 contains an IP 25 isocratic pump an EG 50 eluent generator, a CD 25 conductivity detector, 2 mm AG16/AS16 guard and separation column pair, and an AMMS III suppressor. The column, suppressor, and detector were housed in a LC 30 chromatographic oven. The eluant was 50 mM KOH and the suppressant was 50 mM sulfuric acid. A 1000 µL injection loop was used and the elution time ranged from 9.5 to 10.9 minutes. An eleven-point multi-range internal calibration curve was constructed using duplicate injections over a concentration range from 0.5 to 500 µg/L (Ellington and Evans, 2000). We estimated a reporting level of 1.0 µg/L in water using the methods described above. For data analysis and data plots, any data point below 1.0 µg/L was reported as 0.0 µg/L.

## C. Principal finding and significance

*Occurrences and distribution of perchlorate in Yuma groundwater*

In the first three stages of the survey, the sampling team was able to sample eighty wells consisting of drainage, irrigation, public water supply, and household wells. The range of depth of the wells surveyed varied from 20.0 to 810.0 ft; the average depth of the wells surveyed was 193.0 ft, with the median depth being 220.5 ft. In reporting the depth of the drainage wells, an average value of 220.5 ft was reported for all drainage wells. This was done due to a lack of updating of official records reflecting the changes in the status

of the drainage well. Many of the wells in the original database were redundant, retrofitted, or new-well drilled in the close vicinity of the original well, but the records were not updated and maintained accurately. Thus, it was thought prudent to take the original database regarding the depth of the drainage wells and report the average depth of 220.5 ft (max: 339.0 ft and min: 133.0 ft; n = 16) from the original database as the depth of all drainage wells in data presentation.

Results of the perchlorate analysis of the eighty well water samples collected showed that only 29.0 % of the wells surveyed had detectable levels (≥1.0 μg/L) of perchlorate. The perchlorate concentrations seen ranged from below detection to 12.3 μg/L. The concentrations seen do not exceed the state of Arizona advisory level of perchlorate in drinking water of 14.0 μg/L (Arizona Department of Environmental Quality, 2004). Only three percent of the wells surveyed exceeded perchlorate concentrations of 4.0 μg/L, and only one percent of the wells sampled had perchlorate concentrations greater then 10.0 μg/L. The perchlorate concentration for the first 80 wells surveyed in the Yuma regional aquifer is shown in Figure 2. The perchlorate distribution profile against well depth is shown in Figure 3. There is no correlation between perchlorate concentration and depth of the well for all the wells surveyed that were greater than 20.0 ft in depth.

Based on the results of the 80 wells sampled, the sampling program was further expanded to include shallow observation wells (<20.0 ft in depth) belonging to the US Bureau of Reclamation to ascertain the perchlorate concentration profile of the Yuma aquifer below 20.0 ft. The depth of the observation wells ranged from 3.2 to 19.0 ft, with a mean depth of 8.8 ft; the median depth of the observation wells sampled was 8.2 ft. The perchlorate distribution of the 20 US Bureau of Reclamation observation wells sampled is shown in Figure 4. Of the 20 observation well water samples collected, 55.7% of the wells surveyed had detectable levels (≥1.0 μg/L) of perchlorate. The perchlorate concentrations seen ranged from below detection to 19.6 μg/L. Approximately 16.5% of the wells surveyed exceeded perchlorate concentrations of 4.0 μg/L and 2.3% of the wells sampled exceeded the state of Arizona advisory level of perchlorate in drinking water of 14.0 μg/L. None of the observation well waters exceeded the DWEL of 24.5 μg/L calculated from the reference dose (USEAP 2005) of 0.7 μg/kg per day adopted by the USEPA on recommendation from the National Academy of Science (NAS, 2005). This reference dose is based on a no-observed effect level (NOEL) of 7 μg/kg from a human perchlorate dosing study to which a 10-fold uncertainty factor was applied to address potential sensitive subpopulations (Greer et al., 2002).
The perchlorate distribution profile against well depth for the observation wells are shown in Figure 5. There seems to be no significantly discernable correlation between perchlorate concentration and depth for the observation wells sampled.

It is important to note that even with years of irrigation with Colorado River water containing perchlorate, the Yuma aquifer water is safe with respect to perchlorate concentration. None of the well waters sampled exceeded the calculated EPA DWEL of 24.5 μg/L. All wells sampled with a depth greater than 20.0 ft had perchlorate concentrations less than the state of Arizona advisory level of perchlorate in drinking

water of 14.0 μg/L. This implies that all PWS and domestic wells sampled in the Yuma region are safe with respect to perchlorate exposure and are not a public health concern.

Although within the calculated EPA DWEL of 24.5 μg/L, the presence of perchlorate in well water seems to be more prevalent in the observation wells (Note: the depth of the observation wells ranged from 3.2 to 19.0 ft, with 72.2 % of the wells surveyed having perchlorate concentrations ranging from below detection to 4.0 μg/L). The perchlorate concentration range seen in the observation wells is similar to the range of perchlorate concentrations reported for irrigation water (1.0 to 3.9 μg/L) for the year 2005 reported Figure 1**.** What this may imply is that the zone of influence of the irrigation events may be limited to the shallow depths of the aquifer. As the irrigation water enters deeper depths of the aquifer, the perchlorate concentration is possibly diluted by perchlorate-free water present in the aquifer, and, also, quite possibly, the perchlorate present is reduced by chemical and microbial action. Prior researchers have reported on the potential of chemical and microbial reduction of perchlorate (Coates et al., 1999; Coates et al., 2000; Chauduri et al., 2002). As efforts are underway in eliminating the source of contamination of perchlorate into the Colorado River (Hogue, 2003), the concentrations in the irrigation water should decrease further.

*Quality of Yuma groundwater with respect to pH, conductivity, and taste*

The field data comprised of measurements of pH, temperature, and conductivity. The pH of the well waters sampled ranged from 6.07 to 8.40, with the mean and median values of 7.41 and 7.45, respectively. The median temperature of the well waters sampled was 27.9 $^{o}$C. The specific conductivity ranged from 398 to 1831 uS/cm; the mean and median specific conductivity was 874 and 820 uS/cm, respectively. While sampling domestic wells, a common complaint of the household users was that the water was salty in taste and, therefore, they were not using the well water for drinking. The primary use of the well water was for cleaning, gardening, and cooking. Rural households were more inclined to purchase reverse osmosis treated water from water stations.

*Summary*

This report describes the preliminary investigation of perchlorate occurrence in the groundwater of the Yuma region of Arizona. There is a concern that years of irrigation with perchlorate contaminated Colorado River water may have contaminated the Yuma aquifer with perchlorate, and that communities around the greater Yuma region using groundwater as their source of potable water may be exposed to perchlorate by drinking perchlorate contaminated groundwater.

The results of the survey showed that well waters being used by PWS and rural households are well within regulatory limits of perchlorate. Detectable perchlorate concentrations are mostly limited to wells less than 20 ft in depth, and the concentration ranges seen reflect concentrations seen in the Colorado River water, which is within the calculated EPA DWEL of 24.5 μg/L.

## References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Clark, J.J. Perchlorate toxicology (Chapter 3). In Perchlorate in the Environment, E.T. Urbansky, (ed.). Kluwer/Plenum. New York, NY. **2000**.

Coates, J.D., U. Michaelidiou, S. M. O'Connor, R. A. Bruce, L. A. Achenbach. 2000. The diverse microbiology of (Per)chlorate reduction (Chapter 24). In *Perchlorate in the Environment*, E.T. Urbansky, (ed.). Kluwer/Plenum: New York, NY.

Coates, J.D., U. Michaelidou, R. A. Bruce, S. M. O'Connor, J. N. Crespi, and L. A. Achenbach. 1999. The ubiquity and diversity of dissimilatory (per)chlorate-reducing bacteria. Appl. Environ. Microbiology 65: 5234-5241.

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Hogue, C. 2003. Rocket-Fueled River: Lower Colorado carries perchlorate to millions who drink its water, to those who eat lettuce irrigated with its water, and into a tribal well. Chemical and Engineering News. August 18. 37-46.

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**Figure 2.** **The perchlorate concentration distribution for the first 80 wells surveyed in the Yuma regional aquifer.**

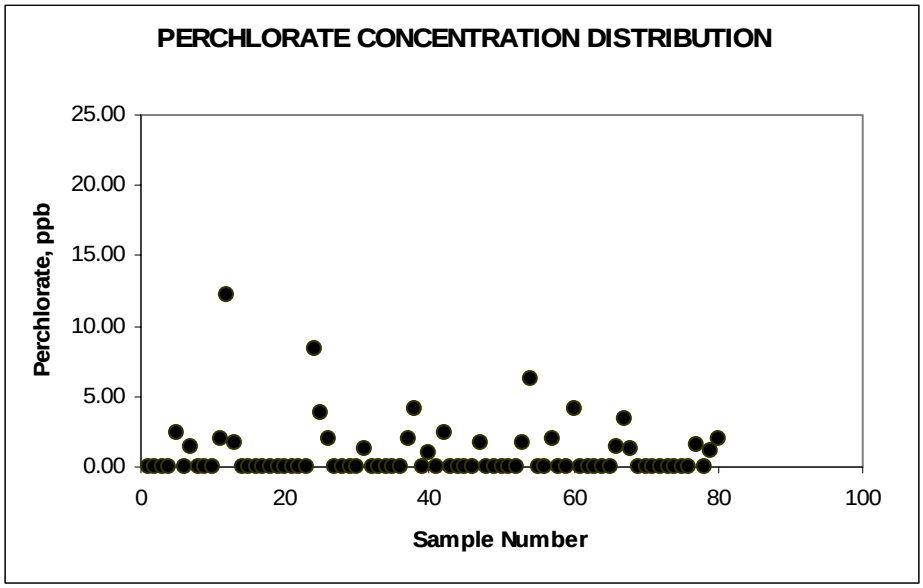



**Figure 3.** **The perchlorate distribution profile against well depth for the first 80 wells surveyed in the Yuma regional aquifer.**

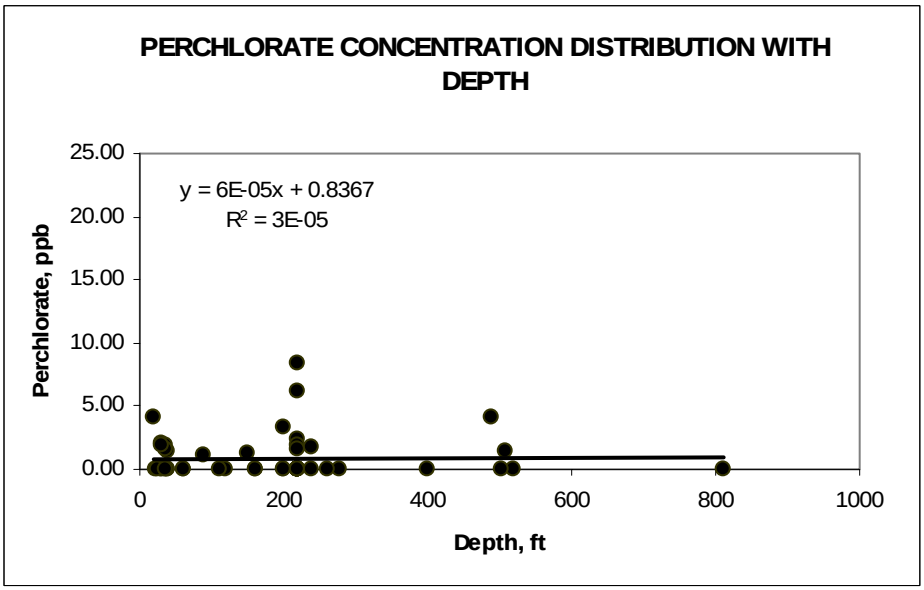


**Figure 4. The perchlorate distribution of the 20 US Bureau of Reclamation observation wells sampled in the Yuma regional aquifer.**

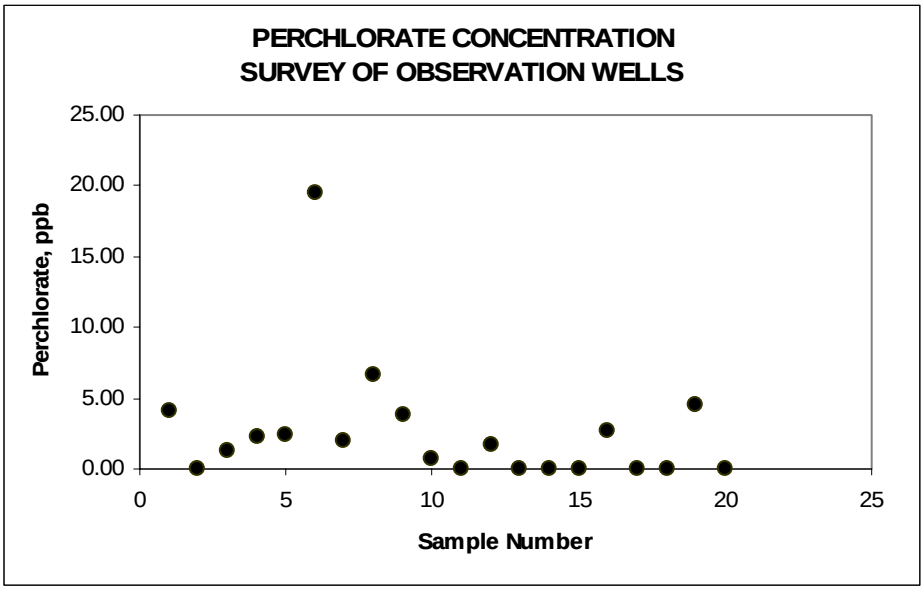
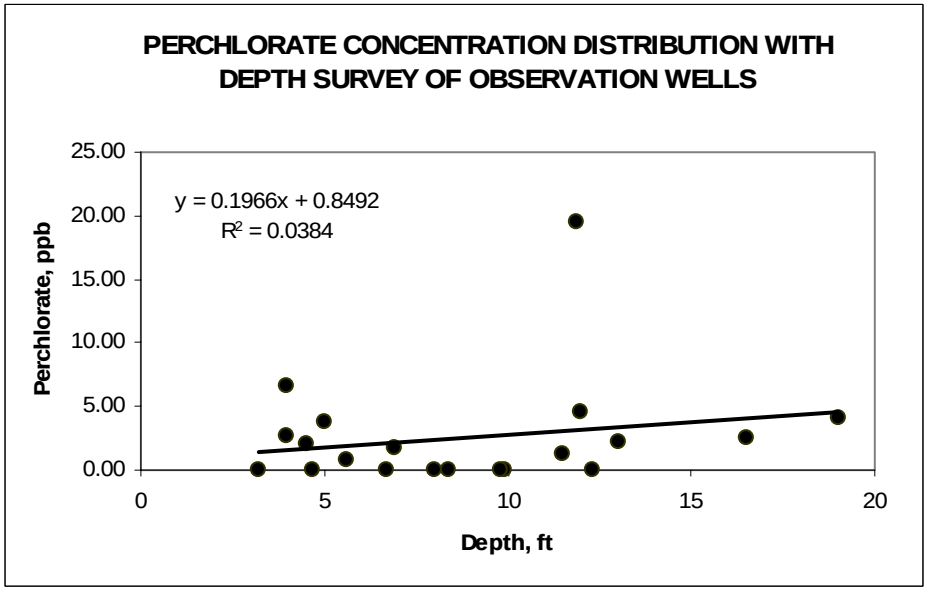



**Figure 5. The perchlorate distribution profile against well depth of the 20 US Bureau of Reclamation observation wells sampled in the Yuma regional aquifer.**



# Treatment of Nitrate in Groundwater with Autotrophic Bioreactors

## Basic Information

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|---|---|
| **Title:** | Treatment of Nitrate in Groundwater with Autotrophic Bioreactors |
| **Project Number:** | 2005AZ81B |
| **Start Date:** | 3/1/2005 |
| **End Date:** | 2/28/2006 |
| **Funding Source:** | 104B |
| **Congressional District:** | 07 |
| **Research Category:** | Engineering |
| **Focus Category:** | Nitrate Contamination, Treatment, Water Quality |
| **Descriptors:** | Nitrate, Groundwater, Bioreactors |
| **Principal Investigators:** | Reyes Sierra, James Field |

## Publication


1. Beristain Cardoso R, Sierra-Alvarez R, Rowlette P, Razo Flores E, Gómez J, Field JA. 2006. Sulfide oxidation under chemolithoautotrophic denitrifying conditions. Biotechnol. Bioengr. (Under review).
2. Sanz JL, Fernández N, Gómez R, Amils R, Field, JA and Sierra-Alvarez R. 2006. Microbiological and structural aspects of granular sludge from autotrophic denitrifying reactors. Water Sci.Technol. (In press).
3. Beristain, R., Sierra-Alvarez, R., Salazar, M., Fernandez, N., Gomez, J., Razo-Flores, E., and Field, J. A. 2005. Autotrophic Denitrification with Elemental Sulfur. VIII Latin American Workshop and Symposium on Anaerobic Digestion. October 2-5, 2005. Punta del Este, Uruguay. Pp. 383-388.
4. Sanz JL,Fernández N, Gómez R, Amils R, Sierra-Alvarez R and Field JA. 2005. Microbiological and structural aspects of granular sludge from autotrophic denitrifying reactors. VIII Latin American Workshop and Symposium on Anaerobic Digestion. October 2-5, 2005. Punta del Este, Uruguay. Pp. 15-20.
5. Fernández, R. Gomez, R. Amils, J.L.Sanz, R. Sierra-Alvarez, J.A Field. Microbial ecology of autotrophic denitrifying reactors. Biomicroworld 2005: Int Conf on Environ, industrial and Applied Microbiology. March 15-18th,2005. Badajoz, Spain.
6. Sierra-Alvarez, R and J. A. Field. 2005. Autotrophic Denitrification For The Treatment Of Drinking Water Fall semi-annual meeting of the NSF Arizona Water Quality Center. Dec. 5th, 2005, Tucson, Arizona. (Abstract).

## A. Problem and Research Objectives

*Statement of Critical Regional or State Water Problems*

Nitrate ($NO_3^-$) is one of the most common groundwater contaminants in Arizona. Over 1,000 wells across the State exceed the maximum contaminant level (MCL) for nitrate in drinking water (10 mg $NO_3$-N $L^{-1}$) set by the US EPA. Nitrate concentrations in groundwater in the West Salt River Valley (WSRV), including areas in Glendale, Mesa, Chandler and Phoenix, are among the highest in the Nation (9). Shallow groundwater from an agricultural area in the WSRV exceeded USEPA drinking-water standards and guidelines for nitrate in more than 78 percent of samples. In this area, groundwater samples from above the clay beds had a median nitrate concentration of 19.0 mg $NO_3$-N $L^{-1}$. High nitrate levels also occur in other areas in the State, including Marana, St. David, Quartzsite, Bullhead City, Lake Havasu City, among others (2). Nitrate in groundwater originates primarily from agricultural fertilizers, septic systems, landfills, and wastewater treatment plants. Nitrate is not significantly attenuated by the soil and it is transported with the groundwater largely unchanged (9).

The high nitrate concentrations in Arizona groundwater resources constitute a public health concern. Nitrate at concentrations exceeding the MCL can cause methemoglobinemia, or "blue-baby disease" (18). Birth defects also have been attributed to high nitrate concentrations (5). In adults, high nitrate levels have been associated with cancer (39, 50).

*Background Information*

Denitrification is an anaerobic microbial process in which nitrate ($NO_3^-$) is converted into dinitrogen gas ($N_2$) in four enzymatic steps via the intermediates nitrite ($NO_2^-$), nitric oxide (NO), and nitrous oxide ($N_2O$) (12,52). The ability to respire nitrate under anaerobic conditions is widespread among several genera of heterotrophic bacteria (23,44,52). Heterotrophic denitrifiers utilize simple organic substances such as methanol, ethanol and glucose, as electron donating substrate (e-donor). Some denitrifying bacteria are chemolithoautotrophic and use reduced sulfur compounds such as elemental sulfur ($S^0$), sulfide ($S^{2-}$), thiosulfate ($S_2O_3^{2-}$), or sulfite ($SO_3^{2-}$) as electron donors (e-donor) (37,42,45). Under chemolithoautotrophic conditions, carbon dioxide or bicarbonate are used as a C source for microbial cell synthesis. The occurrence of denitrification coupled to the oxidation of reduced sulfur compounds has been previously reported in natural environments (27,33,36,41) and sulfur-utilizing chemolithoautotrophic denitrifiers are believed to play an important role in mineral cycling by linking sulfur and nitrogen cycles. Among these, two obligate autotrophic species are known, *Thiobacillus denitrificans* and *Thiomicrospira denitrificans*, which grow at neutral pH (29,37,46).

Denitrification has been studied for the treatment of drinking water (21,35). The process has been applied at full-scale in Europe. The main reasons for slow transfer of the technology to the USA are concerns over bacterial contamination and presence of residual organics used as electron donors. Potential problems associated with residual organics can be avoided if inorganic substances are used as e-donors. The use of hydrogen gas ($H_2$) has been considered (7, 30). Under practical conditions, the application of $H_2$ will require membrane systems (20, 30), which would involve high maintenance and operating costs. A much simpler, low-cost and low-maintenance approach would be to utilize $S^0$ as e-donor for denitrification.

A technology under consideration that utilizes $S^0$ for denitrification is the "*Sulfur − Limestone Autotrophic Denitrification* (*SLAD*)" process, in which elemental sulfur serves as the e-donor to support chemolithoautotrophic denitrification. The stoichiometry of the reaction indicates that acidity is produced.

$$NO_3^- + \frac{5}{6}S^0 + \frac{1}{3}H_2O \quad \rightarrow \quad \frac{1}{2}N_2 + \frac{5}{6}SO_4^{2-} + \frac{2}{3}H^+ \qquad (1)$$

Limestone serves to buffer the generated acidity as well as to supply inorganic carbon for cell synthesis by the denitrifying bacteria. Recommended ratios of $S^0$:limestone range from 3:1 to 1:1 (10,32,51). The SLAD technology was first proposed by Dutch scientists in the year 1987 (38). Since then, a number of studies have reported on its applicability for the removal of nitrate in drinking water (10,16,25,47). The results demonstrate that volumetric loads up to 200 g $NO_3^-N$ $m^{-3}$ reactor $d^{-1}$ can be treated effectively with 95% removal efficiencies. The SLAD process was tested at the pilot-scale in parallel with reverse osmosis and ion exchange for the removal of nitrates from drinking water (11). The physico-chemical methods provided an average nitrate removal efficiency of 85 to 90% and generated waste brines. The SLAD provided an average nitrate removal efficiency of 96% without generating waste brines. All of the previous research has been carried out with relatively high nitrate concentrations (generally 60-500 mg $NO_3^-N$ $L^{-1}$) necessitating the use of the limestone for buffering.

$S^0$ is an apolar mineral, thus mass transfer is expected to be an important rate-limiting factor in the overall process. The specific surface area of $S^0$ is a principal factor governing the kinetics of its biological oxidation (26,43), including oxidation linked to denitrification (24). Surface colonization of $S^0$ particles is essential in the aerobic biooxidation of $S^0$ by *Thiobacillus*. In order to achieve high surface areas, $S^0$ particle sizes may be too small to be suitable for a continuous bioreactor due to washout. However, newly developed puffed $S^0$ products are now available, such as "*Popcorn sulfur"* which could provide the high surface area while maintaining a large particle size, amendable to retention in bioreactors. Biologically produced sulfur is more hydrophilic than mineral $S^0$ (22) and, thus, it would be expected to be more bioavailable.

Therefore, different forms of $S^0$ varying in properties of specific surface area and hydrophobicity still need to be considered to improve the kinetics of the SLAD process.

*Research Objectives*

The objective of this study is to evaluate the application of elemental sulfur as an electron donor for the biological treatment of nitrate in groundwater. Novel forms of $S^0$ of enhanced bioavailability will be tested which are expected to provide more rapid biological conversion rates compared to conventional $S^0$ products.

## *B. Methodology*

*Microorganisms:* The chemolithoautotrophic denitrifying enrichment was obtained in a laboratory-scale anaerobic bioreactor (0.5 L) operated at a hydraulic retention time (HRT) of 8 h in a temperature-controlled chamber at 25ºC for 8 weeks. The reactor was fed a medium composed of 100 mg $L^{-1}$ S as thiosulfate ($S_2O_3^{2-}$), 20 mg $L^{-1}$ as $NO_3^-N$, 1000 mg $L^{-1}$ of bicarbonate (carbon and alkalinity source) and micro-/macronutrients (40). Thiosulfate was selected as electron donor to facilitate enrichment of autotrophic denitrifiers. The bioavailability of thiosulfate, a soluble compound, exceeds many-fold that of elemental sulfur. The reactor was inoculated with anaerobic sludge from a full-scale anaerobic bioreactor treating recycle paper wastewater. Earlier research has shown that a highly active autotrophic denitrifying enrichment can be obtained utilizing the latter consortium as inoculum after approx. 8 weeks of operation (15). The influent of the bioreactor was monitored daily for pH, nitrate and thiosulfate. Parameters monitored in the effluent will include: pH value, nitrate, nitrite, thiosulfate, and sulfate. Biomass cultivated in the laboratory reactor was used as inoculum for batch- and continuous experiments. The autotrophic denitrification activity of the enrichment was determined in bioassays with thiosulfate by following the loss of thiosulfate and nitrate with an ion-chromatograph or appearance of $N_2$ in a helium flushed headspace. Bioassays were set up as described below. Stock cultures were maintained in the mineral medium without thiosulfate and kept under refrigerated conditions (4°C).

*Batch bioassays:* The effect of $S^0$ on denitrification kinetics was determined at 30ºC in shaken anaerobic batch bioassays. Various commercial grades of $S^0$ were tested including biologically-produced sulfur and popcorn sulfur of different particle sizes. To prevent $O_2$ contamination, the bottles were sealed with thick butyl rubber stoppers and aluminum crimp caps and, then flushed thoroughly with helium gas. Subsequently, the flasks were supplied aseptically with a pH 7, $O_2$-free mineral medium (40) containing bicarbonate (1000 mg $L^{-1}$), nitrate (5-25 mg $NO_3$-N $L^{-1}$) and inorganic reduced sulfur (thiosulfate and/or elemental sulfur) at specific concentrations, depending on the aim of the experiment. Then, bacterial

inoculum was added. Anaerobic conditions were established in each bottle by flushing with helium gas. Samples were taken periodically to determine substrate or/an electron acceptor utilization and product formation. Samples of the headspace gas were analyzed for $N_2$ and $N_2O$.

Flow-through columns: A packed bed reactor (0.4 l) was be filled with a mixture of $S^0$ granules (120.8 ml) and limestone grit (128.4 ml) between 5 and 16 mesh. The reactor was inoculated with 1.2 g VSS of enrichment culture granular sludge. The $S^0$ granule particle size was approximately 3.5 mm wide $\times$1 mm thick. The total mass of $S^0$ added to the reactor was 141.3 g. The reactor was fed with an influent containing 7.1 mM $NO_3^-$, 23.8 mM and basal mineral medium containing (g/l): $KH_2PO_4$, 1; $MgSO_4 \cdot 6H_2O$, 0.2; $NH_4CI$, 0.4; Na $HCO_3$, 2; trace element solution (described above), 2 ml/l. The column effluent was recycled back to the top of the reactor until biofilm development on the packing was noticeable. The performance of the reactor was then tested at HRTs ranging from 24 to 1.5 h. HRT are based on the empty bed volume of the reactors. Once fully operational, influent nitrate concentration was decreased to determine if treatment is feasible at concentration range that is realistic for groundwater treatment conditions. The influent was monitored periodically for pH, nitrate and bicarbonate alkalinity. The effluent was also monitored for pH value, nitrate, nitrite, sulfate, and thiosulfate. The composition of the biogas ($CO_2$, $N_2$, $N_2O$) was monitored weekly or as needed.

*Analytical Methods:* Dinitrogen gas ($N_2$), nitrous oxide ($N_2O$) and carbon dioxide ($CO_2$) were quantified in a GC equipped with a thermal conductivity detector. The concentration of nitrate, nitrite, thiosulfate and sulfate in liquid samples were analyzed by ion chromatography (IC) with suppressed conductivity detection using a Dionex DX-500 system equipped with a Dionex AS11-HC4 column (Dionex, Sunnydale, CA) and an eluent containing 15 mM KOH at a flow rate of 1.2 mL $min^{-1}$. Liquid samples were membrane-filtered (0.45 μm) prior to IC analysis. Other parameters, such as volatile suspended solids (VSS) in the biomass and bicarbonate alkalinity in liquid samples were measured according to *Standard Methods for the Examination of Water and Wastewater* (4).

## *C. Principal Findings and Significance*

Chemolithotrophic enrichment cultures have been established that can couple denitrification to the oxidation of $S^0$ (Fig. 1). The role of elemental sulfur and nitrate concentrations on the kinetics and stoichiometry of autotrophic denitrification was investigated. Results of batch bioassays indicated a continued increase in denitrification rates at concentrations far exceeding the stoichiometric requirement (Fig. 2), pointing to the occurrence of mass transfer limitations from solid phase $S^0$ to the aqueous phase. Different grades of $S^0$ were tested and the material providing the best compromise between physical and electron donating properties was selected

for further work. An increased in denitrification rates was observed with decreasing $S^0$ particle size, which can be attributed to the increase in surface area, resulting in better mass transfer.

The rates of chemolithotrophic denitrification in assays utilizing different reduced sulfur compounds as e-donors were compared. The average oxidation state of the sulfur atoms in the three compounds tested, *i.e.,* sulfide, elemental sulfur and thiosulfate, is –2, 0 and +2, respectively. Fig. 3 illustrates the conversion of nitrate and the formation of nitrogen-containing products as a function of time for the various assays. Also plotted in this figure is the time course of conversion for the various reduced sulfur compounds to sulfate. Nitrogenous gas intermediates were not detected in this experiment. Thiosulfate was the most readily utilized electron donor, followed by hydrogen sulfide and elemental sulfur. The rates of nitrate degradation in assays with thiosulfate were 4.6 and 9.5 fold higher compared to sulfide and elemental sulfur, respectively. Similarly, the rates of sulfate generation in assays with thiosulfate were 4.8 and 25.3-fold higher compared to sulfide and elemental sulfur, respectively. Nitrate was recovered as $N_2$ gas in (near) stoichiometric proportions by the end of the experiments. Thiosulfate is readily bioavailable and non-toxic, which could partly explain the high sulfoxidation and denitrification rates detected with this compound. While, $H_2S$ is also bioavailable, it is a well-known inhibitor of a wide variety of microorganisms, including denitrifying bacteria, and its inhibitory impact may account for lower metabolic rates compared to thiosulfate. The lowest rates were observed for chemolithotrophic denitrification of $S^0$ and this is most likely due to the limited mass transfer of substrate from solid phase $S^0$. Elemental sulfur is an apolar mineral, thus mass transfer is expected to be an important rate-limiting factor in the overall process. The specific surface area of elemental sulfur is a principal factor governing the kinetics of its biological oxidation (26), including oxidation linked to denitrification (24).

The feasibility of removing nitrate in continuous bench-scale columns packed with $S^0$ as slow-release e-donor was investigated. A packed bed reactor with an approximate ratio 1:1 of sulfur:limestone ($CaCO_3$) granules was rapidly started up utilizing a chemolithotrophic denitrifying enrichment culture as inoculum. The initial start up concentration was 105 mg N-$NO_3^-$/L. Nitrate concentration in the influent was lowered stepwise to concentrations typical of highly contaminated groundwater resources in Arizona (approx. 20 mg N-$NO_3^-$/L). The performance of the reactor is illustrated in Fig. 4. Results obtained indicate that a bioreactor packed with $S^0$ can successfully treat nitrate with a high efficiency at high volumetric loading (Table 1) and HRTs of only 1.8 h. The maximum nitrate loading rate attained, 237 mg $NO_3^-$-N/$L_{reactor}$-d, is comparable to the fastest rates achieved in the literature with $S^0$ as e-donor. The recovery of N as benign $N_2$ gas was nearly stoichiometric.

The results of this study confirm the effectiveness of microbial chemolithotrophic denitrification linked to oxidation of $S^0$ for the removal of nitrate. In addition, these findings indicate the

potential of sulfur-limestone biofilters for the low-cost, low-maintenance treatment of nitrate-contaminated groundwater.

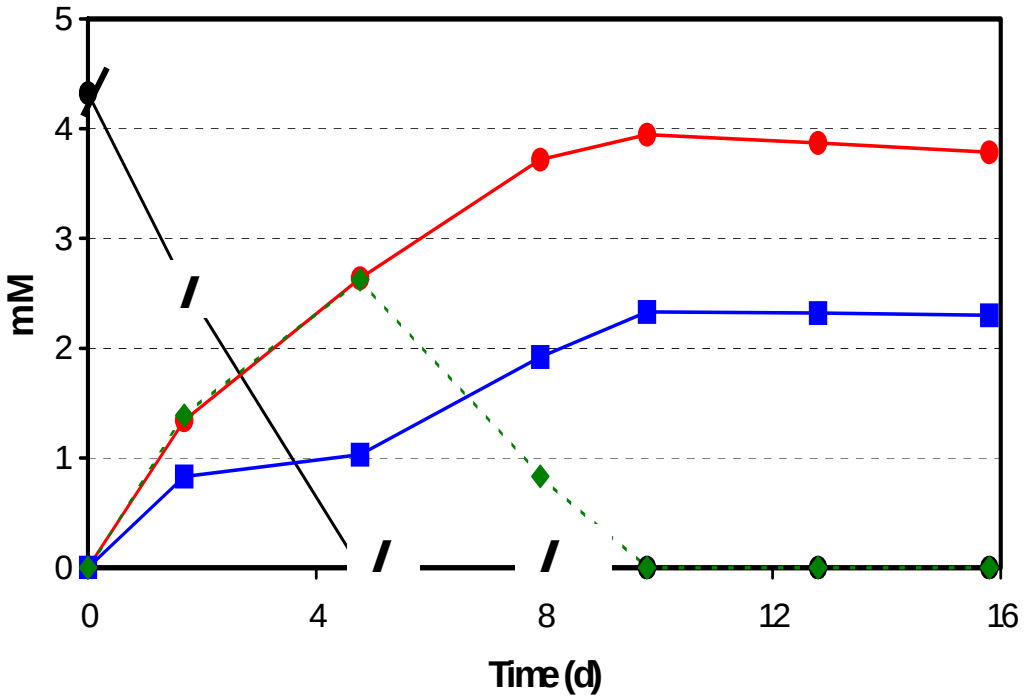


*Figure.1. Time course of denitrification by a chemolithotrophic enrichment culture utilizing elemental sulfur (15.2 mM) as electron donating substrate. The initial nitrate concentration was 3.8 mM, and the sludge concentration was 0.5 g VSS/L. (/) Nitrate; (■) $N_2$ gas; (◆) nitrite; (●) sulfate.*

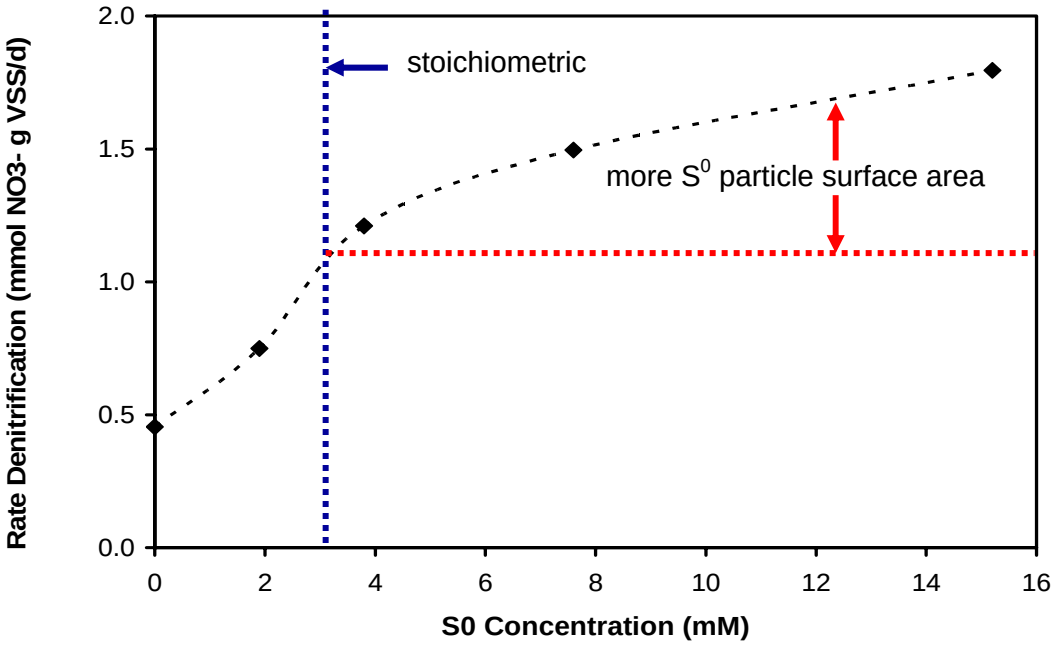


*Figure 2. Effect of the sulfur concentration on the rate of denitrification determined for a chemolithotrophic denitrifying enrichment culture.*

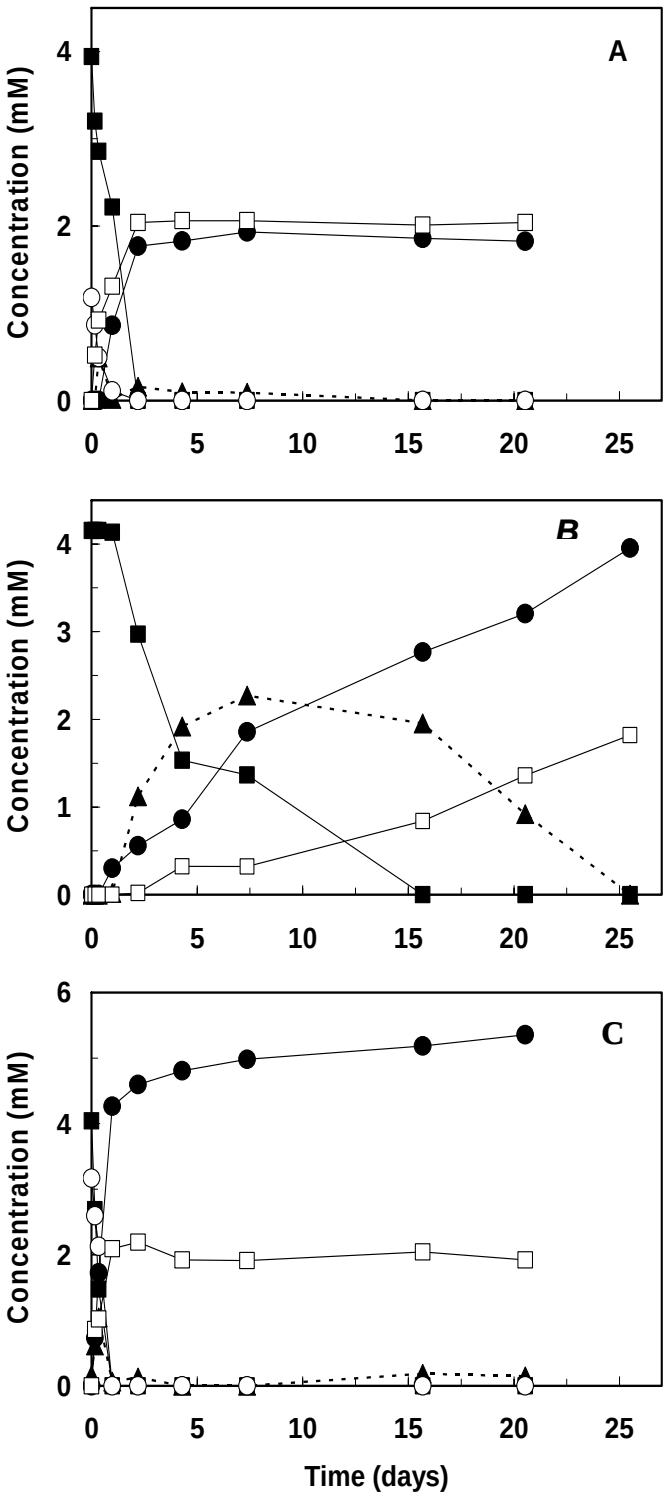


*Figure 3. Time course of denitrification and sulfoxidation by a chemolithotrophic denitrifying mixed culture (0.5 g VSS/L) utilizing hydrogen sulfide ($H_2S$) (Panel A), elemental sulfur ($S^0$) (Panel B), or thiosulfate ($S_2O_3^{2-}$) (Panel C) as electron donors. Electron donor (○); sulfate (●); nitrate (■), nitrite (▲), and dinitrogen gas (□). Bioassays were supplied with 4 mM nitrate and stoichiometric concentrations of the reduced sulfur compounds.*

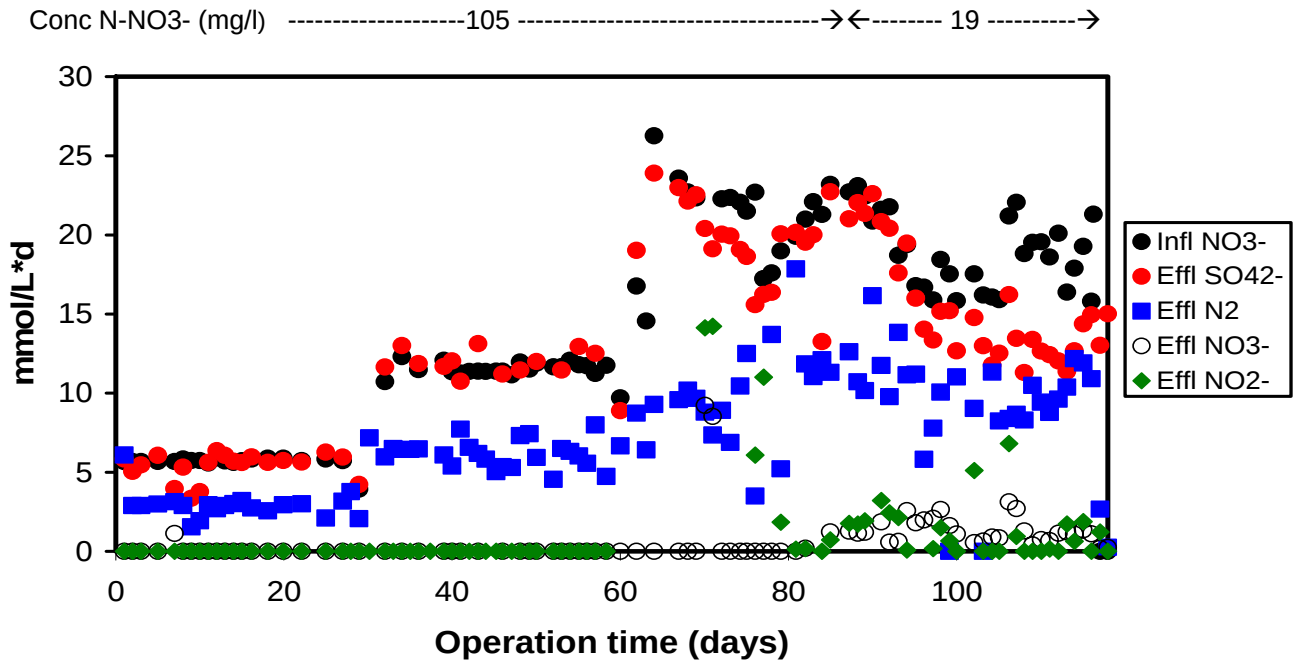


*Figure 4. Nitrate and sulfur conversion in a laboratory-scale reactor packed with sulfur: limestone fed with a simulated groundwater supplied with nitrate.*

**Table 1.** Performance of an Elemental Sulfur Packed Bioreactor Used for the Treatment of Nitrate

| Parameter | Value | Units |
|---|---|---|
| Inlet Concentration $NO_3^-$-N | 19.0 | mg $l^{-1}$ |
| Hydraulic Retention Time | 1.9 | h |
| Volumetric Load $NO_3^-$-N | 237 | mg $l^{-1}_{reactor}$ $d^{-1}$ |
| Removal $NO_3^-$-N | 92.1 | % $NO_3^-$-$N_{in}$ |
| Recovery$NO_2^-$-N | 4.5 | % $NO_3^-$-$N_{in}$ |
| Recovery $N_2$-N | 99.4 | % $NO_3^-$-$N_{in}$ |

## Acknowledgements

This research was supported in part by a grant from the USGS 104B Grant Program and by a National Science Foundation grant (R. S-.A., NSF-0137368 award).

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# Salt River Riparian Ecosystem Restoration

## Basic Information

| | |
|---|---|
| **Title:** | Salt River Riparian Ecosystem Restoration |
| **Project Number:** | 2005AZ83B |
| **Start Date:** | 3/1/2005 |
| **End Date:** | 2/28/2006 |
| **Funding Source:** | 104B |
| **Congressional District:** | AZ 5 |
| **Research Category:** | Biological Sciences |
| **Focus Category:** | Wetlands, Hydrology, Ecology |
| **Descriptors:** | Salt River, Riparian, Ecosystem, Restoration |
| **Principal Investigators:** | Julie Stromberg |

## Publication

## A. Problem and Research Objectives

Riparian ecosystems of southwestern USA have changed extensively since European settlement. Rivers located in urban areas have undergone the greatest transformation, as exemplified by the Salt River in the Phoenix metropolitan area. Damming, water diversion, and stream channelization allowed for agricultural and urban development, but reduced the density of riparian vegetation and narrowed the riparian corridor. The cottonwood-willow forests, mesquite woodlands, shrublands, marshlands and riparian grasslands that were once common have become scarce in this reach of the river, as have patches of desert saltbush that occurred on terraces adjacent to the river (Graf 1982; Rea 1983; Hendrickson and Minckley 1984; Davis 2001). The changes in stream hydrology, geomorphology, and vegetation have reduced wildlife habitat and recreational opportunities and altered other functions including climate moderation and groundwater recharge (National Research Council 2002).

To restore some of the amenities once provided by the Salt River, several multi-million dollar riparian ecosystem restoration projects are planned or underway in the Phoenix metropolitan area. These include the Va Shly'ay Akimel project, Tempe Rio Salado Project, Phoenix Rio Salado Project, Rio Salado O'este, and the Tres Rios Project. These projects are partnerships between the U. S. Army Corps of Engineers and local municipalities. The projects have multiple goals, including increasing wildlife habitat quality and restoring historically-present plant communities while also providing recreational opportunities, maintaining flood water conveyance, and reducing the risk of mosquito-borne disease.

Explicitly, restoration refers to returning a site to a prior historical condition and thus to a pre-degradation state (Bradshaw 2002). The Salt River restoration projects are more accurately rehabilitations, as restoration to pre-dam conditions is not a realistic goal. Current restoration/rehabilitation plans for the Salt River seek to recreate the historical community-level structure of the river's biotic community largely by planting woody vegetation and installing and maintaining drip irrigation systems. Less emphasis is being placed on restoration of the historical physical processes, such as the flows of water and sediment, which are the primary determinants of riparian vegetation structure (Poff et al. 1997; Ward et al. 2001; Rood et al. 2002).

If physical processes such as water flows are restored, plants, animals, and microorganisms may colonize the site of their own accord if source populations are present. Many woody riparian plants, such as Fremont cottonwood (*Populus fremontii*) and Goodding willow (*Salix gooddingii*) trees, are highly fecund, fast growing, disturbance-adapted species that colonize floodplains; each year these trees produce large numbers of short-lived seeds. Many annual or short-lived perennial herbaceous riparian species are also disturbance adapted and often have viable seeds stored in the soil seed bank (Richter and Stromberg 2005); these seeds potentially serve as an *in situ* source for colonization, should water flows be restored to a site.

Some of the Salt River reaches targeted for restoration have been 'accidentally' rewatered in recent decades by storm drain runoff from the urban watershed. Pockets of riparian vegetation have developed at these storm drains, some of which have perennial stream flow and small flood pulses that reflect the climatic signal. These areas, although small, support a productive and diverse riparian flora indicating that riparian plant communities can reestablish where water flows are adequate. In addition, these sites may provide a source of propagules for natural revegetation of other river reaches. If seed sources are present in adequate densities in the seed bank or from standing vegetation, then passive restoration of a site can be a viable and inexpensive option (Briggs 1996).

Our goal was to provide information that could be used to inform restoration planning of the Salt River riparian corridor. Specifically, our research goals were to: 1) determine how the Salt River riparian plant community has been altered by diversion of stream flow from Granite Reef diversion dam; 2) Determine how the pockets of riparian vegetation that have developed naturally at rewatered urban reaches compare to those in a perennial river reach, in terms of diversity and composition of plants in both the soil seed bank and extant vegetation.

## B. Methodology

*Study sites.* Sites were selected within three reaches of the Salt River in and near the Phoenix metropolitan area (Figs. 1 and 2). The uppermost reach, referred to as the rural perennial reach, is located in Tonto National Forest and has perennial flow with suppression of most floods by Roosevelt Dam and other dams. The middle reach, referred to as the suburban diverted reach, is in the city of Mesa, below Granite Reef Dam, and due to water diversion by Salt River Project exhibits ephemeral flow. This reach is to be restored as part of the Va Shly'ay Akimel project. The lower reach flows through the large urban center of Phoenix. This reach, referred to as the urban rewatered reach, is watered in areas by outflows of treated wastewater, storm water, and irrigation runoff. Two sites were selected in this reach. This section encompasses portions of the Phoenix Rio Salado O'Este riparian restoration project area (in the planning stages) and the Phoenix Rio Salado riparian restoration project area (in the development stage).

The most prevalent patch types in each reach were selected for study. In the rural perennial reach, there were two patch types: a hydromesic forest patch type, vegetated by *Populus fremontii, Salix gooddingii*, and *Tamarix chinensis,* and a mesic woodland/shrubland vegetated by *Prosopis velutina* and *Baccharis sarothroides.* Within the suburban diverted reach there was one predominant patch type, a xeric shrubland vegetated mainly by *Bebbia juncea*. Within the urban rewatered reach there were three patch types: hydromesic forest vegetated by a mixture of tree species including *Salix gooddingii, Populus fremontii,* and *Tamarix chinensis,* mesic woodland-shrubland vegetated by *Prosopis velutina, Baccharis sarothroides* and grasses, and xeric shrubland vegetated by *Bebbia juncea*. Within this latter reach, the hydromesic forests were located along the Salt River channel, and also clustered in pockets at storm-drain outfalls.

*Vegetation sampling:* Six independent 100m² quadrats were randomly selected for vegetation and seed bank sampling within each patch type, per river reach, for a total of 36 quadrats. Herbaceous vegetation was sampled in March, June, and September of 2004, within five 1m² plots within each 100m² quadrat. March and September are periods of high seasonal rainfall while June is a seasonal dry period. Percent cover of herbaceous species was visually estimated using Daubenmire cover classes. Woody species were sampled within the 100m² plots for presence/absence. To characterize the overall vegetation structure in each reach, three line transects were established in 2005 within each of the four study reaches spanning the riparian zone. Vegetation patches along the transects were delimited in terms of dominant species and physiognomy, and the width of each patch was measured.

*Seed banks:* Within the quadrats three replicate soil cores were taken at three depths of 0-2.5 cm, 2.5-5 cm and 5-10 cm using a split-core sampler with a radius of 2.5 cm during February 2004. The three replicates of each depth were placed in baggies, labeled for site and depth, and then stored in a cooler for transport to the ASU greenhouse. A total 324 soil samples were taken (36 quadrats x 3 soil depths x 3 replicates). The seed banks were investigated using the seedling emergence method (Roberts 1981). The experiment was carried out in a greenhouse on the main campus of Arizona State University. Samples were spread over the potting soil with a maximum depth of 3 cm. The flats were placed in the greenhouse on three benches using a random block design. Temperatures were regulated to coincide with average temperature in the Phoenix area and were changed on a monthly basis for one year. The samples were bottom watered daily using irrigation. The seed bank was inventoried on two week to one month intervals. Seedlings were allowed to mature until they were able to be identified to species after which they were removed to eliminate competition.

*Data reduction and analysis*: The composition of the above and below ground vegetation community was characterized by classifying species as predominantly annual vs. predominantly perennial, as historically non-native vs. native to the United States (following designation in USDA 2002) and as riparian (species with a wetland indicator class as indicated in USDA 2002) vs. upland (species with no wetland indicator class). Mean plot-level species richness and vegetation abundance (cover for the extant vegetation and density for the soil seed bank) were calculated for each patch. Shannon-wiener diversity coefficients and patch-level species richness also were calculated.

To compare the riparian plant communities above and below Granite Reef diversion dam, two sample t-tests (n=6) were used to test for differences in richness and abundance (of seed bank and extant vegetation) between the xeric patch type in the diverted reach and the upstream hydro-mesic patch, and between the xeric patch types and the upstream mesic patch type. To make comparisons between the rural perennial and urban rewatered reaches, two sample t-tests (n=6) were used to test for differences between the two hydro-mesic riparian forest patch types and between the two mesic woodland/shrubland patch types. To scale up the results from the patch level to the entire riparian zone, patch values were weighted by the proportion of the floodplain the patch occupied. All two sample tests were done in SAS (version 9.1, SAS institute Inc, Cary

NC). Assumptions of normality and equal variance for parametric tests were analyzed by normal probability plots and F-tests for equal variance respectively. Shannon-wiener diversity indices were calculated using estimateS (Colwell 2005).

## C. Principal Findings and Significance

*Effects of river diversion on the riparian plant community.* Below Granite Reef diversion dam, both the above and below ground vegetation communities differed substantially from the above dam reach floodplain (Table 1). Species richness in the soil seed bank was 47% lower and annual extant vegetation richness was 23% lower below the diversion dam (Fig. 3). A 21% reduction in ground cover was recorded and canopy cover above 1 m was nonexistent in the diverted reach. The number of individuals in the soil seed bank was 43% lower below the dam as well. Shifts in composition were also observed in the diverted reach, most evidently in the extant vegetation, which were dominated by upland rather than by riparian species. The soil seeds banks of the dewatered reach also had a smaller proportion of riparian species compared to the above dam reach (Fig. 4).

*Significance*. These results indicate that the long-term diversion of the Salt River stream flow has converted the remaining undeveloped floodplain from a species-rich hydromesic riparian forest/shrub community to a species-poor xeric shrub community. This community reassembly, although to an altered condition, demonstrates the resilience of the ecosystem to respond to a disturbance (i.e., dewatering). Yet, riparian species, such as cattail (*Typha domingensis*) and umbrella sedge (*Cyperus odoratus*), are present in the seed bank of these xeric shrublands. This may facilitate redevelopment of the former riparian state following a new perturbation, i.e., the planned rewatering of the river as part of restoration actions. Support for this hypothesis was observed when a small riparian herbaceous patch developed in the diverted reach following the 2005 flooding of the lower Salt River after a release from the upstream diversion dam. The seed source for the post-flood recruitment of riparian species may have been the soil seed bank or the annual seed rain.

*Effects of rewatering on the urban riparian plant community*. Species richness (Table 2) and diversity (data not shown) of the riparian patches were similar in the urban rewatered and rural perennial reaches for both seed bank and extant vegetation. For the hydromesic patch type, 30 seed bank species were in the urban rewatered reach (per 1062 $cm^2$) compared to 33 in the rural perennial reach (Fig. 5). Respective values for the mesic patch type were 22 (rural perennial) and 30 (urban rewatered) (figure not shown). No significant differences were observed in the mean number of seed bank species per plot between the rural perennial and urban rewatered reaches for the hydromesic patches, but there was a trend for significantly more species in the urban rewatered reach for the mesic patches. Similarly, species richness of the extant vegetation was comparable between the rural perennial and urban rewatered reaches for the hydromesic patches but was greater in the urban rewatered reaches for the mesic patches. In both reaches most species were classified as riparian, both for the soil seed banks and extant vegetation (Fig. 6). Both reaches had a high percentage of exotic species. There was high overlap in the

composition of riparian species observed the soil seed bank in the rural perennial and urban rewatered reaches (Table 3).

*Significance*. These findings of similarities in species richness and composition between riparian patch types of the above-dam perennial and below-dam rewatered reaches have implications for the way riparian restoration is approached. Presently, the riparian restoration efforts that are ongoing and planned for the Salt River entail a large component of tree planting and seeding. Our results indicate that this highly altered urban river has high resilience in the sense of having high capacity to redevelop species-rich riparian plant communities without intervention, given that adequate flows of water and sediment are restored.

The composition of the plant community in the urban reach differs from the historical condition, but this change is inevitable given that the flow of water, seeds, and sediment from upstream sources are restricted, and the physical processes that maintained the historical plant community cannot be restored in an urban setting. In addition, the urbanization of the surrounding watershed is reflected in the riparian plant community. The storm drains appear to be functioning as urban tributaries, providing not only water, sediments and flood disturbance, but also seeds from the urban watershed. It is noteworthy, however, that the proportion of exotic species in both the soil seed bank and the extant vegetation of the urban rewatered reach were similar to the proportion observed in the upstream perennial reach.

Not only do these small riparian storm drain communities provide anecdotal evidence for the resilience of this system, but they are also useful tools for restoration of the Salt River riparian corridor. The species composition of these sites provides an example of a riparian community that can establish and maintain itself with limited intervention under these altered conditions. Also, these communities, if left intact during restoration interventions, could function as source of propagules for the establishment of riparian species in adjacent reaches.

Table 1: Change in vegetation community within the floodplain above and below Granite Reef Diversion Dam

| | Above Dam | Below Dam | Percent Change |
|---|---|---|---|
| Floodplain patch type composition | | | |
| Hydro-mesic | 44% | 0 | -- |
| Mesic | 56% | 0 | -- |
| Xeric | | 100% | |
| Soil seed bank | | | |
| Species richness (1062 $cm^2$) | 28.3 | 15.0 | 47 |
| Abundance (1062 $cm^2$) | 373.7 | 212.0 | 43.3 |
| Mean species richness (177 $cm^2$) | 9.1 | 4.8 | 46.8 |
| Mean abundance (177 $cm^2$) | 66.2 | 34.0 | 48.7 |
| Extant vegetation | | | |
| Species richness (30 $m^2$) | 23.4 | 17.0 | 23.1 |
| Mean species richness (5 $m^2$) | 7.6 | 7.7 | 1.3 |
| Mean percent ground cover | 11.1 | 8.7 | 21.2 |
| Percent canopy cover | 29.2 | 0 | -- |

Table 2: Change in vegetation community within the floodplain in the rural perennial and urban rewatered reaches

| | Rural Perennial | Urban Rewatered | Percent Change |
|---|---|---|---|
| Floodplain composition | | | |
| Hydro-mesic | 44% | 63% | |
| Mesic | 56% | 37% | |
| Soil seed bank | | | |
| Species richness (1062 $cm^2$) | 28.3 | 30.0 | 6.1 |
| Abundance (1062 $cm^2$) | 373.7 | 755.4 | 102.1 |
| Mean species richness (177 $cm^2$) | 9.1 | 10.1 | 11 |
| Mean abundance (177 $cm^2$) | 66.2 | 127.3 | 92.3 |
| Extant vegetation | | | |
| Species richness (30 $m^2$) | 23.4 | 35.93 | 53.5 |
| Mean species richness (5 $m^2$) | 7.6 | 10.393 | 37.3 |
| Mean percent ground cover | 11 | 26 | 132.6 |
| Percent canopy cover | 29 | 16 | -44.9 |

Table 3: Riparian species observed in the soil seed bank of the riparian patch types in the urban rewatered and rural perennial reaches

| | | | | | Urban Rewatered | | Rural Perennial | |
|---|---|---|---|---|---|---|---|---|
| Species | Family | Habit* | Lifespan* | Origin* | Hydro-mesic | Mesic | Hydro-mesic | Mesic |
| *Amaranthus albus* | Amaranthaceae | F | A | N | | X | | |
| *Aster subulatus* | Asteraceae | F | A | N | X | X | X | |
| *Calibrachoa parviflora* | Solanaceae | F | A | N | X | X | X | X |
| *Chenopodium sp* | Chenopodiaceae | F | P | | | X | | |
| *Conyza Canadensis* | Asteraceae | F | A | N | | X | X | |
| *Cynodon dactylon* | Poaceae | G | P | I | X | | | |
| *Cyperus difformis* | Cyperaceae | G | A | I | | X | | |
| *Cyperus involucratus* | Cyperaceae | G | P | I | X | X | | |
| *Cyperus odoratus* | Cyperaceae | G | P | N | X | X | X | |
| *Echinochloa colona* | Poaceae | G | A | I | X | X | | |
| *Eclipta prostrate* | Asteraceae | F | A | N | X | X | | |
| *Eleocharis montevidensis* | Cyperaceae | G | P | N | X | X | X | X |
| *Leptochloa fusca ssp uninervia* | Poaceae | G | A | N | X | X | X | X |
| *Melilotus indicus* | Fabaceae | F | A | I | X | X | X | X |
| *Pluchea odorata* | Asteraceae | SS/S | A/P | N | X | | X | |
| *Polypogon monospeliensis* | Poaceae | G | A | I | X | X | X | X |
| *Portulaca oleracea* | Crassulaceae | F | A | N | X | X | X | X |
| *Prosopis sp* | Fabaceae | T | P | N | X | | | |
| *Rumex crispus* | Polygonaceae | F | P | I | X | | X | X |
| *Schenoplectus americanus* | Cyperaceae | G | P | N | X | | | X |
| *Sonchus asper* | Asteraceae | F | A | I | X | X | X | X |
| *Typha domingensis* | Typhaceae | F | P | N | X | X | X | X |
| *Veronica anagallis aquatica* | Scrophulariaceae | F | P | N | X | X | X | X |

*Forb, Graminoid, Subshrub; Annual, Perennial; Native, Introduced

Figure 1. Location of study sites (stars) along the Salt River.

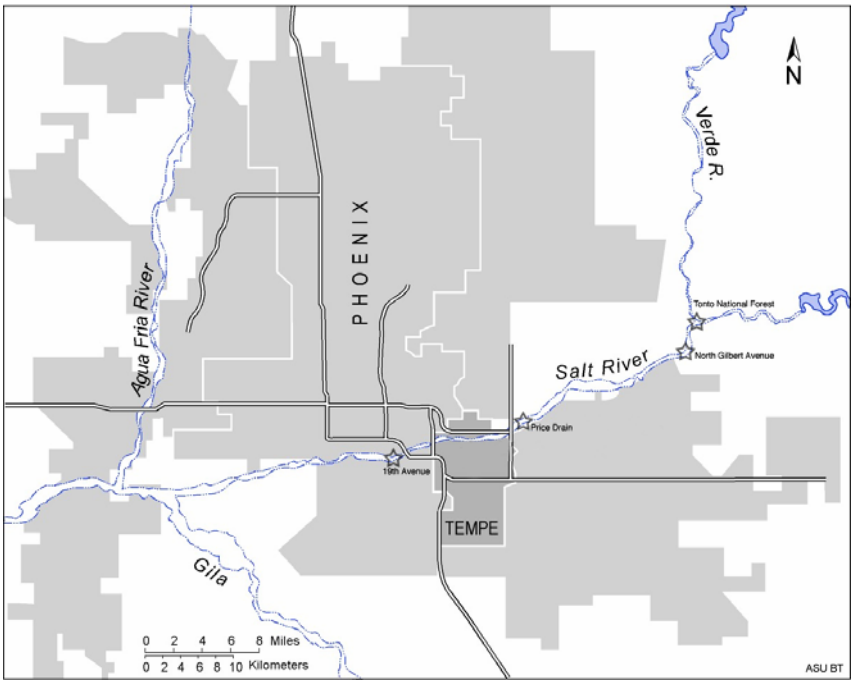


Fig. 2. (following page) Photographs of the Salt River in a reach upstream of Granite Reef Diversion dam (top), a reach downstream of the dam (middle), and a reach receiving storm drain inflow (bottom).

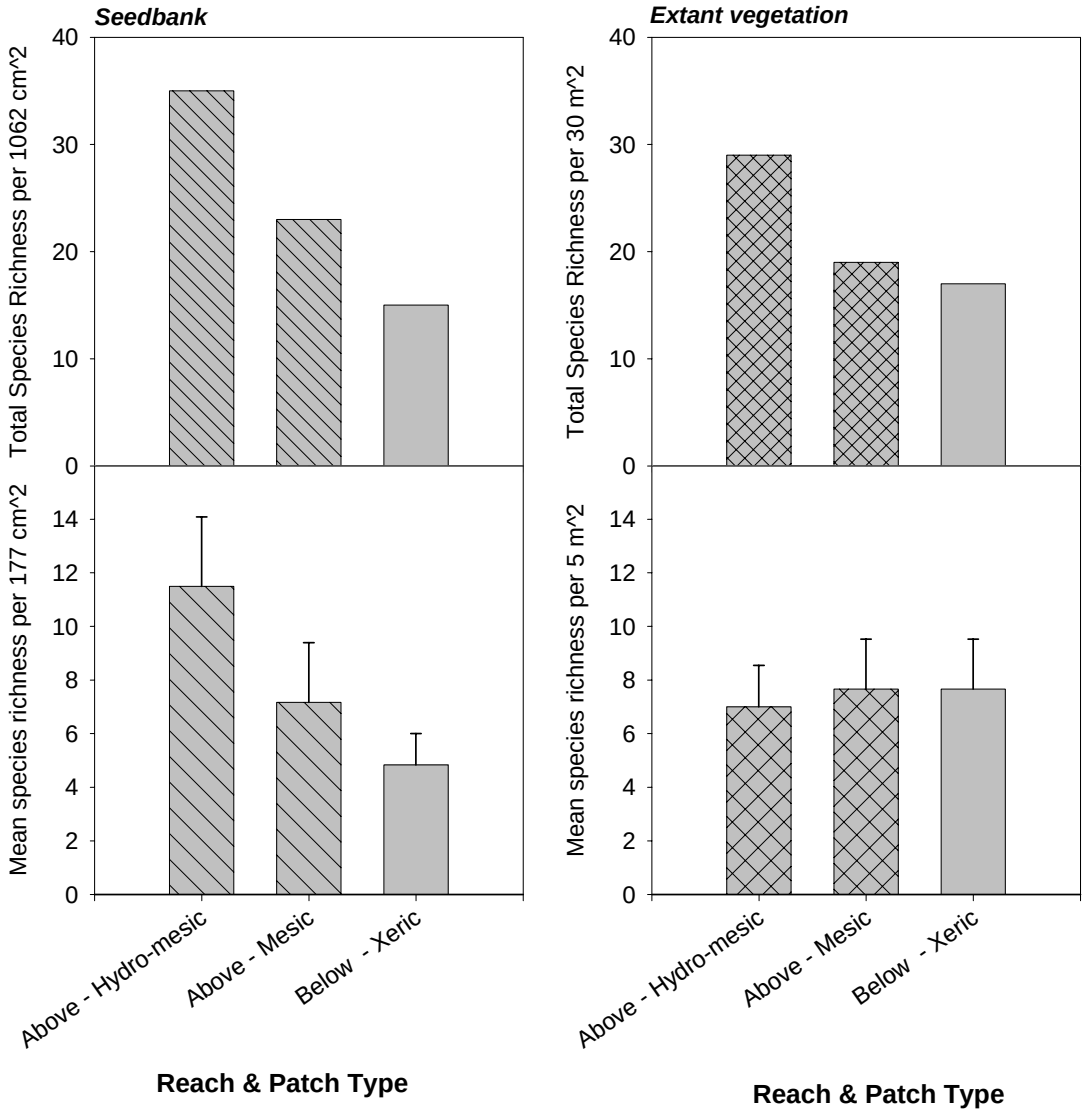


Figure 3: Species richness in the soil seed bank and extant vegetation above and below Granite Reef Diversion Dam. Patterned bars represent above dam patch types and solid bars represent below dam patch types. Top panel displays overall richness across all plots. Lower panel displays mean richness per plot ± one standard deviation.

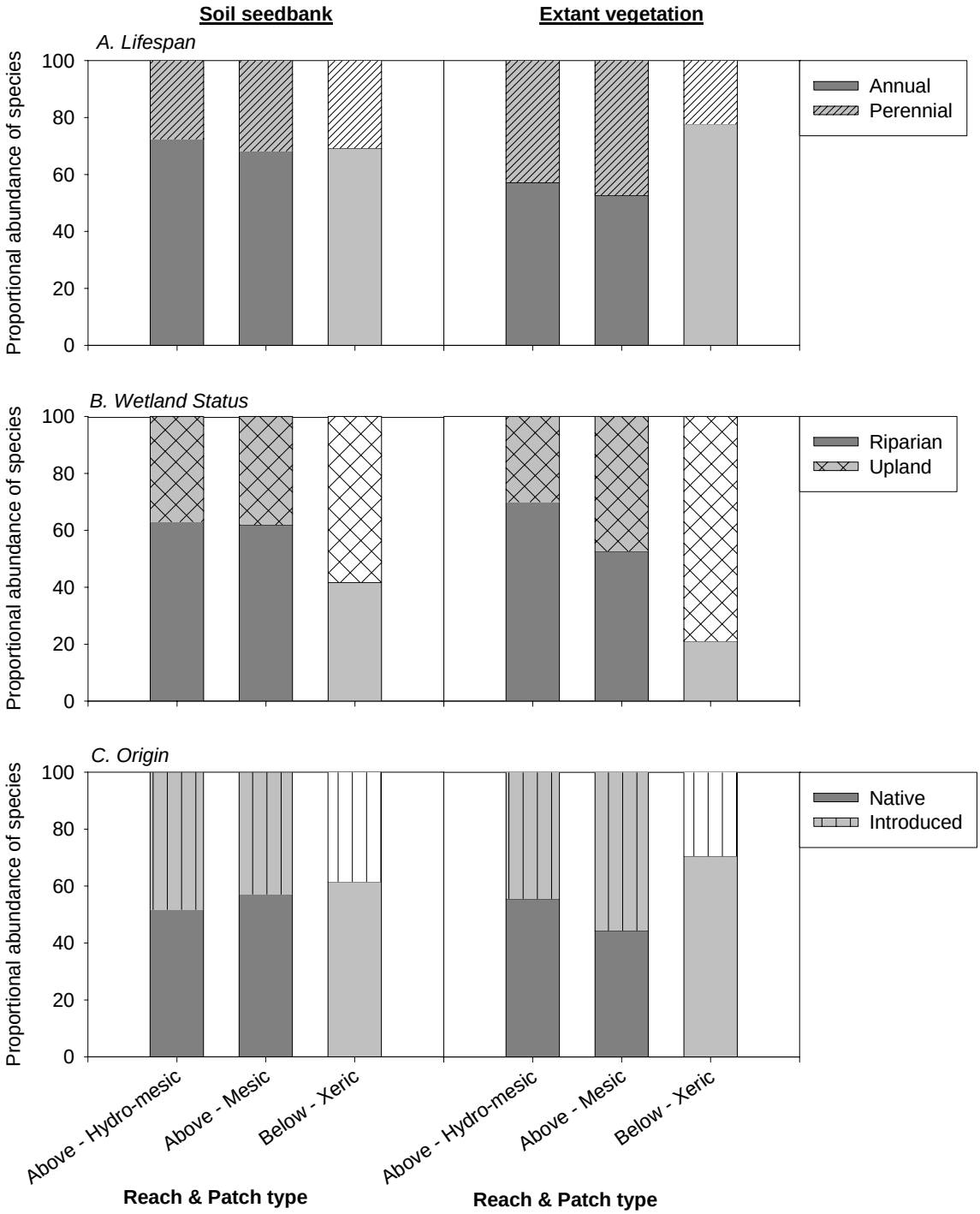


Figure 4: Composition of species in the soil seed bank and extant vegetation of patch types located above and below Granite Reef Diversion Dam. Darker shaded bars indicate patch types above the dam and lighter colored bars indicate patch types below the dam.

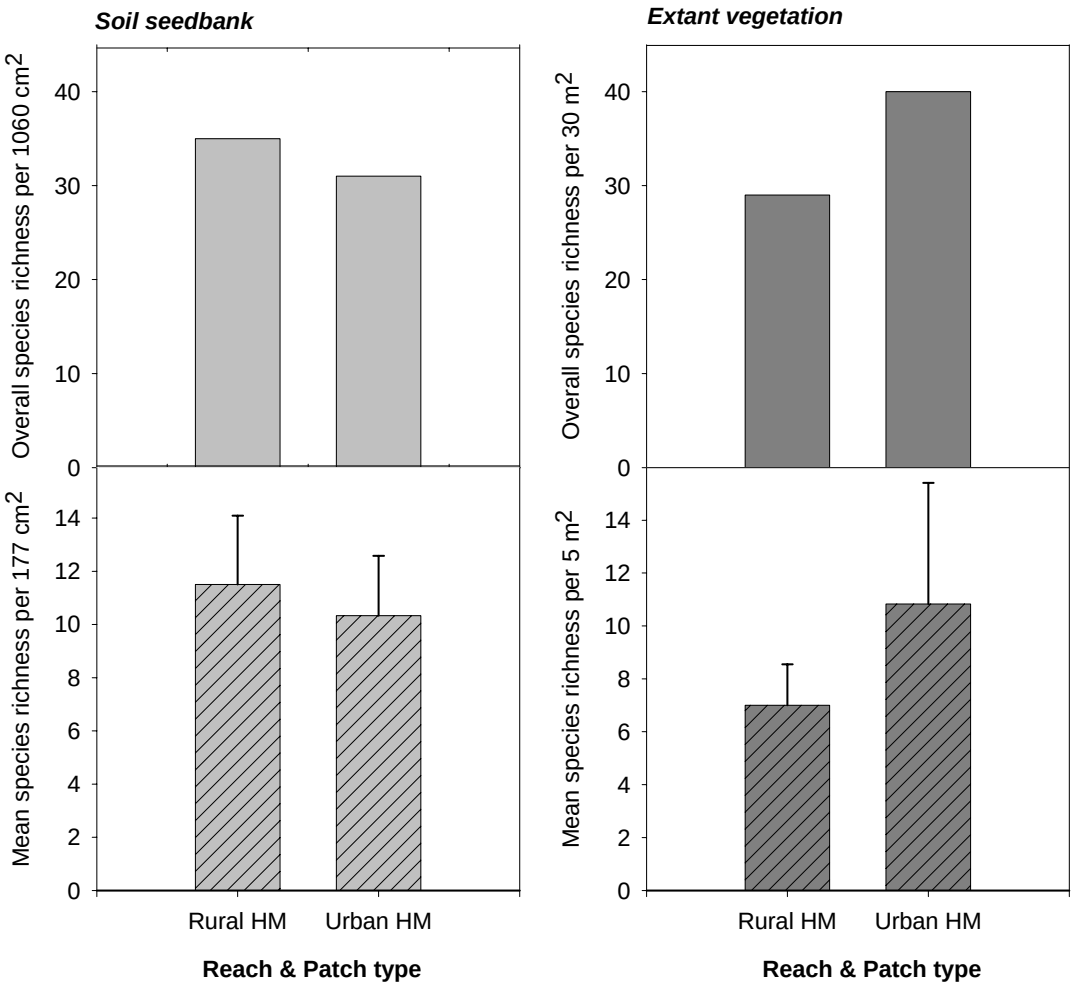


Figure 5: Species richness in the hydro-mesic (HM) patch type soil seed bank and extant vegetation of two reach types. Top panel displays overall richness across all plots. Lower panel displays mean richness ± one standard deviation.

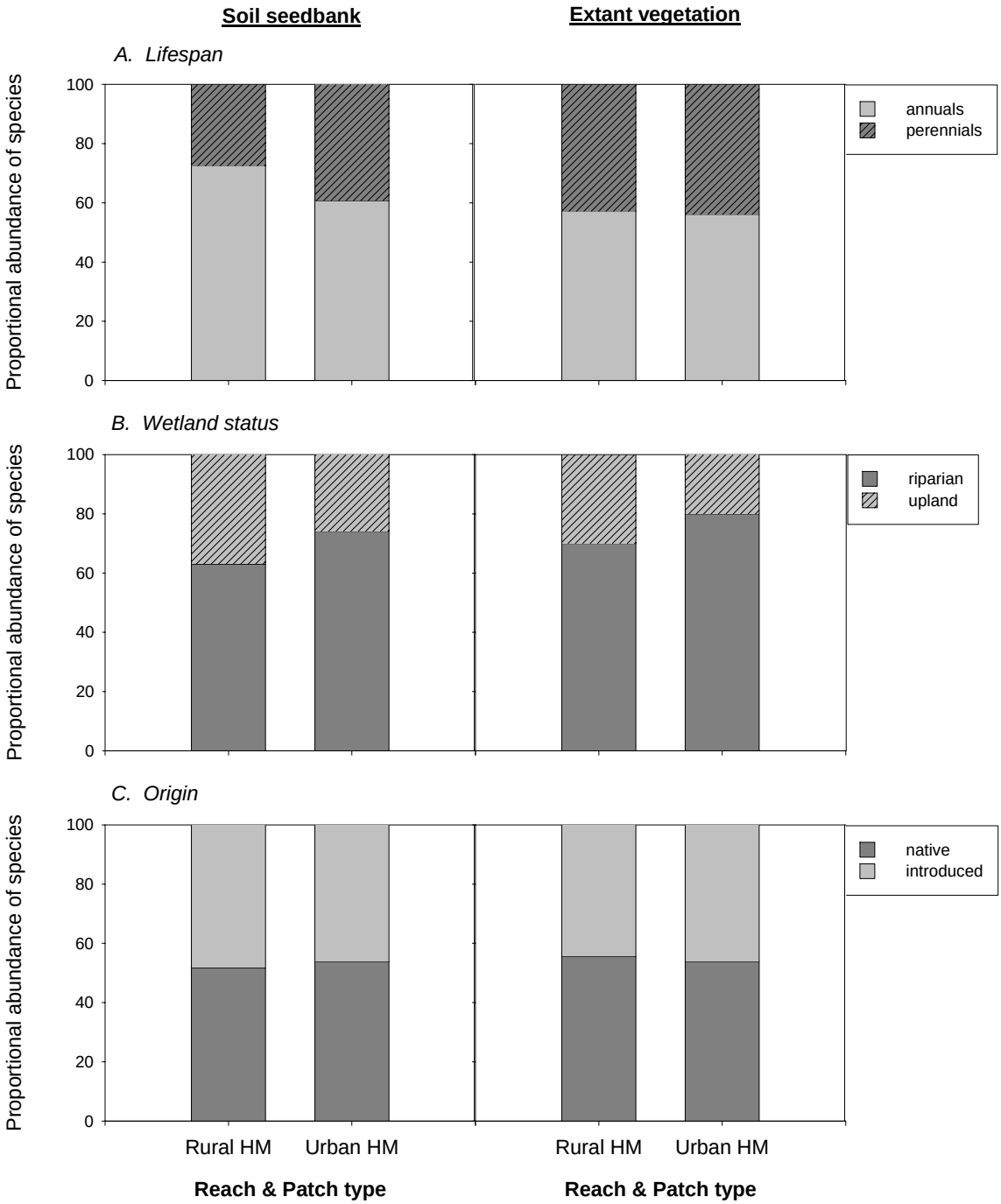


Figure 6: Composition of species in the soil seed bank and extant vegetation of the hydro-mesic (HM) forest patch type for rural and urban reaches.

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# Big Chino Basin 3-D Digital Hydrogeologic Framework Model

## Basic Information

| | |
|---|---|
| **Title:** | Big Chino Basin 3-D Digital Hydrogeologic Framework Model |
| **Project Number:** | 2005AZ89B |
| **Start Date:** | 3/1/2005 |
| **End Date:** | 2/28/2006 |
| **Funding Source:** | 104B |
| **Congressional District:** | 1 |
| **Research Category:** | Ground-water Flow and Transport |
| **Focus Category:** | Models, Groundwater, None |
| **Descriptors:** | Groundwater, Big Chino Basin, Hydrology, Models |
| **Principal Investigators:** | Abe Springer |

## Publication


1. Springer, A.E. and M.C. Fry. 2005. 3-D Visualization of Aquifers of Arizona Using the GeoWall. Annual meeting of the Arizona Hydrological Society, September 22-25, Flagstaff, AZ.
2. Springer, A.E. and M.C. Fry. 2005. Using the GeoWall to Visualize Aquifers of Arizona in Three Dimensions. Annual meeting of the Geological Society of America, October 16-19, Salt Lake City, UT.

## A. Problem and Research Objectives

*Abstract*

The Upper Verde River is one the largest remaining free-flowing, perennial rivers in the Southwestern U.S. The headwaters of the Verde River are a series of springs fed by groundwater from a large regional aquifer(s) which are the primary source of water for the first 29 miles of the upper perennial reach of the Verde River. These 29 miles of river support approximately 800 riparian acres with an average discharge of approximately 25 $ft^3$/sec. Although the location and discharge of the springs which form the Verde Headwaters is known, it has remained uncertain what the source areas of these springs are. The headwaters occur at the intersection of some very complicated geologic terrain at the intersection of Big Chino Basin, Little Chino Basin, Lower Granite Creek area, and bedrock of Big Black Mesa.

The subsurface geology of Big Chino Basin has only recently been defined. A complex combination of interfingering Tertiary sediments, sedimentary rocks, and basalt flows exist, along with faulting of carbonate rocks along the northeastern boundary of the basin. Understanding and quantifying groundwater flow in the Big Chino Basin aquifer first required a complete understanding of the geology of this region.

A Digital Hydrogeologic Framework Model (DHFM) has been constructed using EarthVision, a three-dimensional (3-D) geographic information system (GIS) software. The DHFM will be displayed with a 3-D viewer. Well logs contained in the ADWR well database were interpreted for lithology, and accurately located using GPS or parcel numbers contained within the well logs. These along with contacts digitized from a geologic map (DeWitt, in press) have served as the basis for the DHFM.

*Problem*

The lack of subsurface geologic knowledge in Big Chino Basin equates to a lack of understanding of the hydrogeologic properties of the region. These factors must be understood for effective use and conservation of groundwater in the region. Preliminary assumptions are that basin-fill sediments, fractured basalt flows, and adjacent/underlying carbonate rocks contain substantial groundwater storage for continued development in the area, but these assumptions largely lacked scientific evidence to support such claims. The primary concern lied in the groundwater contribution of this region to springs that form the Upper Headwaters of the Verde River.

Water managers in this region need to understand the geology and hydrogeology of the area in order to develop their resources effectively, and without causing unnecessary negative environmental impacts. The city of Prescott has pursued the possibility of developing Big Chino Basin as an additional source of water, but has been unable to do so, because the effects of high levels of groundwater pumping in the area have remained unclear. This study has served as one of the first steps in clarifying this issue.

*Objectives*

The final product is a georeferenced DHFM of the Big Chino Basin aquifer. It will assist local water managers in the region to effectively manage groundwater resources. It will also be used by the USGS Arizona Water Science Center in Tucson to construct a numerical groundwater flow model. The data, model, and study conclusions will be presented in public forums to relevant stakeholders during summer 2006 and will be available on-line.

## B. Methodology

*Model Area*

Selection of the model area boundaries were made with consideration to assumed hydrogeologic boundaries. The northeastern boundary is coincident with the anticlinal axis of Big Black Mesa and approximates a groundwater divide. Northeast of the boundary, stratigraphic units dip to the northeast, and are connected to aquifer(s) on the Colorado Plateau. Southeast of the boundary, beds dip to the southeast, and are connected to the Big Chino Basin aquifer. The northwestern model boundary was selected to incorporate regions of Big Chino Basin critical to understanding the applicable hydrogeologic properties, and at a lineation where all groundwater flow paths within the basin are perpendicular to the model boundary. The southwestern boundary of the model was selected to incorporate all critical areas of the Big Chino Basin. The southwestern model boundary was selected as to overlap a previously existing DHFM compiled by the principal investigators, which models the Lower Granite Creek and the Verde Headwaters bedrock region. The model dimensions are approximately 15 by 30 miles and contain approximately 450 square miles of the Big Chino Basin aquifer.

*Lithologic Interpretation*

Drillers well logs from over 80 wells contained in the ADWR database have been re-interpreted for lithology by the authors. Interpreted logs were also utilized from a recently published USGS report (Wirt and others, 2004). The tops of respective hydrogeologic units were selected from the interpreted logs and compiled into a database for insertion into EarthVision. Parcel numbers from the well logs and parcel layers obtained from the Yavapai County GIS office were used in ArcGIS v.9 (ESRI, Redlands, CA) to determine accurate locations for the wells.

*Selection of Hydrogeologic Units:*

Hydrogeologic units were selected based on their respective hydrogeologic properties and the amount of data available to accurately model their extent in the subsurface. The breakdown of hydrogeologic units is as follows:

Quaternary sediment (Qs):

Composed of predominantly alluvium along Big Chino Wash and gravels sourced from the Sullivan Buttes latites and other uplands surrounding the basin. These units are presumed to be of intermediate permeability and allow recharge via precipitation and surface water flow. These units are not considered to be part of the basin fill aquifer.

Tertiary sediments and sedimentary rocks (Ts):

Facies lumped into this category range from high permeability gravels to low permeability playa deposits (Wirt and others, 2004). Because of the limited well data available in all but the southeastern most extent of Big Chino Basin, these facies remain undifferentiated in this model. These facies comprise the majority of the volume of the basin-fill aquifer.

Tertiary volcanics (Tv):

At least three distinct subsurface basalt flows have been identified in Big Chino Basin. The flows have been dated to between 4 to 6 Ma and locally contain interbedded cinders. Geographically, the basalt flows are located in the southeastern reach of the basin, and flowed to the northwest during basin development. Primary porosity of basalt is low, but evidence suggests these units are highly fractured, based on field observations of degassing wells made by the authors. Wirt and others, 2004, suggest water discharging at the Upper Verde River springs may have traveled through significant amounts of basalt, due to elevated silica levels at the springs. The authors suggest the subsurface basalt flows as a potential link between the Big Chino Basin aquifer and the springs of the Upper Verde River.

Paleozoic sedimentary rocks (Ps):

Either the Mississippian Redwall Formation or the Devonian Martin Formation or both, underlie much of Big Chino Basin. Both units are limestones or dolomitic limestones and can have high secondary permeability via dissolution. Regionally, these two units are the predominant aquifer units, and are assumed to be directly linked to and form part of the basin fill aquifer.

Proterozoic basement (Xb):

The Proterozoic crystalline basement is comprised mainly of the Mazatzal Quartzite. Also lumped into this category are the younger Tertiary Latite intrusives of the Sullivan Buttes, and the Cambrian Tapeats sandstone. Primary porosity of the above units is low to very low, but locally fractures may create intermediate permeability (Wirt and others, 2004). These units comprise the lower confining unit for the basin-fill aquifer.

*Field Methodology*

Conflicting well locations were field checked to insure accuracy of the locations. Also, previously existing geologic map (DeWitt, in press) was field checked where there were uncertainties. Where possible, the surface traces of faults as exist on the geologic map were supplemented via field observations of fault plane dip and fault offset. These exercises served as a measure of QA/QC for data that was input into EarthVision.

*Laboratory Methodology*

Unit contacts were digitized from a geologic map (DeWitt, in press) in ArcGIS v.9 were compiled into the database created from interpreted well logs, for insertion into EarthVision (Dynamic Graphics, Alameda, CA). A surface was created for the top of each unit in EarthVision, using a minimum tension trend gridding algorithm. Where geologic data was absent or unsubstantial to properly represent strata based on the author's opinions of the subsurface geology, control points were added. A recently published geophysics report by the USGS was used to help the authors determine unit geometry in the subsurface (Langenheim and others, 2005).

## C. Principal Findings and Significance

*Principal Findings*

The extent and geometry of subsurface basalt flows and latite intrusions have been determined in this report. The extent and geometries of these basalt flows have allowed the authors to identify these flows as a potential groundwater link between the Big Chino Basin aquifer and the springs of the Upper Verde River.

*Significance*

This report has been able to improve on the understanding of the hydrogeologic conditions of the Big Chino Basin aquifer. More importantly, it has produced a georeferenced 3-D DHFM which can be interactively presented to water managers and the general public in order to increase their understanding of the system. Understanding the system is crucial in making water use and policy decisions in the region which have the potential to have significant environmental impacts on the Upper Verde River.

It is the opinion of the authors that continued groundwater pumping of the Big Chino Basin aquifer may impact the discharge of the springs of the Upper Verde River, though the impact is still unable to be quantified at this time. Continued research utilizing the findings of this report, principally the groundwater flow model being constructed by the Water Resources Division of the USGS in Tucson will help to further clarify this issue.

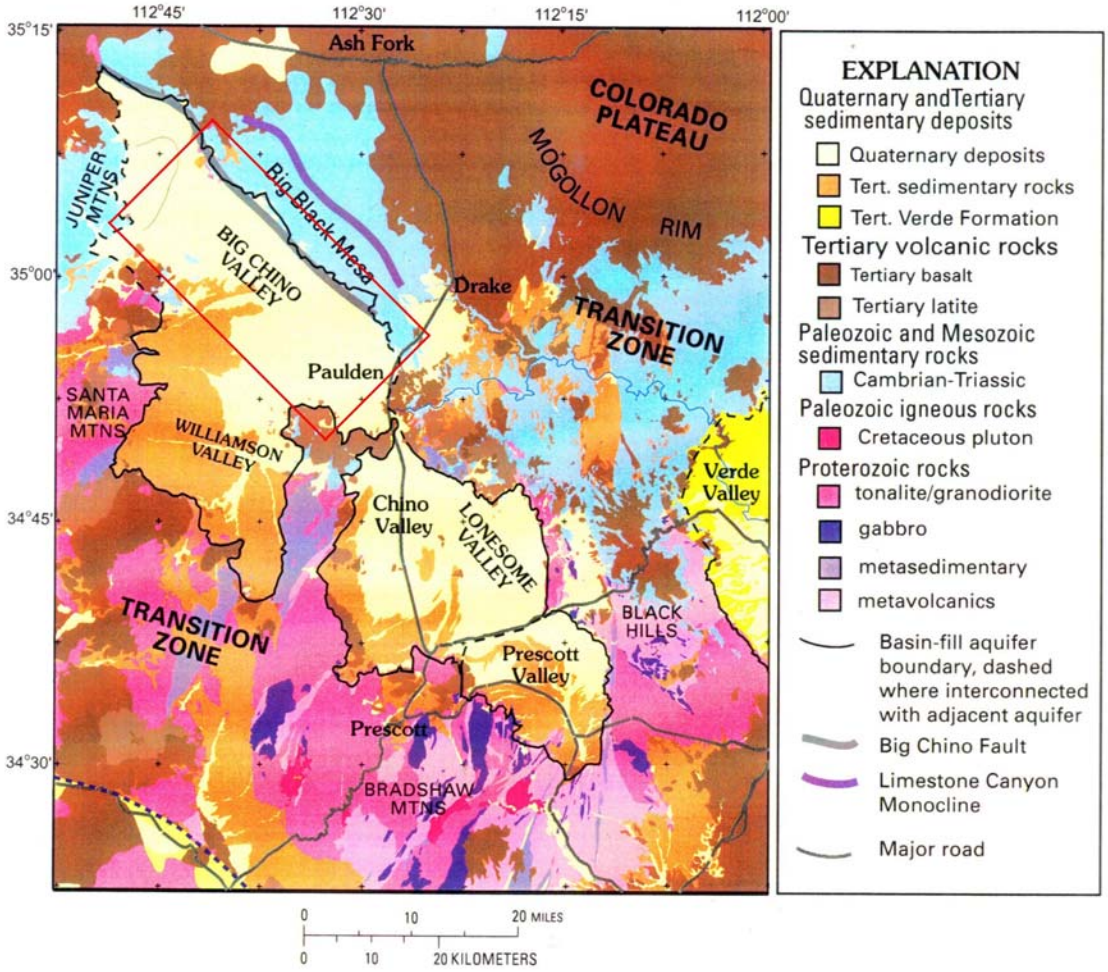


Figure 1: Simplified geologic map of the Verde Headwaters region. Model area shown in red rectangle. Modified from Wirt and others, 2004.

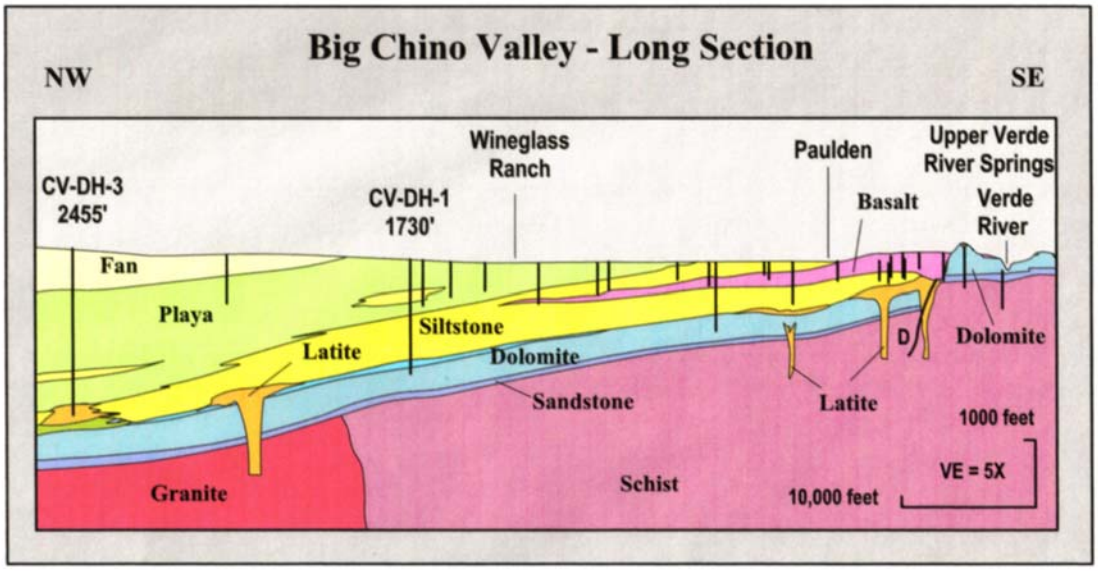


Figure 2: Big Chino Basin long axis cross-section (DeWitt, unpublished).

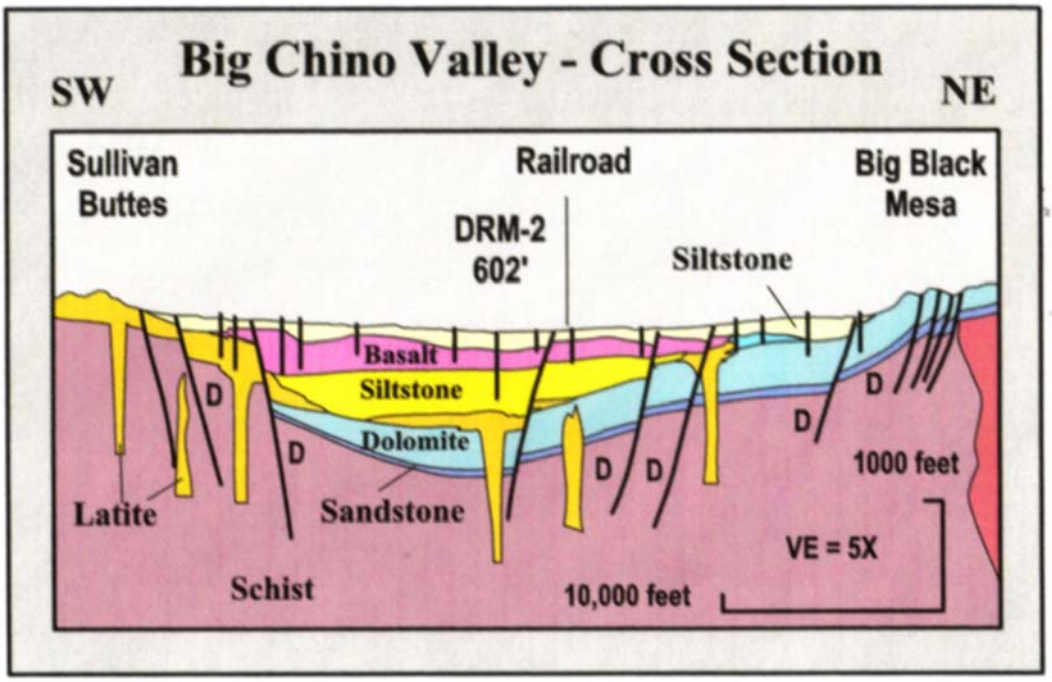


Figure 3: Big Chino Basin short axis cross-section (DeWitt, unpublished).

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# An Outdoor Multi-Stage, Continuous-Flow Photobioreactor for Bioremediation of Nitrate-Contaminated Groundwater

## Basic Information

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|---|---|
| **Title:** | An Outdoor Multi-Stage, Continuous-Flow Photobioreactor for Bioremediation of Nitrate-Contaminated Groundwater |
| **Project Number:** | 2005AZ90B |
| **Start Date:** | 3/1/2005 |
| **End Date:** | 2/29/2006 |
| **Funding Source:** | 104B |
| **Congressional District:** | 5, 6 |
| **Research Category:** | Water Quality |
| **Focus Category:** | Groundwater, Nitrate Contamination, Treatment |
| **Descriptors:** | Nitrate, Groundwater, Bioremediation |
| **Principal Investigators:** | Qiang Hu, Milton Sommerfeld |

## Publication


1. Bellefeuille, Mike (2005) Use of Selected Microalgae for Environmental Bioremediation, MS Thesis, School of Life Sciences, College of Liberal Arts and Sciences, Arizona State University, Tempe, AZ, pp. 74.
2. Case, Natalie (in progress) Screening and characterization of high-performance microalgae for bioremediation of nitrate-contaminated waters, Ph.D. Dissertation, Department of Applied Biological Sciences, Arizona State University, Mesa, AZ.
3. Hanson, Karyn (in progress): Removal of Nutrients from CAFO Wastewater by Microalgae, M.S. Thesis, School of Life Sciences, Arizona State University, Tempe, AZ.
4. Hanson, Karyn, Qiang Hu and Milton Sommerfeld (2006). Prospects for nutrient removal from dairy wastewater by microalgae. Oral presentation at the Fourth Annual Graduate Student Research Conference (April 14, 2006), Arizona State University at the Polytechnic campus, Mesa, AZ 85212, Proceedings, Page11.
5. Case, Natalie, Milton Sommerfeld and Qiang Hu (2006). Utilizing microalgae to remove nitrate-contaminated groundwater. Poster presented at the Fourth Annual Graduate Student Research Conference (April 14, 2006), Arizona State University at the Polytechnic campus, Mesa, AZ 85212, Proceedings, Page12.
6. Bellefeuille, Michael, Qiang Hu and Milton Sommerfeld (2006). Use of the green microalgae

Scenedesmus for nitrate bioremediation, poster presented in Fourth Annual Graduate Student Research Conference (April 14, 2006), Arizona State University at the Polytechnic campus, Mesa, AZ 85212, Proceedings, Page18.

7. Bellefeuille, Michael, Qiang Hu and Milton Sommerfeld (2006). Biological nitrate removal from contaminated agriculture runoff water in an outdoor photobioreactor by the microalga Scenedesmus sp. Oral presentation at the Arizona-Nevada Academy of Science 50th Anniversary Meeting (April 7-8, 2006), University of Arizona, Tucson, Arizona, Proceedings, Page 11.

## A. Problem and Research Objectives

Groundwater contributes more than 40% of the Arizona's drinking water supply, making it a precious, yet vulnerable resource critical to Arizonans' health, and to the State's and region's economic prosperity. Nitrate contamination in groundwater has been identified by several federal agencies (e.g., USEPA, USDA) as one of the most widespread and severe environmental problems in the state of Arizona and many other parts of the country. In Arizona, over 10 percent of the groundwater wells tested (more than 1,000 wells) have been reported to exceed the maximum recommended concentration of 10 milligrams per liter (mg $L^{-1}$) of nitrate as nitrogen ($NO^{-3}$-N) in drinking water (Pontius 1993). This is equivalent to 45 mg $L^{-1}$ of nitrate ($NO^{-3}$). The major groundwater pollutant sources include agricultural activities, industrial waste, leaking underground storage tanks, septic tanks, landfills, mining and wastewater treatment plants. Many of the groundwater quality problems are located in the Phoenix and Tucson metropolitan areas, although groundwater quality problems are found in all of Arizona's 10 watersheds. Particularly large portions of the aquifers within the Salt River Valley, including areas of Glendale, Mesa, Chandler and Phoenix, contain groundwater with nitrate concentrations high enough to render the water unfit for potable use. In addition, high nitrate levels occur in Marana, St. David, Quartzsite, Bullhead City, Lake Havasu City and other areas. Animal feeding operations and septic tank discharges are common nitrate sources in rural areas of Arizona and have contaminated many drinking water wells. Quartzsite, Bullhead City and Lake Havasu City are just a few locations with documented nitrate problems from septic tanks (*ADEQ's FY '02 Groundwater Assessment*, http://www.azdeq.gov).

High concentrations of nitrate in groundwater can pose a serious health risk to the State's residents, particularly in places where residents rely on groundwater supplies for drinking water. It has been shown that high levels of nitrate can be fatal to infants when nitrate is reduced to nitrite in the stomach, and the latter combines with hemoglobin in the blood to form methemoglobinanemia, leading to a condition known as "blue baby syndrome" (Gangolli et al. 1994). Reduction of nitrate to nitrite can also represent a risk to adults deficient in glucose-phosphate dehydrogenase (Pontius 1993). Moreover, nitrite can react with secondary amines or amides in water or food to form *N*-nitroso compounds that are potential animal carcinogens (Gangolli et al. 1994). Long-term consumption of drinking water containing nitrate concentrations of $\geq$ 18 mg $L^{-1}$ has also been reported to contribute to the risk of non-Hodgkin's lymphoma (Ward et al. 1996).

Shortage of surface water supplies, especially due to the continuing drought across the State in the recent years, coupled with rapid increase in population, has already placed heavy pressure on Arizona's cities and water supply utilities to treat available groundwater. To convert nitrate-contaminated groundwater into acceptable human drinking water resources, a number of treatment options have been proposed or tested. These options include microbial-based nitrification and denitrification, and chemically and physically-based technologies, such as ion exchange, reverse osmosis, electrodialysis and catalytic denitrification (Kapoor and Viraraghavan 1997). However, these treatment processes are often difficult to accomplish on a large scale and very expensive. They require not only a large capital investment, but also have high operation and maintenance

costs. Additionally, input of chemical additives (e.g., organic carbon sources, salts, acids, or base solutions) generates concentrated waste-streams that then must be treated and properly disposed. High costs have prevented many cities, especially those small communities in remote areas from adopting these treatment strategies to treat their groundwater. Therefore, development of innovative, environmentally-friendly, and cost-effective sustainable technologies for treating nitrate-contaminated groundwater is becoming increasingly critical.

A novel photobioreactor-based algal biotechnology has been proposed by the ASU investigators for high efficient, sustainable removal of nitrate and possibly other contaminants from groundwater, while concomitantly producing renewable biomass. Two critical challenges to this concept were identified: 1) finding high performing algal species that can thrive in groundwater and take up nitrate at rates substantially higher than those previously reported; and 2) developing a large-scale photobioreactor to accelerate the biological process in a sustainable manner. In a previous Water Resources Research Center research grant (Grant No. 01-HO-GR-0113), the first critical challenge has been successfully met. As a result, four high-performance algal species have been isolated and evaluated in terms of nitrate removal rate. It was demonstrated that one of the species, a *Scenedesmus* strain, can remove 50 mg $L^{-1}$ nitrate as nitrogen from groundwater within 24 hours, a record high rate set for algae-based nitrate removal potential. In this grant research (Grant Number: 01-HQ-GR-0113), we have focused on the second challenge, i.e., development of a highly efficient, cost-effective photobioreactor. A Multiple-stage, Continuous-Flow Photobioreactor (MCP) has been designed, fabricated, and operated under outdoor conditions. The high-performance *Scenedesmus* strain was used as a model organism to evaluate the performance of microalgae in the MCP. Future R&D issues about the system scale-up, automation, and optimization are also discussed.

## B. Methodology

*Photobioreactor:* A prototype MCP system consisted of 6 flat-plate bioreactor units arranged in a linear fashion and located at a series of heights with one end bioreactor unit being at the highest position, whereas the other end bioreactor unit being at the lowest position. Individual bioreactor units were made of glass measuring ca. 210 cm long, 50 cm height, and 15 cm depth, and having a volume capacity of about 150 liters. The total culture volume capacity of the MCP prototype was slightly over 900 liters. Culture mixing was provided by a compressed air stream containing 0.5-1.0% $CO_2$ through tubing submerged at the bottom of the tank. All 6 bioreactor units were connected by piping, through which the culture suspension can overflow or cascade down from one reactor unit to another by gravity. An evaporative cooling system was installed in the MCP and cooling water was collected and reused.

*Organism*: The high-performance *Scenedesmus* strain was used to evaluate the performance of the prototype MCP. *Scenedesmus* cells were grown in the lab-scale bioreactor to generate sufficient inoculum. Then, the *Scenedesmus* cells were transferred

into the outdoor MCP system to pre-culture for up to 2 to 3 days to allow adaptation of the algal cells to the natural conditions before starting trials.

*Growth measurement*: Algal growth was measured using optical density. Optical density of the culture was measured with a UV-Vis spectrophotometer at a wavelength of 750 nm.

*Nutrient analysis*: $NO_3^-$ measurement wwas performed on a Bran-Luebbe TrAAcs 800 Autoanalyzer, a continuous flow wet chemistry autoanalyzer using the cadmium reduction method (APHA, #4-89). The instrument was operated according to the standard operating procedure provided by the manufacturer. The standards and reagents were prepared fresh the day of analysis. The standards were made from a 100 ppm concentration of sodium nitrate ranging from 0.01, 0.02, 0.05, 0.2, 0.8, 2.0, and 5.0 ppm. After every six samples, the blank and drift were measured.

*Nitrate uptake rate*: Cellular nitrate uptake rate of individual algal species was calculated using the following equation:

*Nitrate uptake rate* (mg N $L^{-1}$ $h^{-1}$) = $(LnN_2 – LnN_1)/(t_2 – t_1)$
Where $t_1$ and $t_2$ represent different time points, and $N_1$ and $N_2$ represent nitrate concentration in the growth medium at time $t_1$ and time $t_2$, respectively.

## C. Principal Findings and Significance

Fabrication and Installation of the Prototype Photobioreactor Module

*Overview*: The prototype MCP module is shown in **Figure 1**. The MCP module consisted of 6 separate, identical culture tanks. The volume capacity of the MCP module was 900 liters. The module is supported on a welded steel frame, which rests on 9 small concrete pads. Six glass tanks were set on the frame in stair-step fashion to allow full solar illumination. The tanks were each aerated continuously and $CO_2$-rich air was fed into the aeration system from a $CO_2$ cylinder during daylight hours. Evaporative cooling was provided by spraying water on the front surface of the tanks. Culture flowed from the top tank through intermediate tanks to the lowest tank, where it was harvested. Essentially nitrate-free water from the last reactor unit will be subjected to a separation process where algal biomass will be removed from the water by means of centrifugation, sand/membrane filtration or dissolved air flotation. The purified groundwater can then be discharged into rivers, canals or delivered into the conventional water treatment plant for regular water treatment. While a small portion of harvested algal cells can be used as inoculum for the individual reactor units, bulk quantities of algal biomass generated from the process will be subjected to downstream processing to obtain wet/dry algal biomass for various potential applications.

**Figure 1.** an outdoor multi-stage, continuous-flow photobioreactor (MCP) for groundwater nitrate removal.

Design and construction details are presented below:

*Support Frame***:** The module was supported on a welded steel frame made of 1" square tubing, primed and painted to retard corrosion. Tube ends are sealed shut to exclude moisture (**Figure 2**). The frame is supported on 9 small concrete pads to prevent movement with wet soil or erosion (**Figure 3**). Six reactor units were set on the frame in stair-step fashion to allow full solar illumination. Reactor units had a narrow base, and each one was attached to an upright post on the frame, to prevent tipping.

**Figure 2.** A welded steel frame made of 1" square tubing for bioreactor support.

**Figure 3**. Nine small concrete pads to support the steel frame.

*Reactor tank units***:** Each tank is measuring ca. 210 cm long, 50 cm height, and 15 cm depth. Standard window glass is used, with a thickness of ¼ inch (**Figure 4**). All sides were glass, but the top was open. Glass panes were cemented together with silicone adhesive, and the glass portion was self-supporting as many aquariums are. A hole was provided in the bottom pane for plumbing. Tanks had a protective trim around the top and bottom edges, and flexible pads isolated them from the steel frame, to accommodate thermal stresses and unevenness in the frame. Clear plastic top covers were provided to reduce airborne contamination and water evaporation, while allowing additional solar radiation to enter algal culture to enhance photosynthesis (**Figure 5**). A PVC piping

system allowed water to flow from higher to lower tanks as groundwater was added, and the piping system had provision for draining the tanks when needed.

**Figure 4.** A glass tank.

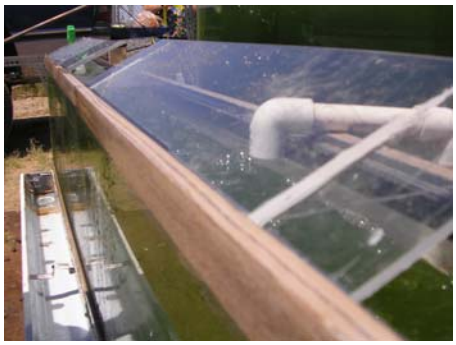

**Figure 5.** Clear polycarbonate sheet cover on tope of the glass tank.

*Aeration***:** Aeration was necessary to maintain algal cells in suspension for effective photosynthesis and also to facilitate cellular nutrient uptake and enhance gas mass transfer for $O_2$ and $CO_2$. Aeration was provided by a Sweetwater regenerative blower at 70 cfm. The blower was bolted to a concrete pad behind the reactor (**Figure 6**). The blower was shaded to limit overheating. The potential for overheating of the blower on hot summer days needs to be evaluated.

**Figure 6**. A Sweetwater regenerative blower for culture aeration.

**Figure 7**. A PVC manifold system conveys compressed air into individual glass tanks for aeration.

Compressed air from the blower was delivered to individual tanks through a PVC manifold system at a pressure of 1 psi. The amount of air entering the tank was controlled by a valve installed right above the tank (**Figure 7**). A Fiskar 5/8" garden soaker hose was placed at the bottom of the tank to deliver air bubbles in individual tanks. Fiskar soaker hose is used for pond aeration applications and produces medium-size bubbles. The hose weighted with steel rod encased in PVC tubing, and it was removable from the top of the tank for service. Carbon dioxide from a gas cylinder was added into the inlet of the air manifold system connected to the blower. Injection of $CO_2$ into the air stream at a final concentration of ca. 0.1 liter of $CO_2$ per liter of air per minute (vvm) took place during the daylight hours. A timer shut off the $CO_2$ supply at night (**Figure 8**).

**Figure 8.** $CO_2$ supply system.

*Cooling System***:** A cooling system is an important design component of an outdoor photobioreactor to prevent potential overheating of algal culture by solar radiation and to a lesser extent, heat from aerated air contributes to water heating. A low-cost, simple evaporative cooling system was used to maintain the culture temperature below 36$^{o}$C. Misting heads spray water on the front faces of the glass tanks, cooling the tanks as it evaporates. Water was sprayed from a distance of 25 cm in front (south) of each tank, using six standard misting heads per tank (**Figure 9**). The misting heads were mounted on ½" copper tube. Excess water running down the glass flowed into a standard rain gutter and was recycled through a 7.5 cm drain line that flowed into a 170-liter underground reservoir (**Figure10**). A sump pump in the reservoir pressurized the water for delivery to the copper tube. Periodically, water was added to the reservoir to maintain the constant water level by an automatic water leveling device. A thermostat in one tank actuated the cooling pump to feed cooling water when the temperature rises above 35$^{o}$C. It appears that the evaporative cooling system installed in this bioreactor was sufficient to maintain the culture temperature at an optimal temperature range of 25 to 35$^{o}$C during daylight hours.

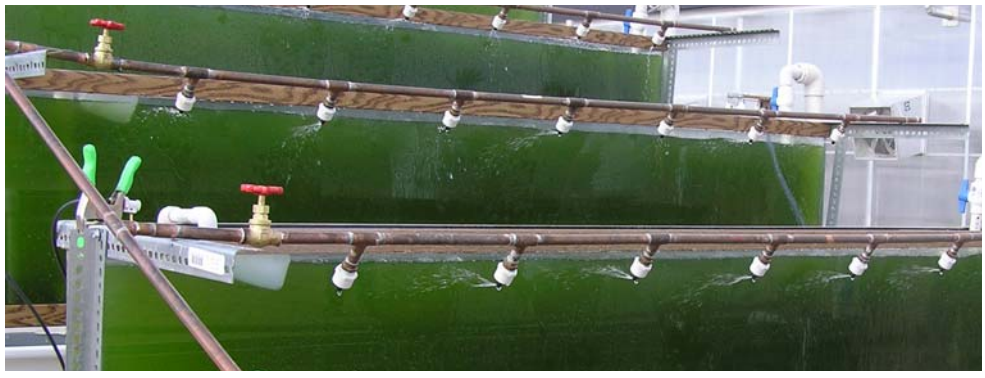

**Figure 9.** A evaporative cooling system for culture temperature control.

**Figure 10**. A underground cooling water reservoir receives the water from and delivers to glass tanks using a sump pump.

***Power*:** Electricity was required for the aeration pump, cooling pump, and control boxes. Power was provided through a standard 15-amp 1-phase 120V line.

Performance of Algal Culture in the Prototype MCP Module

*Scenedesmus* cells were first grown in a column photobioreactor outdoors for four days and then inoculated into the MCP module in a 1 to 10 ratio (one part seed culture added into 10 parts of groundwater). **Figure 11** shows a *Scenedesmus* culture maintained in the prototype MCP module two days after inoculum. Our preliminary results indicate that algal cells grew more rapidly in the MCP module than the column photobioreactor which was also developed by our research team (data no shown). **Figure 12** shows growth and the nitrate removal of *Scenedesmus* cells grown in the column photobioreactor in March 2006. The cell concentration in the culture, as indicated by optical density measured at a wavelength of 730 nm, increased gradually over a six-day period (**Figure 12A**). Note that the increase in cell concentration occurred during the daylight only, indicative of obligate photoautotrophic nature of the algal strain. As alga proliferated, nitrate levels in the groundwater decreased from 54 mg $NO_3^-$-N $L^{-1}$ to below 10 $NO_3^-$-N $L^{-1}$ within four days, representing a daily nitrate removal rate of ca. 10 $NO_3^-$-N $L^{-1}d^{-1}$ (**Figure 12B**). Little nitrate removal occurred during the night, confirming that the nitrate assimilation is photosynthesis/growth-dependent. The nitrogen removal rate of 10 $NO_3^-$-N $L^{-1}d^{-1}$ is at least 300% more efficient than other algal culture devices have reported previously (Blier et al. 1996). The higher the photosynthetic activity and growth, the higher the nitrate uptake rate. It is anticipated that the MCP module will facilitate superior algal performance and thus higher nitrate removal potential than other reactors. Quantitative evaluation of growth and nitrate uptake rate of *Scenedesmus* cells maintained in the prototype MCP module is continuing.

**Figure 11.** Operation of the multi-stage, continuous-flow photobioreactor for groundwater nitrate removal by *Scenedesmus* cells.

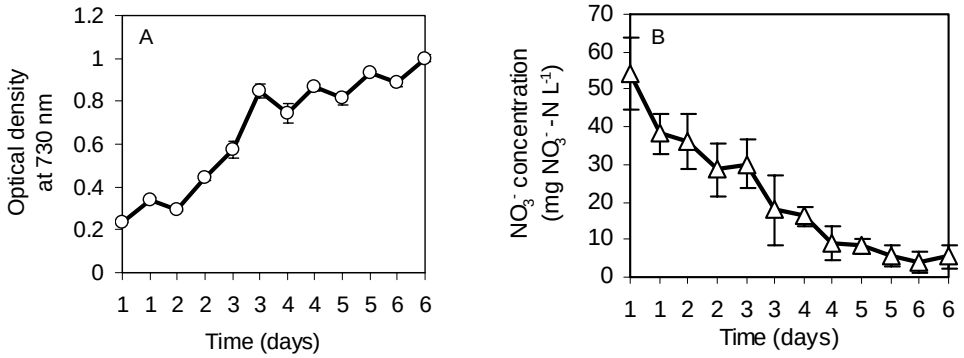


**Figure 12.** Growth (A) and nitrate removal (B) of *Scenedesmus* sp. grown in an outdoor column photobioreactor fed with groundwater.

Significance of the Prototype MCP Module Development

The major objective of the research project has been successfully fulfilled, and the work represents a major milestone in the effort to design, construct, and operate a commercial large-scale MCP modular photobioreactor. Continuation of this research using the prototype MCP module developed in this project and future designed large-scale MCP modular system is necessary to demonstrate that photobioreactor-based algal biotechnology has potential as an advanced engineered biological system for large-scale sustainable nitrate bioremediation.

**ACKNOWLEDGEMENTS**

The authors gratefully acknowledge the valuable advice and technical assistance of Professor Alvin Post in the Department of Mechanic & Manufacturing Engineering at Arizona State University. This research was supported by grants from the USGS 104B Grant Program.

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# Chemolithotrophic denitrification: The missing link in the biogeochemical cycle of arsenic

## Basic Information

| | |
|---|---|
| **Title:** | Chemolithotrophic denitrification: The missing link in the biogeochemical cycle of arsenic |
| **Project Number:** | 2005AZ114G |
| **Start Date:** | 9/1/2005 |
| **End Date:** | 8/31/2007 |
| **Funding Source:** | 104G |
| **Congressional District:** | AZ05 |
| **Research Category:** | Biological Sciences |
| **Focus Category:** | Toxic Substances, Groundwater, Non Point Pollution |
| **Descriptors:** | Denitrification, Arsenic, Non Point Pollution |
| **Principal Investigators:** | Reyes Sierra, James Field, Ronald S Oremland |

## Publication


1. Sierra-Alvarez, R., W. Sun, P. Rowlette, I. Cortinas and JA Field. 2005. Anoxic Oxidation of Arsenite Linked to Denitrification. Eighth International In Situ and On-Site Bioremediation Symposium. June 6-9, 2005. Baltimore, MD. (Conference proceedings).

# PROGRESS PROJECT REPORT

## USGS-National Competitive Grant Program

**Title**: **Chemolithotrophic Denitrification: The Missing Link in the Biogeochemical Cycle of Arsenic**. Federal Grant # 2005AZ114G. (09/01/2005 to 08/31/2007)

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**Background:** The most abundant forms of inorganic arsenic (As) in the natural aqueous environment are arsenate ($As^{V}$), encountered under aerobic conditions, and arsenite ($As^{III}$), which predominates in reduced anaerobic environments. Of the two commonly occurring species, $As^{III}$ is clearly more toxic to humans as well as microorganisms (1,2). Likewise, $As^{III}$ is generally more mobile than $As^{V}$ since it is less tightly adsorbed by aluminum- and iron-bearing minerals in soil and sediments (3-4).

Microorganisms play an important role in the conversion of As between the two commonly occurring oxidations states (5). Likewise, microorganisms catalyze conversions between solid ferric iron oxide and soluble ferrous iron ($Fe^{II}$), impacting the sorption of arsenic. The onset of reducing conditions has been recognized as one of the most important triggers associated with As contamination of groundwater. Naturally occurring- or anthropogenic sources of organic matter support the microbial catalyzed reductive dissolution of iron oxides as well as the microbial reduction of $As^{V}$ to $As^{III}$. These processes enhance the mobility and toxicity of arsenic. Conversely, the microbial oxidation of $As^{III}$ and $Fe^{II}$ is associated with decreased mobility and a lower toxicity of As in soils and sediments.

Chemolithotrophic microorganisms are well known that utilize dissolved oxygen (DO) to gain chemical energy from the oxidation of $As^{III}$ to $As^{V}$ (5) or the oxidation of $Fe^{II}$ to $Fe^{III}$ (6). However, the modest inventory of DO in water (max. 9 ppm) may be consumed by organic matter, sulfides, and other reducing compounds, preventing oxidation of $As^{III}$ and $Fe^{II}$ by aerobic chemolithotrophs. Recently, it has come to light that nitrate can be utilized by anaerobic microorganisms to gain energy from $As^{III}$ (5) and $Fe^{II}$ oxidation (7). Nitrate is very soluble and, therefore, can potentially occur in water at concentrations far exceeding the electron accepting capacity of water saturated with D.O. Evidence is increasing as to the importance of nitrate as a controlling factor of As mobility in the environment. Results from an urban lake have shown that nitrate levels in the anoxic zone are positively correlated with the formation of both $As^{V}$ and $As^{V}$ -adsorbing hydrous ferric oxides (8). Nitrate injected into As-contaminated groundwater of Bangladesh was shown to effectively lower the aqueous As concentration (9).

**Research Objectives:** The objective of this study is to evaluate the importance of chemolithotrophic denitrifying bacteria in the biogeochemical cycle of arsenic. Two types of biogeochemical impacts will be distinguished: *i)* the microbial oxidation of $As^{III}$ with nitrate; *ii)*

the microbial oxidation of $Fe^{II}$ linked to denitrification and the subsequent adsorption of $As^{V}$ by the iron oxides formed. The central question to be addressed in this proposal is whether anoxic oxidations of $As^{III}$ and $Fe^{II}$ are ubiquitous process in groundwater and surface waters. Our hypothesis is that these anoxic bio-oxidations are indeed ubiquitous.

The proposal will determine if the conversions linked to denitrification occur at significant rates. Likewise the project will attempt to identify the diversity of microorganisms involved and the nature of the chemolithotrophic reactions they carry out. Another pertinent question is whether the iron oxides generated by anoxic oxidation of $Fe^{II}$ will have the same high capacity for the sorption of $As^{V}$ as iron oxides generated by aerobic oxidation. Lastly, the project will investigate the natural attenuation of dissolved $As^{III}$ in continuous-flow sediment columns operated under denitrifying conditions.

**Summary of Progress to Date:** Considerable progress has been attained during the first 8 months of the current project. Results of batch experiments indicated that microorganisms capable of oxidizing $As^{III}$ with nitrate appear to be widely distributed (Table 1). Six out of the nine inocula screened, which included sludge from high-rate anaerobic wastewater treatment reactors and anaerobic sludge digestors, a denitrifying enrichment fed thiosulfate and anaerobic sediments from various sources, tested positive for anoxic $As^{III}$. Anoxic oxidation of arsenite was not detected with the only aerobic inoculum tested, municipal activated sludge. The ability of microorganisms in the Pinal Creek sediments to oxidize arsenite with nitrate could not be confirmed as $As^{III}$ was also oxidized in the absence of nitrate. The Pinal Creek sediments contain high levels of manganese. Formation of $As^{V}$ in the absence of nitrate was probably linked to reduction of manganese, a process that can be mediated both by chemical and microbial mechanisms.

**Table 1.** Biological anoxic oxidation of 500 µM of As(III) by different sources of environmental inoculum.

| **Environmental Sample** | **As(V) formation** | | **Time**[†] |
|---|---|---|---|
| | $+NO_3^-$ | $-NO_3^-$ | (d) |
| Anaerobic bioreactor sludge, distillery | + | - | <4 |
| Anaerobic bioreactor sludge, paper mill | - | - | |
| Municipal anaerobic digester sludge | + | - | 10 |
| Thiosulfate-denitrification enrichment | + | - | 10 |
| Municipal aerobic active sludge | - | - | |
| Duck pond sediments | + | - | <5 |
| Winogradsky column sediment | + | - | <5 |
| Pinal Creek sediments (high Mn) | + | + | |
| Groundwater | - | - | |

[†] Time to oxidize 500 µM As(III) to As(V) linked to denitrification

Anoxic chemolithotrophic enrichment cultures have been established that can couple denitrification to the oxidation of arsenite. Figure 1 illustrates the time course of anoxic $As^{III}$ oxidation with nitrate in municipal anaerobic digester sludge. $As^{III}$ (500 µM) was stoichiometrically oxidized to $As^{V}$ in the assays with living sludge. $As^{III}$ remained constant in the abiotic assays supplemented with heat-killed sludge and in control assays devoid of nitrate.

Flow-through laboratory-scale bioreactors were started up to investigate the anoxic oxidation of $As^{III}$ linked to chemolithotrophic denitrification. Figure 2 illustrates the time course of anoxic $As^{III}$ (3.75 mM) oxidation in periods when the reactor feed was supplemented with nitrate (day 440-510 and day 540-600) and in periods when nitrate addition was omitted (day 510-540).

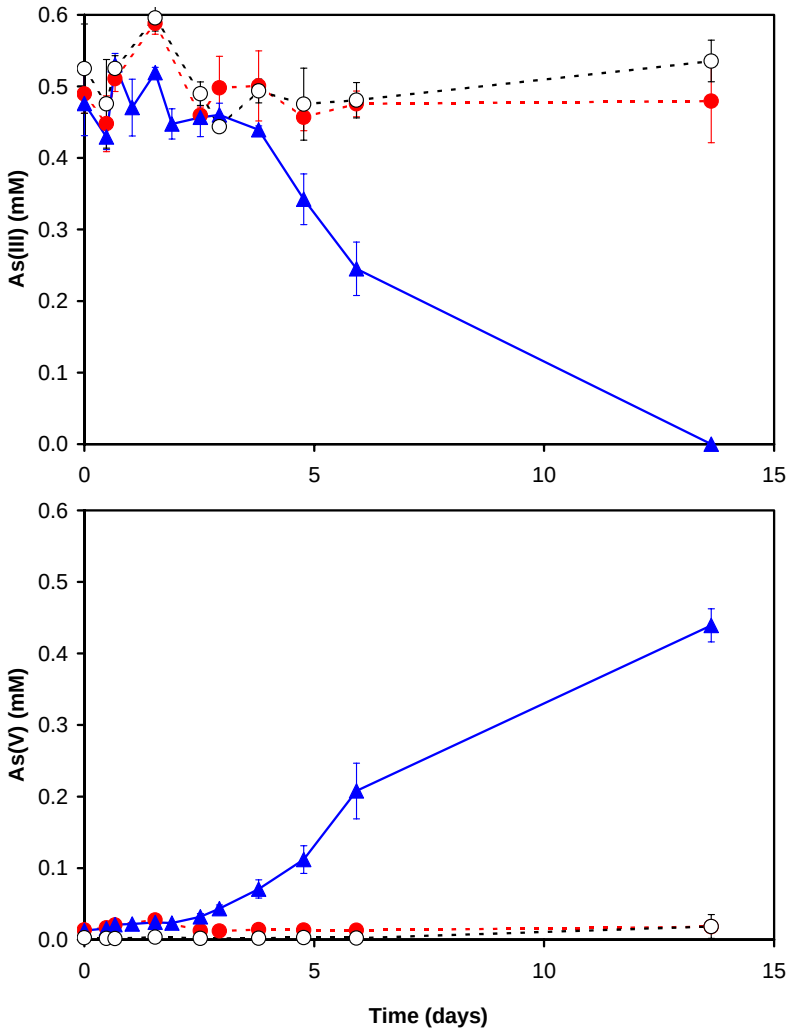


**Figure 1.** Anoxic oxidation of As(III) (0.5 mM) linked to denitrification in municipal anaerobic digester sludge. Upper panel shows time course of added As(III) concentration; lower panel shows time course of biologically formed As(V) concentration. Legend: living sludge and nitrate (10 mM), solid triangles; living sludge and no nitrate added, solid circles; heat killed sludge and nitrate added, open circles.

$As^{III}$ was almost completely oxidized to $As^{V}$ when nitrate was supplemented in the bioreactor influent. In contrast, no arsenite oxidation was observed when nitrate addition ceased. Nitrate removal exceeded the theoretical stoichiometric requirements for $As^{III}$ oxidation, suggesting that a fraction of the nitrate was utilized for endogenous metabolism. The disappearance of nitrate corrected for endogenous respiration coincided closely with the expected stoichiometry for complete denitrification with $As^{III}$. Batch bioassays performed with sludge withdrawn from the bioreactor and supplied with 3.5 mM $As^{III}$ confirmed that the net production of dinitrogen gas ($N_2$) corresponded to the formation of arsenate. In contrast with the only previously described isolate that was reported to reduce nitrate to nitrite (10), these findings indicate that the enrichment cultures developed in this study were capable of complete denitrification of nitrate to $N_2$.

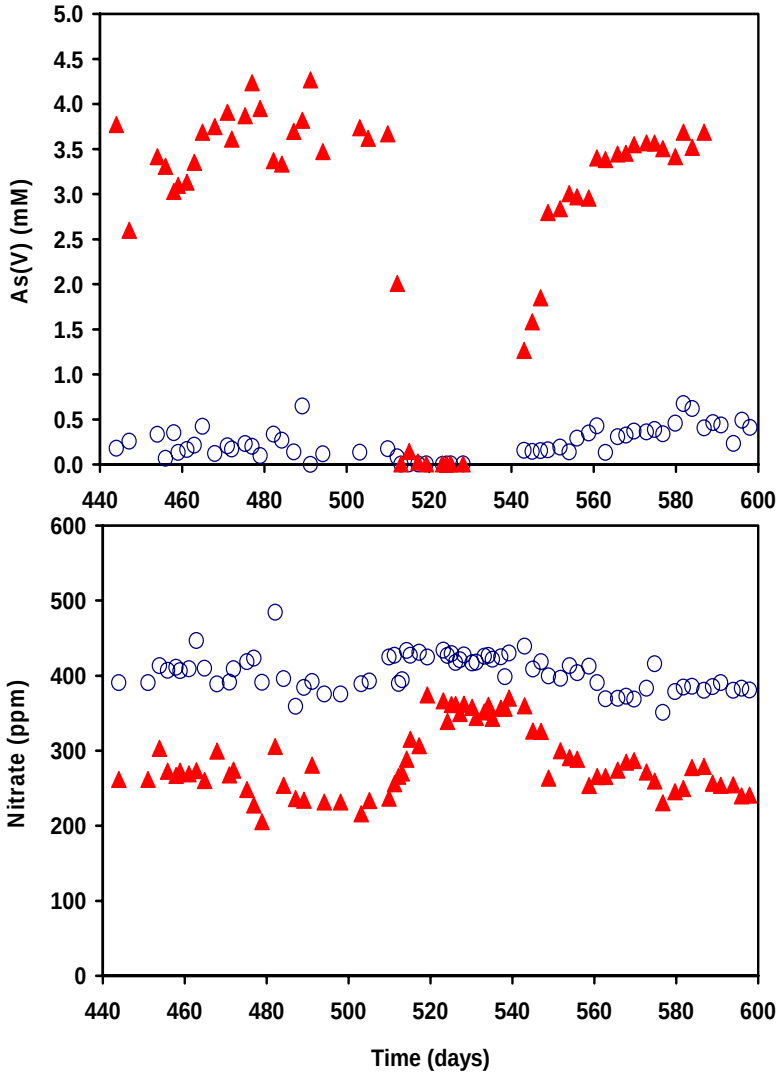


**Figure 2**. The effect of removing As(III) from the feed on nitrate removal in the continuous flow bioreactor which links the anoxic oxidation of 3.75 mM As(III) to denitrification. Upper panel shows the influent and effluent As(V). The lower panel shows the influent and effluent nitrate. Legend: influent, open circles; effluent, filled triangles.

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# Agricultural Chemicals as a Major Non-Point Source of Arsenic: Microbial Transformation of Organic Arsenicals

## Basic Information

| | |
|---|---|
| **Title:** | Agricultural Chemicals as a Major Non-Point Source of Arsenic: Microbial Transformation of Organic Arsenicals |
| **Project Number:** | 2002AZ9G |
| **Start Date:** | 9/1/2002 |
| **End Date:** | 8/31/2005 |
| **Funding Source:** | 104G |
| **Congressional District:** | AZ05 |
| **Research Category:** | Water Quality |
| **Focus Category:** | Toxic Substances, Agriculture, Non Point Pollution |
| **Descriptors:** | Agricultural Chemicals, Arsenic, Transformation of Organic Arsenicals |
| **Principal Investigators:** | James Field, A. Jay Gandolfi, John R Garbarino, Reyes Sierra, Robert L Wershaw |

## Publication


1. Cortinas, I., J. A. Field, M. Kopplin, J. R. Garbarino, A. J. Gandolfi and R. Sierra-Alvarez. 2006. Anaerobic biotransformation of roxarsone and related N-substituted phenylarsonic acids. Environ. Sci. Technol. (in press).
2. Sierra-Alvarez, R., U. Yenal, J. A. Field, M. Kopplin, A. J. Gandolfi and J. R. Garbarino. 2006. Anaerobic Biotransformation of organoarsenical pesticides, monomethylarsonic acid (MMAV) and dimethylarsinic acid (DMAV). J. Agric. Food Chem. (in press).
3. Sierra-Alvarez, R., I. Cortinas, U. Yenal and J. A. Field, 2004. Methanogenic inhibition by arsenic compounds. Appl. Environ. Microbiol. 70:5688-5691.

## A. Problem and Research Objectives:

### Introduction

Large quantities of arsenic (As) enter into environment through agricultural activity. Most of the widely used As-containing agricultural chemicals are organic (Figure 1). Monosodium methanearsonate (MSMA), disodium methanearsonate (DSMA), which are salts of monomethylarsonic acid (MMA(V)), and cacodylic acid (CA also known as demethylarsinic acid, (DMA(V)) are utilized as defoliants and herbicides. MSMA is among the top pesticides utilized in the USA with approximately 2.6 million kg applied annually to 3.8 million acres (26) which equates to 1.2 million kg of As. In addition, approximately 35 thousand kg of arsenic from DMA(V) are applied annually as defoliant (4). Roxarsone (3-nitro-4-hydroxyphenyl-arsonic acid) and p-arsanilic acid (4-amino-phenylarsonic acid) are used as growth promoting and antibiotic agents in poultry (and to a lesser extent swine). Based on broiler production and roxarsone feed dosage data, it has been estimated that approximately 900 metric tons of roxarsone are released annually into the environment in the U.S., which is equivalent to 250 metric tons of arsenic per year (12).

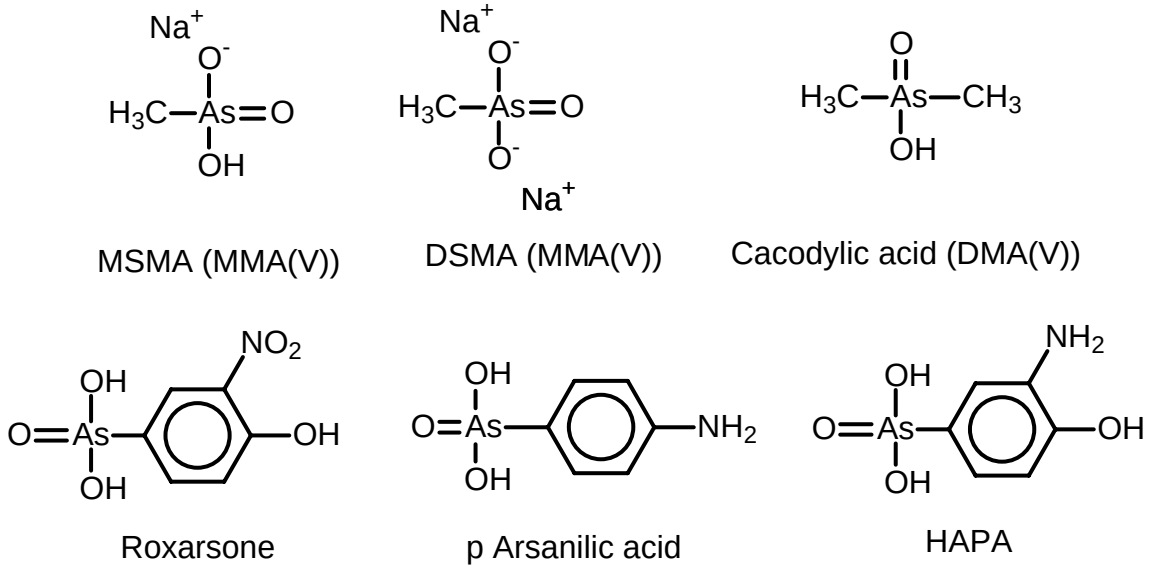



**Figure. 1.** Important organic arsenicals utilized in agriculture

These organoarsenical pesticides have been measured in the environment. In cotton producing areas, MMAV and DMAV are detected in surface water, groundwater and rivers (3, 4). The highest levels were observed in stagnant surface water, where concentrations reached as high as 10 and 100 ppb, of DMAV and MMAV, respectively. These pesticides were also detected in soil 1 to 1.5 y after their application to experimental field plots (1, 32). MMAV applied to golf course greens was detected in percolate water (9). The arsenic content of poultry litter ranges from 14 to 48 mg $kg^{-1}$ (2, 12, 15, 16). The environmental impact of land applying poultry litter is potentially significant when considering that this arsenic laden material is spread onto relatively small land

areas in the direct vicinity of poultry houses. Soil samples from field sites having received broiler poultry litter for years contain significantly higher concentrations of water extractable arsenic compared to similar soils with no history of poultry litter addition (7).

### Research Objectives

The objectives of this project were to study the microbial conversion of organic arsenicals that are introduced into the environment through agricultural practices. The main target agricultural chemicals are roxarsone and p-arsanilic acid utilized as a feed additives in poultry as well as MMA(V) and DMA(V) utilized as herbicides and defoliants in cotton. The project aims to identify major metabolites accumulating in the environment from the bioconversion of these organic arsenicals as well as evaluate their toxicity. Microbial processes responsible for key conversions will be studied to gain better insight on the mechanisms responsible for the biotransformation of organic arsenicals in the environment.

## B. Methodology

### Microorganisms

Anaerobic methanogenic sludge was used as inoculum in the assays. It was obtained from an industrial upward-flow anaerobic sludge blanket (UASB) treatment plant treating recycled paper wastewater (Industriewater, Eerbeek, The Netherlands) The sludge was washed and sieved to remove fine particles before use in the tests. The content of volatile suspended solids (VSS) in the Eerbeek was 12.9 %. The anaerobic sludge was stored under nitrogen gas at 4°C.

### Batch bioassay

Anaerobic biotransformation of organoarsenical compounds was performed in batch bioassay flasks that were incubated in a climate-controlled chamber at 30±2˚C in an orbital shaker (75 rpm). Serum flasks (160 ml) were supplied with 75 ml of a basal mineral medium (pH 7.0 - 7.2) containing (in mg $l^{-1}$): $NH_4Cl$ (280); $NaHCO_3$ (2000); $CaCl_2.2H_2O$ (10), $MgSO_4.7H_2O$ (100); $K_2HPO_4$ (600); $NaH_2PO_4.2H_2O$ (795); yeast extract (20) and 1 ml $l^{-1}$ of a trace element solution. The medium was also supplemented with organoarsenicals (concentrations indicated in Tables and Figures). The sludge inoculum was supplied at 1.5 g VSS $l^{-1}$, unless otherwise indicated. A mixture of volatile fatty acids (VFA) (concentration (as mM): acetate (7.5), propionate (6.1), butyrate (5.1)) equivalent to 2.5 g chemical oxygen demand (COD) $l^{-1}$was provided as a co-substrate in one experiment. Selected assays also received $SO_4^{2-}$ or $NO_3^-$ in order to support the sulfate reducing or nitrate reducing bacteria, respectively. Those assays also received the methanogenic inhibitor, 2-bromoethane sulfonate (30 mM final concentration). The final pH value of all media was adjusted to 7.0-7.2 with NaOH or HCl, as needed. Each experiment included several controls. Abiotic controls were prepared without adding microbial inoculum. Killed sludge controls were prepared by adding inoculum and subsequently placing the bottles in an autoclave for 1h at 120 ºC, the contents were subsequently allowed to cool down and then sealed aseptically. All flasks were sealed with butyl rubber stoppers and aluminum crimp seals. The liquid contents and headspace were flushed with $N_2$:$CO_2$ gas (80:20, v/v) to exclude oxygen from the assay. All assays were conducted in triplicates.

**Analytical Methods.** Inorganic arsenic species ($As^{III}$ and $As^{V}$), and organic arsenic species (roxarsone, roxarsone metabolites, $MMA^{V}$, $DMA^{V}$, $MMA^{III}$ and $DMA^{III}$) in liquid samples were analyzed by high performance liquid chromatography - inductively coupled plasma - mass spectrometry (HPLC-ICP-MS) using a method adapted from Gong *et al.* (12). Total arsenic concentration in liquid samples was determined by direct injection into the ICP-MS. All liquid samples were centrifuged and membrane filtered (0.45 μm) immediately after sampling and stored in polypropylene vials (2 ml). The filtered samples were then stored at -20°C until the analysis was performed in order to reduce changes in arsenic speciation. Volatile arsenic species were determined by flushing headspace of the biological assays bottles with $N_2$ gas for 20 to 24 h. The gas was bubbled through 20 ml of 2 M nitric acid used as scrubbing fluid. Samples of the scrubbing fluid were analyzed for total arsenic.

The sludge extraction was performed for the two of three replicated experimental assay bottles by using a sodium hydroxide (NaOH) extraction method. An aliquot of 20 ml of 1.0 M NaOH was added to sludge that was prewashed by decanting with demineralized water. The sealed serum flasks were shaken vigorously for 6 hrs in a water bath at 80 °C. A 1 ml of sample of the extractant was diluted with 9 ml of 0.1 M HCl. After centrifuging an aliquot in eppendorf, a final dilution was made in order to measure with the HPLC-ICP-MS.

The methane content in the headspace of the serum flasks was determined by gas chromatography using an HP5290 Series II system (Agilent Technologies, Palo Alto, CA) equipped with a flame ionization detector (GC-FID). Sulfate was determined by ion chromatography with suppressed conductivity using a Dionex system equipped with a Dionex AS11-HC4 column (Dionex, Sunnydale, CA) and a conductivity detector.

## C. Principal Findings and Significance

### Biotransformation of MMA(V) and DMA(V)

Anaerobic biotransformation of MMA(V) under methanogenic conditions. Several experiments were conducted in which anaerobic sludge was incubated with MMA(V) under methanogenic conditions. In a typical experiment in which 2.7 mM MMA(V) was added with either anaerobic sludge or heat killed sludge there is no decline in MMA(V) concentration in the heat-killed control, while there is a significant decline of the MMA(V) concentration in the living treatment (Figure 2A). At the end of the incubation of 236 days, 49.3% of MMA(V) was removed. From day 112 onwards, there was no further decline in the MMA(V) concentration suggesting inhibition by a biotransformation product. MMA(III) was identified as a biotransformation product with a maximum concentration of MMA(III) observed after 112 days, corresponding to a molar yield of 28.9% of MMA(V) removed (Figure 2B). MMA(III) was not detected in the heat-killed controls.

The biotransformation of MMA(V) was also tested at a higher concentration (14.3 mM). Even at this high concentration, some removal was observed, albeit only 24.0% after 236 days (experiment 1-2 in Table 1). Removal of MMA(V) completely ceased at day 112. The data listed in Table 1 show that MMA(III) was observed as a biotransformation product. Due to suspect inhibition of biotransformation products, all further tests were conducted with lower initial concentrations of MMA(V). The lower concentrations generally permitted greater extents of MMA(V) removal (experiments 4-2, 6-5 and 7-2 in Table 1). MMA(III) was consistently observed as a biotransformation product of MMA(V).

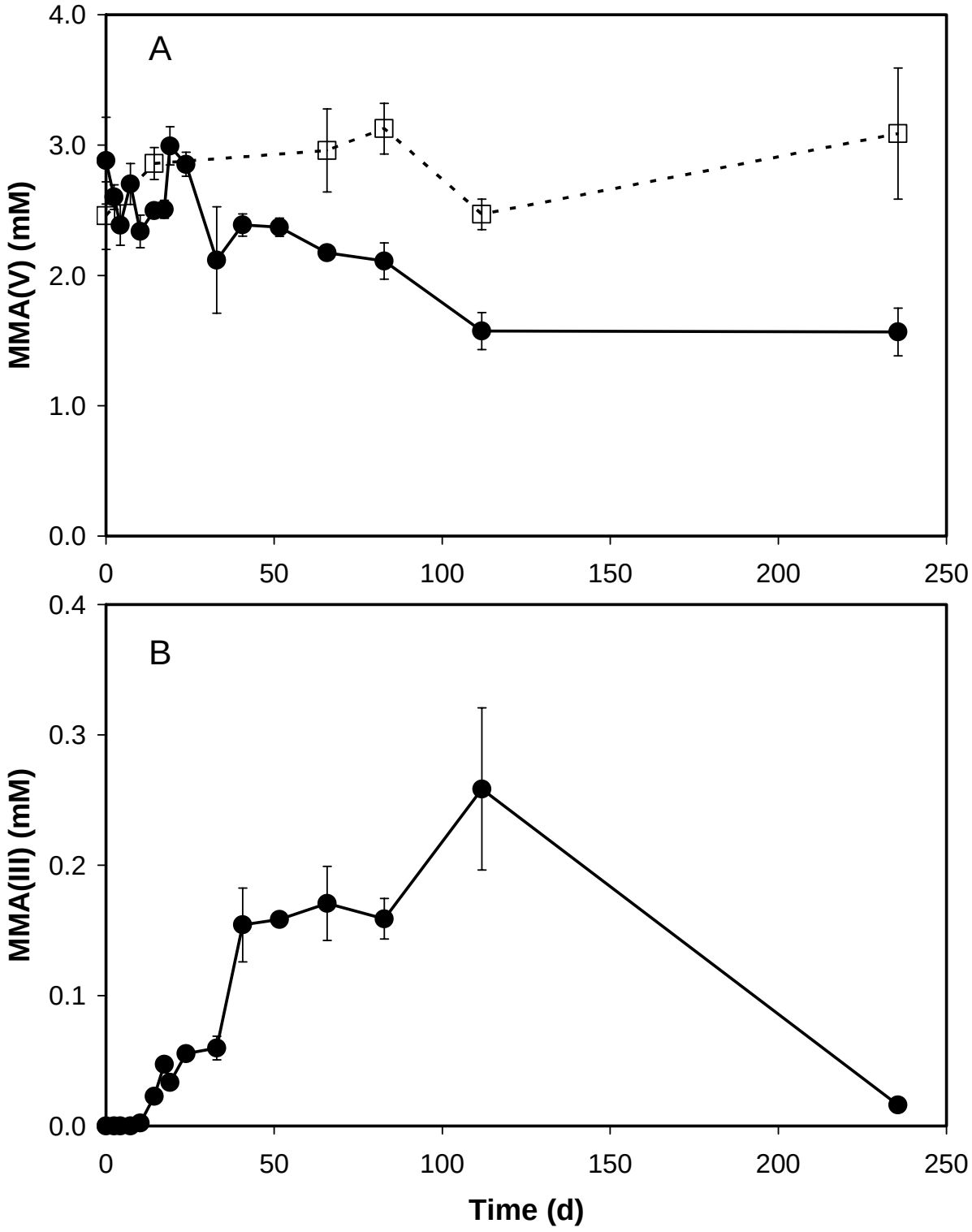



**Figure 2.** The biotransformation of MMA(V) (2.7 mM) during incubation with anaerobic sludge under methanogenic conditions. **A)** Removal of MMA(V); **B)** Formation of the intermediate, monomethylarsonous acid (MMA(III)). Legend: (open squares), heat killed control; (filled circle), biologically active.

**Table 1**. Extent of degradation, first order rate constants (*k*), and molar yield of detected intermediates during the biotrnsformation of MMAV (Part 1) and DMAV (Part 2).

| Exp. (#) | Duration (d) | Conc. (mM) | Redox Condition. | Added $e^-$ Donor | Degradation[1] (% ) | *k* ($d^{-1}$) | Intermediate | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | Compound | Conc. (mM) | Max. Molar Recovery[2] (%) | Time[3] (d) |
| Part 1. MMAV as Substrate | | | | | | | | | | |
| Exp.1-1 | 235.5 | 2.85 | Methanogenic | No | 49.3 | | MMA(III)[a] | 0.258 | 28.9 | 111.8 |
| Exp.1-2 | 235.5 | 14.25 | Methanogenic | No | 24.0 | | MMA(III)[a,b] | 0.281 | 5.3 | 111.8 |
| Exp.3-3 | 216.7 | 1.50 | Methanogenic | No | 49.4 | | MMA(III) | 0.048 | NA[4] | 47.9 |
| Exp.3-4 | 216.7 | 1.50 | Methanogenic | VFA | 26.5 | | MMA(III) | 0.053 | NA[4] | 47.9 |
| Exp.4-2 | 136.8 | 0.70 | Methanogenic | No | 80.0 | 0.0112 | MMA(III) | 0.045 | 13.5 | 80.8 |
| Exp.6-4 | 56.9 | 0.70 | Methanogenic | No | 39.6 | 0.0155 | MMA(III) | 0.052 | 19.4 | 29.8 |
| | | | | | | | DMA(V) | 0.005 | 2.5 | 56.9 |
| Exp.6-5 | 56.9 | 0.70 | $SO_4^{-2}$ reduct. | No | 95.8 | 0.0408 | MMA(III) | 0.089 | 15.6 | 29.8 |
| | | | | | | | DMA(V) | 0.009 | 2.0 | 56.9 |
| Exp.7-2 | 142.8 | 0.70 | $SO_4^{-2}$ reduct. | No | 74.1 | 0.0106 | MMA(III) | 0.279 | 46.7 | 142.8 |
| Part 2. DMAV as Substrate | | | | | | | | | | |
| Exp.2-1 | 207.0 | 1.00 | Methanogenic | No | 95.3 | 0.0126 | MMA(V) | 0.157 | 33.0 | 7.0 |
| Exp.2-2 | 81.1 | 5.00 | Methanogenic | No | 32.6 | | MMA(V) | 0.716 | 65.0 | 27.8 |
| Exp.3-1 | 216.7 | 1.00 | Methanogenic | No | 95.4 | 0.0148 | MMA(V) | 0.113 | 21.7 | 17.9 |
| Exp.3-2 | 216.7 | 1.00 | Methanogenic | VFA | 75.4 | | MMA(V)[a] | 0.084 | 12.9 | 23.9 |
| Exp.4-1 | 44.0 | 0.50 | Methanogenic | No | 97.8 | 0.0983 | MMA(V) | 0.001 | 0.2 | 44.0 |
| Exp.6-1 | 56.9 | 0.50 | Methanogenic | No | 99.8 | | MMA(V) | 0.038 | 12.3 | 56.9 |
| Exp.6-2 | 56.9 | 0.50 | $SO_4^{-2}$ reduct. | No | 100.0 | | MMA(V) | 0.049 | 9.9 | 20.8 |
| Exp.7-1 | 46.8 | 0.50 | $SO_4^{-2}$ reduct. | No | 99.6 | 0.0881 | As(V)[c] | 0.034 | 7.8 | 21.0 |

[1] % degradation = 100 * (killed control$_{tx}$ - treatment$_{tx}$) / killed control$_{tx}$;

[2] Max. Molar Recovery = 100 * intermediate$_{tx}$ / (killed control$_{tx}$ - treatment$_{tx}$)

[3] Time of maximum molar recovery; [4]Cannot calculate due to unreliable values of control

[a] As(III) is detected as an intermediate in trace amount; [b] As(V) is detected as an intermediate in trace amount; [c] MMA(V) is detected as an intermediate in trace amount

Anaerobic biotransformation of MMA(V) under different redox conditions. An experiment was conducted comparing the anoxic degradation of MMA(V) (0.7 mM) under methanogenic, sulfate reducing, and denitrifying conditions (Figure 3A). The results indicate bioconversion of MMA(V) under the methanogenic and sulfate reducing conditions and no conversion under denitrifying conditions. The results illustrate a significant decrease in the MMA(V) concentration in the active treatments compared to non-inoculated controls and controls with heat-killed inoculum. Sulfate reducing conditions promoted a more extensive conversion of MMA(V) compared to methanogenic conditions. The sulfate-reducing assay removed 95.8% of the MMA(V) by the end of the experiment (day 57). MMA(III) was the most important biotransformation product identified (Figure 3B). The maximum molar yields of MMA(III) ranged from 15.6 to 19.4% (experiments 6-4 and 6-5 in Table 1). No MMA(III) was observed in the controls or in the denitrifying culture. To a lesser extent DMA(V) was also observed as a metabolite of MMA(V) conversion at the end of the assay; however, its molar yield was only 2.0 to 2.5%. Some DMA(V) (12 μM) was also detected in the denitrifying culture.

A second experiment was conducted to confirm biotransformation of MMA(V) under sulfate reducing conditions (experiment 7-2 in Table 1). Extensive biologically mediated removal of MMA(V) was again observed. Likewise, MMA(III) was an important product of the biotransformation.

Anaerobic biotransformation of DMA(V) under methanogenic conditions. The anaerobic biodegradability of DMA(V) by methanogenic sludge was also tested in a variety of experimental conditions. Results from a typical experiment are shown in Figure 4a in which 1.0 mM was incubated with either anaerobic sludge or heat-killed sludge. There is no decline in DMA(V) concentration in the heat-killed control, while there is a rapid decline in the DMA(V) concentration in the living treatment (Figure 4a). At the end of the incubation of 207 days, 95.3% of DMA(V) was removed. MMA(V) was identified as an important biotransformation of DMA(V) (Figure 4b). The maximum accumulation of MMA(V) occurred on day 7 at which time, the molar yield was 32.6% of the DMA(V) removed. Only minor amounts of MMA(V) were incidentally detected in the heat-killed control.

The biotransformation of DMA(V) was also tested at a higher concentration of (5 mM). Biologically mediated removal was also observed at the higher concentration, but only 32.6% was removed after 81 days (experiment 2-2 in Table 1). Removal of DMA(V) decreased after the first week and finally ceased at day 52, suggesting the occurrence of a toxic intermediate. All further tests were conducted with initial concentrations of DMA(V) of 1.0 mM or lower to avoid inhibition problems. Working at the lower concentrations permitted extensive degradation of DMA(V) (Table 1). MMA(V) was observed as the biotransformation intermediate of DMA(V) in most of the experiments conducted.

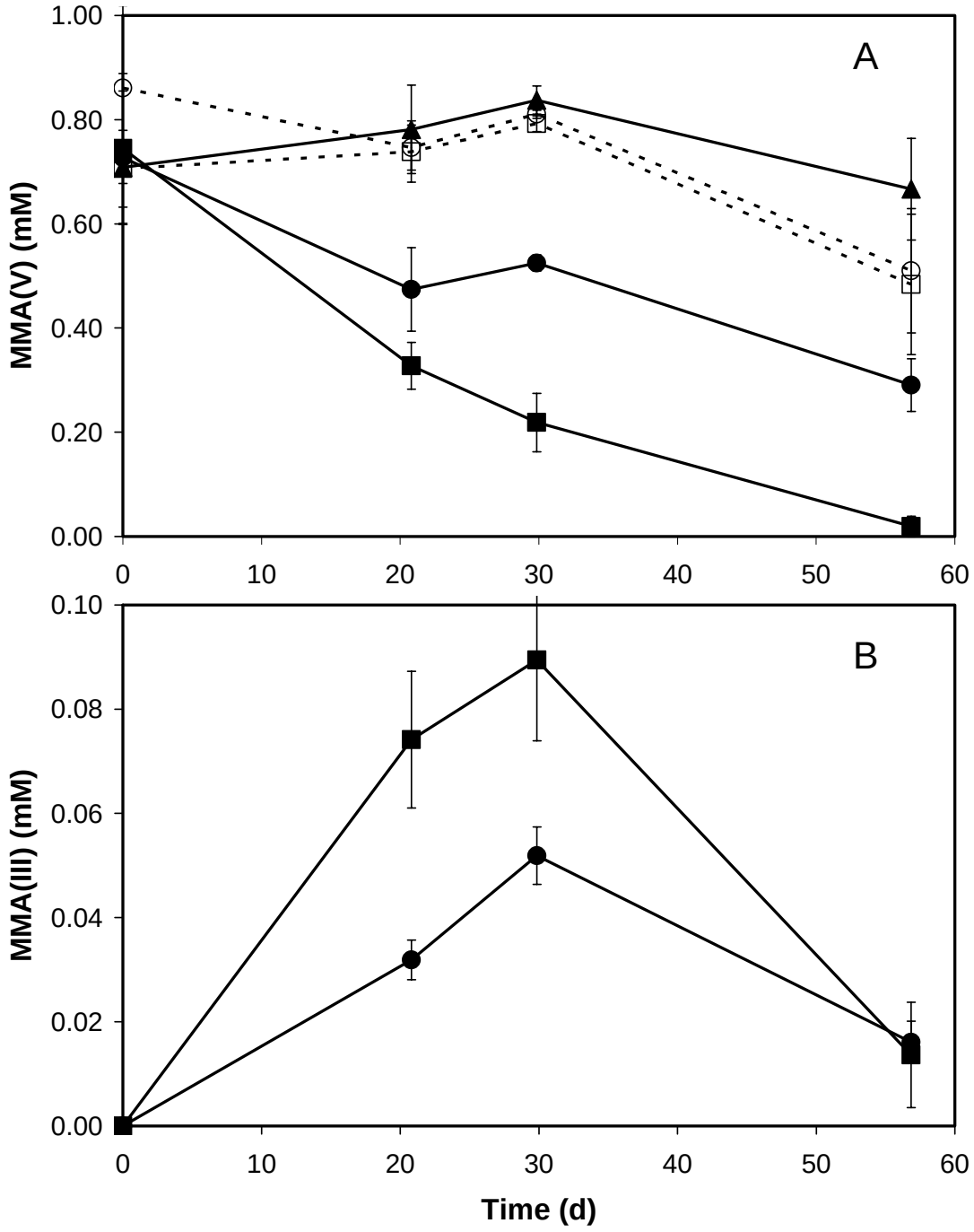



**Figure 3.** The biotransformation of MMA(V) (0.7 mM) during incubation with anaerobic sludge under methanogenic, sulfate reducing or denitrifying conditions. **A)** Removal of MMA(V); **B)** Formation of the intermediate, MMA(III). Legend: (open circles), non-inoculated control; (open squares), heat killed control; (filled circle), biologically active under methanogenic conditions; (filled squares), biologically active under sulfate reducing conditions; (filled triangles), biologically active under denitrifying conditions

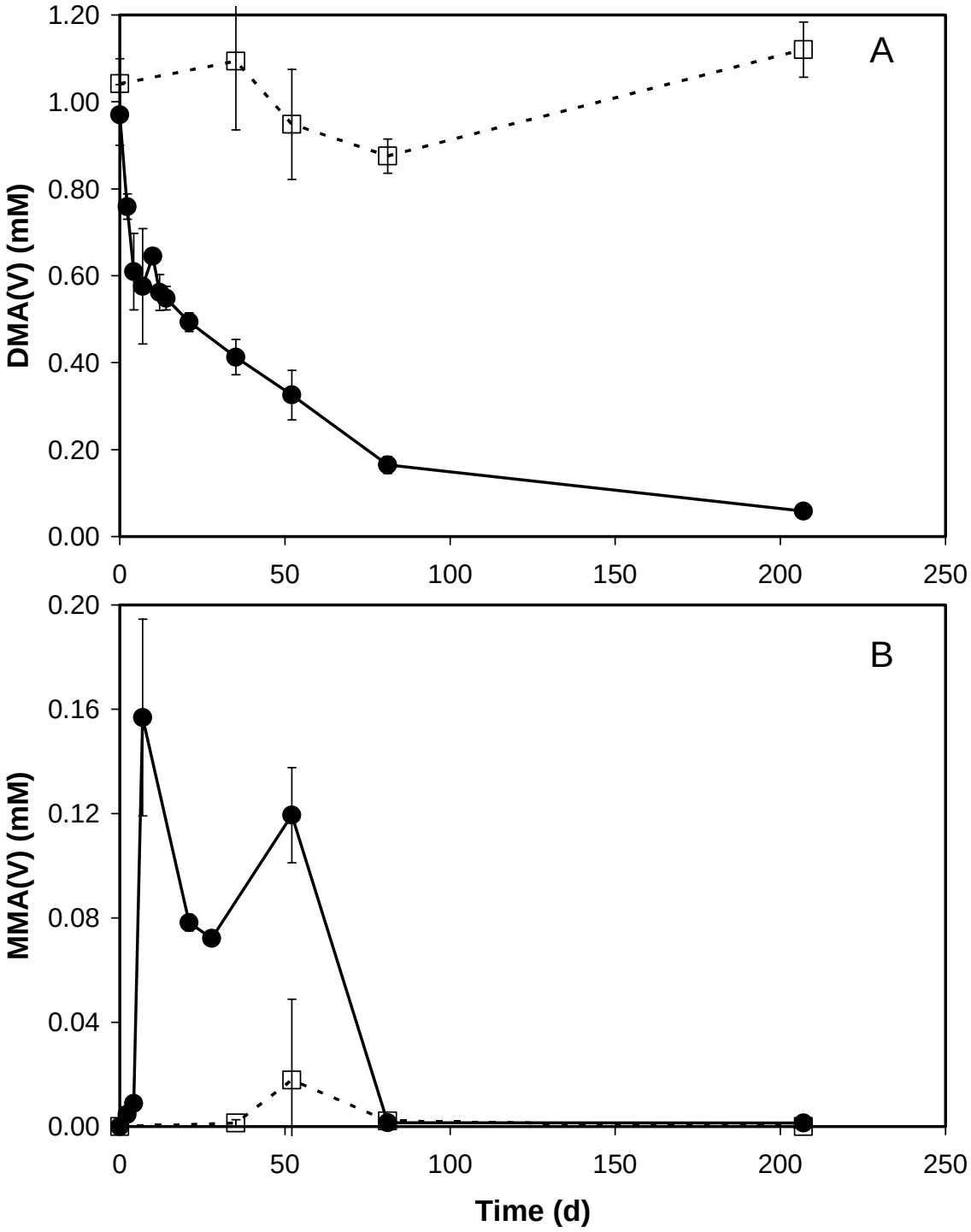



**Figure 4.** The biotransformation of DMA(V) (1.0 mM) during incubation with anaerobic sludge under methanogenic conditions. **A)** Removal of DMAV; **B)** Formation of the intermediate, MMA(V). Legend: (open squares), heat killed control; (filled circle), biologically active.

Effect of cosubstrate on anaerobic biotransformation of DMAV conditions. The impact of cosubstrate on the biotransformation of DMAV was evaluated by incubating the organoarsenical in the presence and absence of a mixture of volatile fatty acids (VFA). The addition of VFA had no major effect on DMAV removal during the first 48 days. However, VFA decreased the long-term biodegradability of DMAV from day 48 onwards. The formation of MMAV as biotransformation product was evident during the first 30 days of the experiment. The presence of VFA lowered the maximum accumulation of MMAV to a small extent and corresponded to a lower molar yield compared to the treatment lacking VFA (experiments 3-1 and 3-2 in Table 1).

Anaerobic biotransformation of DMA(V) under different redox conditions. An experiment was conducted comparing the anoxic degradation of DMA(V) (0.5 mM) under methanogenic, sulfate reducing and denitrifying conditions. The results indicate that complete removal of DMA(V) under the methanogenic and sulfate reducing conditions had occurred already by the first sampling point (Figure 5A). The figure also indicates the lack of any major conversion of DMA(V) in the denitrifying culture, non-inoculated control and heat-killed inoculum control. MMA(V) accumulated to similar extents in the methanogenic and sulfate reducing assays (Figure 5B). MMA(V) concentrations in the biologically active treatments were significantly greater than an incidental detection in the heat killed control. A similar experiment was repeated under sulfate reducing conditions which showed that the extensive degradation of DMA(V) could be reproduced; however, AsV was observed as the biotransformation product instead of MMA(V) (experiment 7-1 in Table 1).

Mass balance of anaerobic MMA(V) and DMA(V) conversion. A mass balance was conducted for selected experiments in which volatile arsenic in the headspace, residual arsenic in the sludge and total arsenic in the liquid phase was measured (Table 2). The total recovery of arsenic ranged from 55 to 76% for MMA(V) and 53 to 68% for DMA(V). The mass balance indicates only minor speciation to volatile arsenic compounds, which only accounted for 0.0002 to 0.0345 % of the added arsenic. Identifiable arsenic species extracted from the sludge with NaOH accounted for 0.5 to 5.5% of added arsenic of which inorganic arsenicals only accounted for 0.02 to 0.2% of the added arsenic. The bulk of the recovered arsenic was present in the liquid. The sum of identifiable species in the liquid was generally in agreement with total arsenic of the MMA(V) experiments; however in one experiment the identified species were only 75% of the total. This observation was in agreement with the occurrence of unidentified peaks in the HPLC-ICP occurring at retention times (RT) of 6.5 and 18.4 min. (for comparison the RTs of MMA(III) and MMA(V) were 1.9 and 3.7 min.). In the DMA(V) experiments, the sum of identifiable species in the liquid was generally only a small fraction of the total arsenic in the liquid (7 to 31%). Several unidentified peaks in the HPLC-ICP were observed that might account for the difference. These included peaks at RTs of 4.4, and 6.5 min. as well as smaller peaks at 7.8 and 12.8 min. (for comparison the RT of DMA(V) was 2.5 min.).

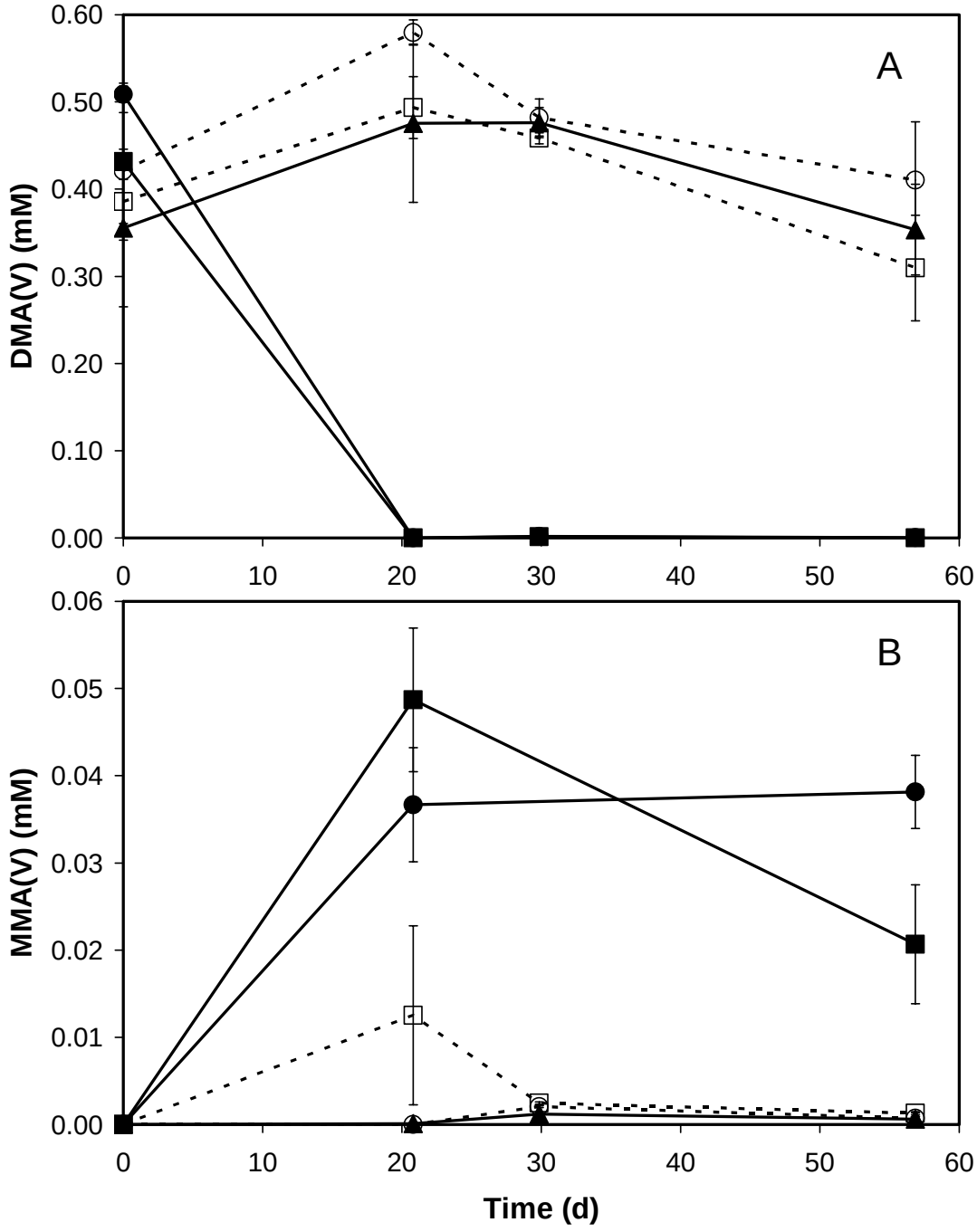



**Figure 5.** The biotransformation of DMA(V) (0.5 mM) during incubation with anaerobic sludge under methanogenic, sulfate reducing, or denitrifying conditions. **A)** Removal of DMA(V); **B)** Formation of the intermediate, MMA(V). Legend: (open circles), non-inoculated control; (open squares), heat killed control; (filled circle), biologically active under methanogenic conditions; (filled squares), biologically active under sulfate reducing conditions; (filled triangles), biologically active under denitrifying conditions.

**Table 2**. Balance of arsenic in headspace (volatiles), sludge (NaOH extractable) and liquid from selected experiments with either MMAV (Part 1) or DMAV (Part 2) as substrates. Unless indicated otherwise, values are expressed as mg arsenic per bottle. Volatile and liquid fractions are averages of triplicates; whereas as sludge values are averages of duplicates.

| Exp. (#) | Duration (d) | Volatiles | Sludge | Liquid | | Sum (vol+slu+liq*) | Input As | Recovery (%) |
|---|---|---|---|---|---|---|---|---|
| | | ICP Only | NaOH Extraction (HPLC-ICP) | HPLC-ICP | ICP Only | | | |
| | | | | Identified Species | Total | | | |
| **Part 1. MMAV** | | | | | | | | |
| Exp.1-1 | 235.4 | 9.27E-06 | 0.12 | 2.1 | 2.80 | 2.92 | 5.34 | 54.68 |
| Exp.1-2 | 235.4 | 4.15E-05 | 0.34 | 16.41 | 14.64 | 14.98 | 26.69 | 56.13 |
| Exp.3-3 | 216.7 | 5.30E-04 | 0.30 | 3.18 | 3.16 | 3.46 | 5.62 | 61.51 |
| Exp.3-4 | 216.7 | 4.04E-04 | 0.31 | 4.59 | 3.96 | 4.27 | 5.62 | 76.00 |
| **Part 2. DMAV** | | | | | | | | |
| Exp.2-1 | 208.3 | 1.94E-03 | 0.24 | 0.29 | 3.20 | 3.44 | 5.62 | 61.24 |
| Exp.2-2 | 208.3 | 5.71E-05 | 0.13 | 18.22 | 18.98 | 19.11 | 28.10 | 68.01 |
| Exp.3-1 | 216.7 | 5.24E-04 | 0.15 | 0.13 | 1.83 | 1.98 | 3.75 | 52.82 |
| Exp.3-2 | 208.3 | 2.91E-05 | 0.13 | 0.67 | 2.13 | 2.26 | 3.75 | 60.39 |

* Sum of arsenic in the volatile fraction (vol), sludge (slu) and liquid (liq) fractions, the value from the liquid fraction was based on the total As (ICP only) measurement

Biotransformation kinetics. DMA(V) and MMA(V) were observed to degrade according to first order kinetics. Based on the first order rate expression:

$C_x = C_0 e^{-kt}$

where: $C_x$ = concentration organoarsenical at a given time;
$C_0$ = initial concentration;
$k$ = first order rate constant;
t = time;

$k$ was estimated from natural logarithmic plots of the data wherever feasible as shown in Table 1. The $k$ of MMA(V) ranged from 0.011 to 0.041 $d^{-1}$; whereas the $k$ of DMA(V) ranged from 0.013 to 0.098 $d^{-1}$. The higher $k$ values for DMA(V) removal observed in experiments 4-1 and 7-1 are associated with a newer sample of the methanogenic sludge utilized for the experiments. Only a few direct comparisons of kinetics were feasible. DMA(V) and MMA(V) biodegradation were compared in parallel during experiments 4-1 and 4-2 as well as 7-1 and 7-2. In those experiments, the rates of DMA(V) removal were from 8.3 to 8.8-fold higher than those of MMA(V). On two occasions, $k$ values of MMA(V) removal were obtained in parallel under methanogenic and sulfate reducing conditions. In experiment 6-4 and 6-5, the $k$ value under sulfate reducing conditions was 2.6-fold higher. However, the $k$ values in a second comparison (experiment 4-2 and 7-2) were similar under methanogenic and sulfate reducing conditions.

Discussion on biodegradation of MMA(V) and DMA(V). In this project, the degradation of MMA(V) and DMA(V) was demonstrated under strict anaerobic conditions. These organoarsenicals were largely removed in the presence of biologically active sludge while they were not significantly removed in non-inoculated or heat-killed sludge controls. Degradation percentages of MMA(V) and DMA(V) ranged from 24 to 96% and 33 to 100%, respectively. Degradation occurred either under methanogenic or sulfate reducing conditions but not in the presence of nitrate. This constitutes the first report of extensive degradation of MMA(V) and DMA(V) under methanogenic and sulfate reducing redox conditions. Previous studies conducted with marine sediments under strict anaerobic conditions have only demonstrated minor conversions of MMA(V) and DMA(V) (14). Several investigators have studied MMA(V) and DMA(V) bioconversion in soils at high moisture contents that presumably correspond to anaerobic or low oxygen conditions. When compared to fully aerobic conditions, the saturated soils provide improved removals of these compounds (1, 11, 33). Up to 85% removal of DMA(V) was observed after 70 days of incubation in wet soil (11).

Little is known about the bacteria responsible for metabolizing MMA(V) and DMA(V). The few isolates evaluated so far have been tested under aerobic conditions. *Mycobacterium neoaurum* was shown to convert MMA(V) by 30% to AsV and AsIII during growth on rich microbial medium (18). Two DMA(V) metabolizing strains were isolated from lake water (19). Strain 12M16, closely related to *Aeromonas hydrophila*, and strain 10M1, closely related to *Comamonas testosterone*, removed DMA(V) by 78 and 34%, respectively; resulting in the formation of inorganic arsenicals and MMA(V) (19).

In this study, MMA(III) was consistently observed as an important metabolite of MMA(V) degradation and it was recovered in molar yields ranging from 5 to 47%. This is the first time that MMA(III) has been reported as a microbial biotransformation product of MMA(V). The occurrence of MMA(III) suggests that reduction of MMA(V) is an important

mechanism of the biotransformation. The only known enzyme responsible for the reduction of MMA(V) to MMA(III) is glutathione-S-transferase ω hGSO1-1 from mammals (34). In this project, an important biotransformation product from DMA(V) metabolism identified was MMA(V), which was recovered in molar yields ranging from 8 to 65%. This observation points to demethylation as an important mechanism of the biotransformation. Evidence for demethylation of DMA(V) under strict anaerobic conditions in marine sediments was previously observed based on the appearance of an unresolved metabolite peak that corresponded to either MMA(V), As(V) or As(III) that coeluted at the same retention time (14). MMA(V) was detected as a product of the biotransformation of DMA(V) in wet soils (11, 32). The evidence in the literature suggests that DMA(V) is mineralized to As(V) and $CO_2$ under aerobic conditions (11, 19, 32, 33), while under anaerobic conditions DMA(V) is largely converted to biotransformation products (11, 32). This pattern seems to hold true under the strict anaerobic conditions tested in this project, where little evidence of any inorganic arsenical formation was observed.

As(V), MMA(V) and DMA(V) are known to be microbially converted to volatile arsine and methylarsine compounds (5). However, such compounds are usually only formed at extremely low yields. After incubating DMA(V) with aerobic and flooded soils for 60 to 70 days, only 0.002 to 1.5% was converted to alkylarsines (11, 31). Yields of volatile arsines are also low (0.00015 to 0.4%) in pure cultures of methanogens or methanogenic consortia administered with either As(V) (20, 21) or with AsIII (25). Consistent with these observations, volatile forms of arsenic volatiles in this study only accounted for 0.0002 to 0.035% of the added arsenic.

Implications. The results of this study suggest that the biotransformation of organoarsenical pesticides, MMA(V) and DMA(V), occurs in methanogenic and sulfate reducing consortia. An important biotransformation product from MMA(V), was found to be MMA(III) and the methane production from the decay of endogenous substrate in the sludge inoculum ceased after the initial formation of MMA(III). This observation agrees with the high toxicity of MMA(III) to methanogenesis. The 50% inhibiting concentration of MMA(III) was found to be 9.1 μM, which is greater than 550-fold more toxic than MMA(V) (25). Aside from microbial toxicity, MMA(III) is a concern to public health. MMA(III) is more toxic to various human cell lines compared to AsV and AsIII. Furthermore, MMA(III) has genotoxic activity (27). The literature evidence suggests that MMA(V) is largely mineralized to As(V) under aerobic conditions albeit with a slower removal rate compared to anoxic conditions (11, 18, 29). Considering the elevated toxicity of the anaerobic biotransformation product, compared to the relatively benign fate in aerobic environments, regulators should carefully assess the use of MMA(V) in areas adjacent to anaerobic environments (shallow aquifers, water bodies, wetlands *etc*).

The biotransformation products detected during anaerobic degradation of DMA(V) was MMA(V) and several unidentified metabolites. The methanogenesis of the endogenous substrate was not inhibited during the degradation assays with DMA(V) indicating a much lower toxicity of its biotransformation products compared to those from degradation assays with MMA(V) as the parent compound. Anaerobic bioconversion of DMA(V) was faster than MMA(V) as evidenced by an approximately 9-fold higher first order rate constant.

**Biodegradation of Nitrogen-Substituted Phenylarsenates.**

Reduction of roxarsone**.** Figure 6 shows the results of an experiment where roxarsone was incubated with anaerobic sludge under methanogenic, sulfate reducing and denitrifying conditions as well as methanogenic conditions supplemented with lactate. Under denitrifying conditions, only negligible removal of roxarsone occurred during the 21-day experiment. On the other hand, under methanogenic and sulfate reducing conditions, rapid decreases in the roxarsone concentration were observed. Supplementation of lactate increased the rate of roxarsone elimination. Biologically mediated reactions are implicated since roxarsone was not removed in the control lacking added sludge. The slow removal of roxarsone in the heat-killed sludge controls indicated that there are also some abiotic mechanisms of roxarsone removal due to components present in the sludge. HAPA was identified as a major biotransformation product of roxarsone degradation, indicating that the main reaction was a reductive transformation of the nitro group to an amino group. HAPA was recovered with a molar yield of approximately 75%.

An experiment was conducted evaluating the impact of different electron-donating substrates on roxarsone biotransformation by anaerobic sludge incubated under methanogenic conditions as shown in Figure 7. The endogenous-substrate control and the acetate-supplemented treatments had the lowest rates of roxarsone reduction. All other electron donating substrates significantly stimulated the roxarsone biotransformation rates. Hydrogen, glucose and lactate, increased the rates of roxarsone biotransformation by 2.4-, 1.7 and 1.5-fold, respectively, compared to the endogenous substrate control. The molar yields of HAPA in the biologically active treatments ranged from 67 to 92% of the roxarsone removed.

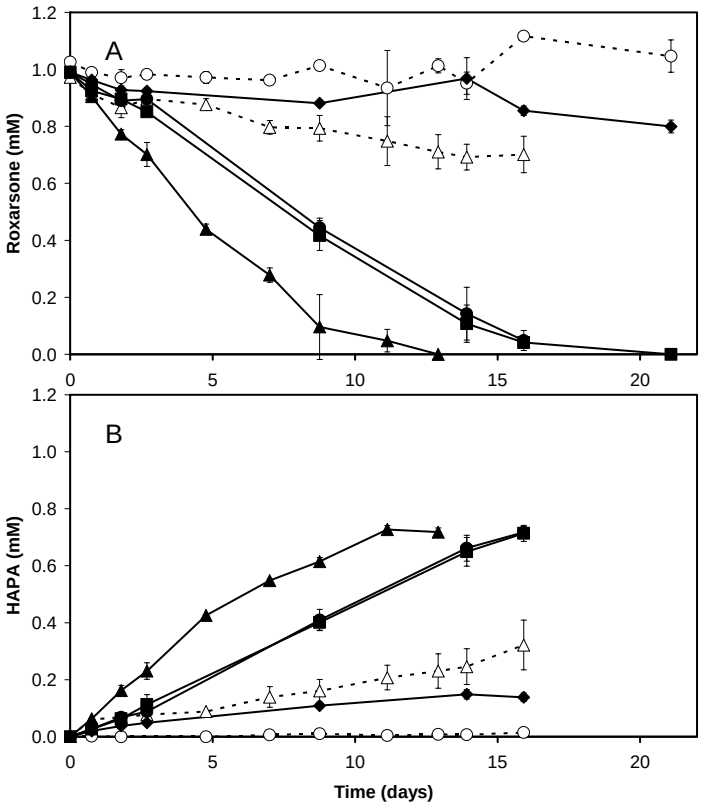
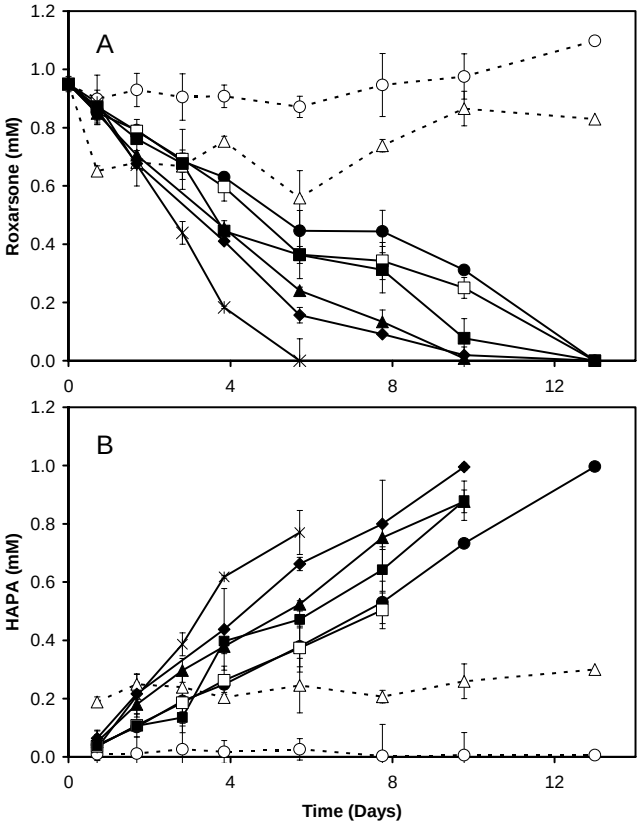



**Figure 6.** Anaerobic biotransformation of roxarsone by methanogenic sludge under different redox conditions. Experiments were incubated on a reciprocal shake table. **A**. Roxarsone concentrations determined by HPLC-DAD. **B**. HAPA concentrations determined by HPLC-DAD.

**Legend**: abiotic control ( - - O - - ); heat killed sludge control ( - - Δ - - ); methanogenic ( —— ● —— ); sulfate reducing ( —— ■ —— ); denitrifying ( —— ◆ ——); and methanogenic + 10 mM lactate added ( —— ▲ — ).

**Figure 8.** Effect of electron donors on the anaerobic biotransformation of roxarsone by methanogenic sludge under methanogenic redox conditions. Experiments were incubated on a reciprocal shake table. **A**. Roxarsone concentrations determined by HPLC-DAD. **B**. HAPA concentrations determined by HPLC-DAD.

**Legend**: abiotic control ( - - O - - ); heat killed sludge control ( - - Δ - - ); no electron donor ( —— ● —— ); acetate, 10 mM ( —— □ —— ); volatile fatty acid ( —— ■ —— ); glucose, 10 mM ( —— ◆ ——); $H_2$, 1.2 atm ( ——∗ ——); and lactate, 10 mM ( —— ▲ — ).



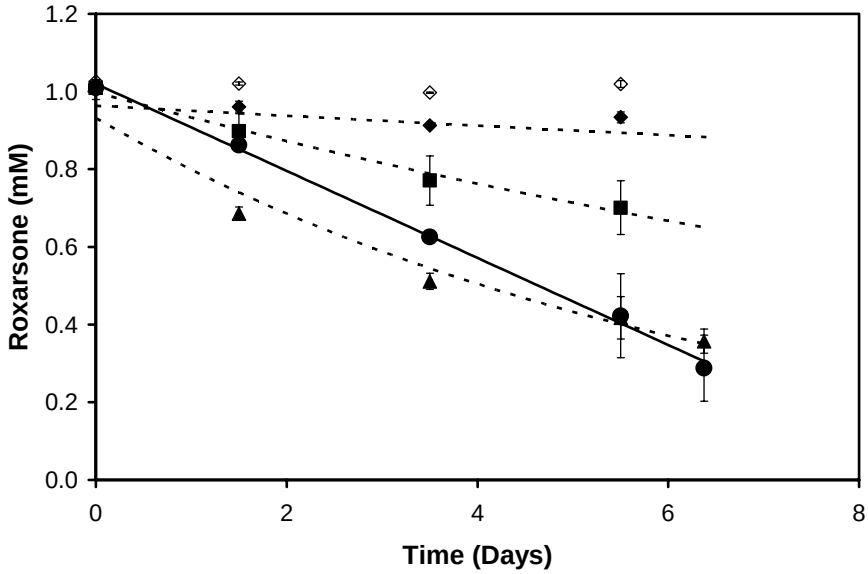




**Figure 8.** The effect of reducing agents on the reduction of roxarsone. **Legend**: roxarsone incubated in basal medium in the absence of heat killed sludge (◊); roxarsone incubated in basal medium in the presence of heat killed sludge (◆); roxarsone incubated in basal medium with 6 mM $FeSO_4$ in the presence of heat killed sludge (▲); roxarsone incubated in basal medium with 3 mM $Na_2S$ in the presence of heat killed sludge (■); roxarsone incubated in basal medium with living sludge and $H_2$ (●).

Ferrous iron ($Fe^{2+}$) and sulfides are inorganic reducing agents present in anaerobic environments. Therefore the ability of stoichiometric amounts of FeSO4 (6 mM) and Na2S (3 mM) to reduce roxarsone (1 mM) abiotically in the presence of heat killed sludge and the basal mineral medium was evaluated (Figure 8). Under these conditions, $Fe^{2+}$ and to a lesser extent $S^{2-}$ supported the abiotic reduction of roxarsone. On day 5 the molar yield of HAPA was 72 and 22% of roxarsone eliminated by $Fe^{2+}$ and $S^{2-}$; respectively. The abiotic reactions followed first order kinetics (dashed lines); whereas the biologically catalyzed reaction with $H_2$ as electron donor followed zero order kinetics (solid line).

Long-term biodegradability of roxarsone. An experiment was conducted to determine the long-term biodegradability of roxarsone. The results after 10 days were in agreement with the findings of the first experiment (Figure 6) in that roxarsone was largely converted to HAPA and the extent of the conversion was greatest in the treatment receiving lactate. The molar yield of HAPA was 86% of roxarsone removed.

After 120 days, roxarsone was completely removed in the two biologically active treatments as well as the heat-killed sludge treatment. The result indicates that the slow abiotic conversion of roxarsone in the heat-killed sludge treatment was sustained over a long period of time so that eventually roxarsone was completely converted to HAPA. The HAPA, which accumulated, was recovered with a molar yield of 83%. In the two biologically active treatments, the HAPA intermediate was subjected to further biodegradation as evidenced by the dramatically lower HAPA levels on day 120 compared to levels that had previously accumulated on day 10. The HPLC-ICP-MS data revealed that part of the HAPA eliminated accumulated as As(III) and As(V) (Table 3). As(III) was the most important inorganic arsenic compound and it had significantly accumulated up to 17.9% of the arsenic supplied . The soluble inorganic arsenicals (corrected for background) detected in the endogenous substrate and lactate-fed treatments correspond to a molar yield of 18 and 23% of the phenylarsonic acid compounds removed, respectively. The data of arsenic speciation was compared with the total arsenic measured in liquid samples (Table 3). In the biologically active treatments, the sum of identified arsenic species (Σ sp) accounted for about a third of the arsenic. Most of the unidentified arsenic was present in the liquid.

Long-term biodegradability of HAPA. The results of an experiment to verify the anaerobic biodegradability of HAPA are presented in Figure 9. Under methanogenic and sulfate reducing conditions, HAPA was significantly removed compared to the abiotic and heat-killed sludge controls. Lactate supplementation had no significant effect on the rates of degradation under methanogenic conditions. The most important biodegradation product observed was As(III). On days 113 to 134, there was some temporal accumulation of As(V). The molar yield of soluble inorganic arsenicals (corrected for background inorganic arsenic) in the methanogenic and sulfate-reducing treatments ranged from 15 to 21% of HAPA removed on day 132 and ranged from 19 to 28% of HAPA removed on day 229. No significant removal of HAPA occurred in uninoculated or heat-killed sludge controls. Likewise there was no significant production of inorganic arsenicals in these controls. These observations confirm that the removal in the methanogenic and sulfate reducing treatments was due to biological mechanisms.

The heat-killed control and the methanogenic treatments were incubated up to day 310, at which time HAPA was removed by 98.9% and As(III) corrected for background levels, accounted for 26% of the HAPA removed in the biologically active treatment. By comparison,

HAPA was removed by only 38.8% in the heat killed treatment with negligible production of As(III) after the extended incubation (results not shown).

Biodegradation under denitrifying conditions was delayed and slower. By the end of the experiment (day 229), there was still no significant decrease of HAPA beyond the levels in the controls.

The fraction of total arsenic lost from the liquid is indicated by the difference between total arsenic in the abiotic control and that of the biologically active treatments (the "not in liquid" fraction in Table 4). Up to day 132, the loss of total arsenic was minor, ranging from 10-19% in the methanogenic and sulfate reducing treatments. However, by the end of the experiment on day 229, the loss of total arsenic was quite substantial, accounting for 41-53% of the arsenic in the abiotic control. The data of arsenic speciation was compared with the total arsenic measured in liquid samples (Table 4). In the methanogenic and sulfate reducing treatments, the sum of identified species (Σ sp) accounted for 59 to 63% of the arsenic on day 132 and for 30 to 42% of the arsenic on day 229.

Long-term biodegradability of p-arsanilic acid (4-APA). p-Arsanilic acid **(**4-APA) is structurally related to HAPA and is also used as a feed additive in poultry. An initial experiment was conducted to evaluate the biodegradabilty of 4-APA by anaerobic sludge under several redox conditions. In this experiment, the HPLC-DAD was used to monitor the 4-APA concentration. 4-APA was subject to immediate anaerobic biodegradation in the methanogenic and sulfate reducing treatments. At the end of the experiment (day 68), 4-APA degradation reached 71.3 to 82.8% (results not shown). Partial elimination of 4-APA occurred under denitrifying conditions after a lag phase of 41 days. No significant removal of 4-APA occurred in the uninoculated or heat-killed sludge controls, indicating that the removal in the biologically active treatments was due to biodegradation.

A second experiment was conducted with the methanogenic treatment and controls in order to obtain data about the release of inorganic arsenicals. 4-APA was also reliably biodegraded under methanogenic conditions in the second experiment (Figure 10). 4-APA biodegradation was accompanied by significant production of As(III) as well as a lower production of As(V). These inorganic arsenicals (corrected for background) were produced with a molar yield of 24% compared to 4-APA eliminated on day 174. Total As measurements indicate that about half of the arsenic was removed from the liquid at the end of the experiment (results not shown).

**Table 3**. Balance of arsenic on day 120 in the experiment evaluating the long-term incubation of roxarsone under methanogenic conditions.

| Treatment | Roxarsone | | HAPA | | As(V) | As(III) | Σ sp[†] | Total As | Unidentified[‡] | |
|---|---|---|---|---|---|---|---|---|---|---|
| | HPLC-DAD | HPLC-ICP | HPLC-DAD | HPLC-ICP | HPLC-ICP | HPLC-ICP | | ICP | in liquid | not in liquid |
| | ---------- % As roxarsone abiotic ---------- | | | | | | | ------ % total As abiotic ------ | | |
| Killed | 0.5±0.2 | 0.4±0.1 | 83.2±6.0 | 123.6±24.2 | 4.3±1.0 | 1.6±0.4 | 89.6 | 97.9±8.7 | 8.3 | 2.1 |
| Meth | 0 | 0 | 20.2±7.0 | 30.2±12.2 | 1.7±1.5 | 13.6±10.9 | 35.5 | 96.9±34.6 | 61.4 | 3.1 |
| Meth+Lact | 0 | 0 | 6.0±5.2 | 6.0±1.7 | 4.0±1.5 | 17.9±10.0 | 27.9 | 76.7 | 48.8 | 23.3 |

[†]sum of identified species, utilizing HPLC-DAD data for roxarsone and HAPA

[‡]Unidentified As in liquid = Total As – sum of identified species; Unidentified As not in liquid = 100 – Total As

**Table 4.** Balance of arsenic on days 132 and 229 in the experiment evaluating the long-term incubation of HAPA under methanogenic conditions (Figure 9).

| Treatment | HAPA | As(V) | As(III) | Σ sp[†] | Total As | Unidentified[‡] | |
|---|---|---|---|---|---|---|---|
| | HPLC-ICP | HPLC-ICP | HPLC-ICP | | ICP | in liquid | not in liquid |
| | ---------- % As HAPA abiotic ---------- | | | | ------ % total As abiotic ------ | | |
| **Day 132** | | | | | | | |
| Killed sludge | 93.6±7.1 | 0.6±0 | 1.1±0.2 | 95.3 | 96.6±6 | 1.3 | 3.4 |
| Meth | 38.2±7.6 | 5.6±4.5 | 19.4±2.8 | 63.3 | 85.9±9.8 | 22.6 | 14.1 |
| Meth+Lact | 33.2±5.9 | 6.5±2.1 | 19.5±2.5 | 59.2 | 84.5±7.7 | 25.3 | 15.5 |
| $SO_4^{2-}$ | 43.7±9.8 | 6.6±0.9 | 13.1±3.1 | 63.4 | 94.3±6.9 | 30.9 | 5.7 |
| $NO_3^-$ | 110.5±9 | 4.3±1.1 | 0.6±0.1 | 115.5 | 87.4±4.1 | -28.1[*] | 12.6 |
| **Day 229** | | | | | | | |
| Killed sludge | 77.7±12.1 | 1.3±0.3 | 1.5±0.1 | 80.4 | 91±9.1 | 10.7 | 9.0 |
| Meth | 8.1±2.8 | 1.3±0.3 | 20.1±2.6 | 29.5 | 47.4±2.9 | 17.9 | 52.6 |
| Meth+Lact | 7.9±2.7 | 1.0±0.1 | 25.6±2.2 | 34.4 | 59.2±6.8 | 24.8 | 40.8 |
| $SO_4^{2-}$ | 13.9±8.9 | 1.3±0.5 | 26.7±12.6 | 42.0 | 58.4±13.5 | 16.4 | 41.6 |
| $NO_3^-$ | 72.9±15.0 | 5±1.9 | 0±0.1 | 78.0 | 58.7±9.9 | -19.3[*] | 41.3 |

[†]sum of identified species

[‡]Unidentified As in liquid = Total As – sum of identified species; Unidentified As not in liquid = 100 – Total As

[*]negative number due to an apparent overestimation of HAPA concentration by the HPLC-ICP-MS compared to total As measurement by ICP-MS

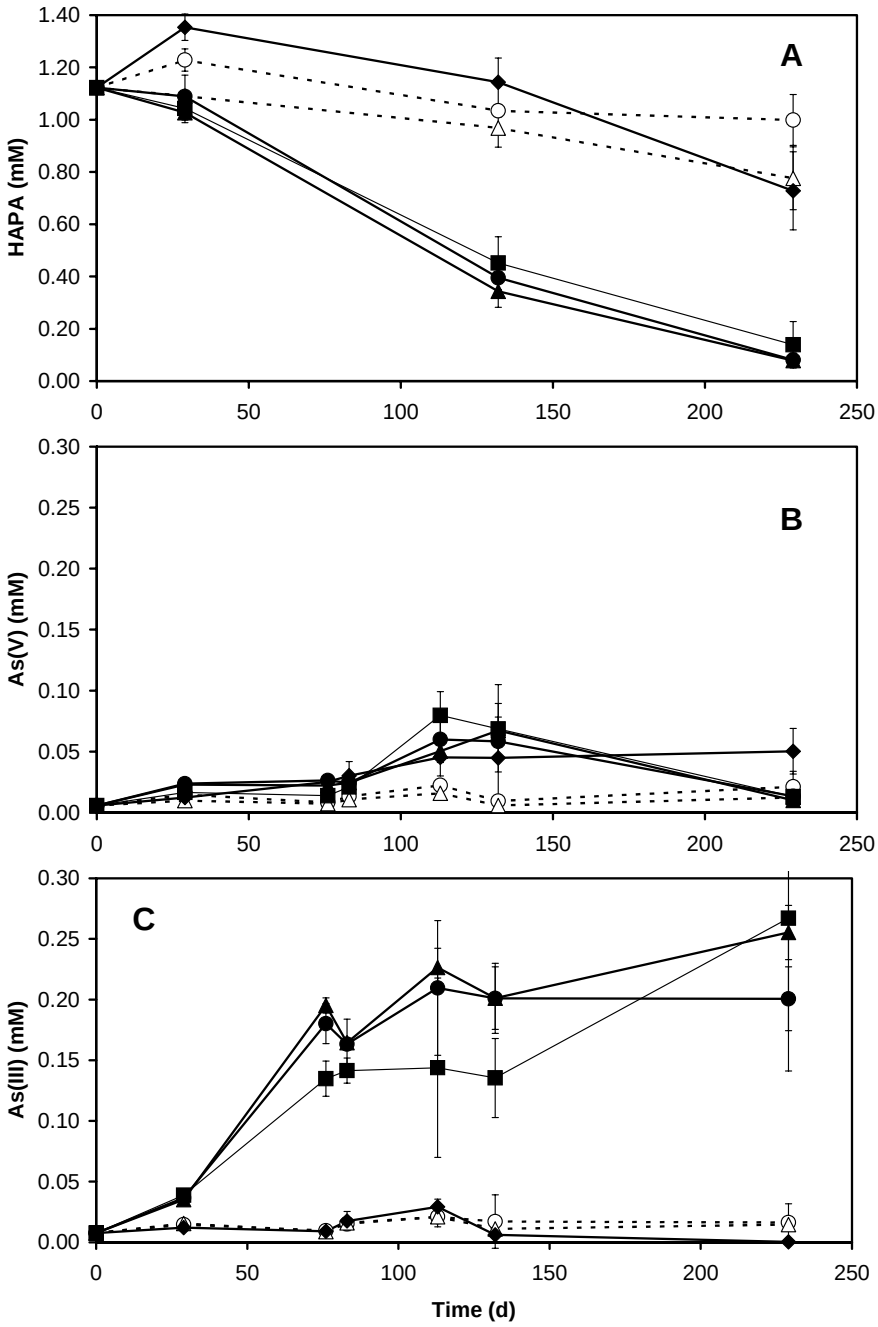



**Figure 9.** Anaerobic biotransformation of HAPA during a long-term incubation with methanogenic sludge. **A**. HAPA concentrations determined by HPLC-ICP-MS. **B**. As(V) concentrations determined by HPLC-ICP-MS. **C**. As(III) concentrations determined by HPLC-ICP-MS. **Legend**: abiotic control ( - - O - - ); heat killed sludge control ( - - Δ - - ); methanogenic ( —— ● —— ); sulfate reducing conditions ( —— ■ —— ); denitrifying conditions ( —— ◆ ——); and methanogenic conditions with 10 mM lactate added ( —— ▲ — ).

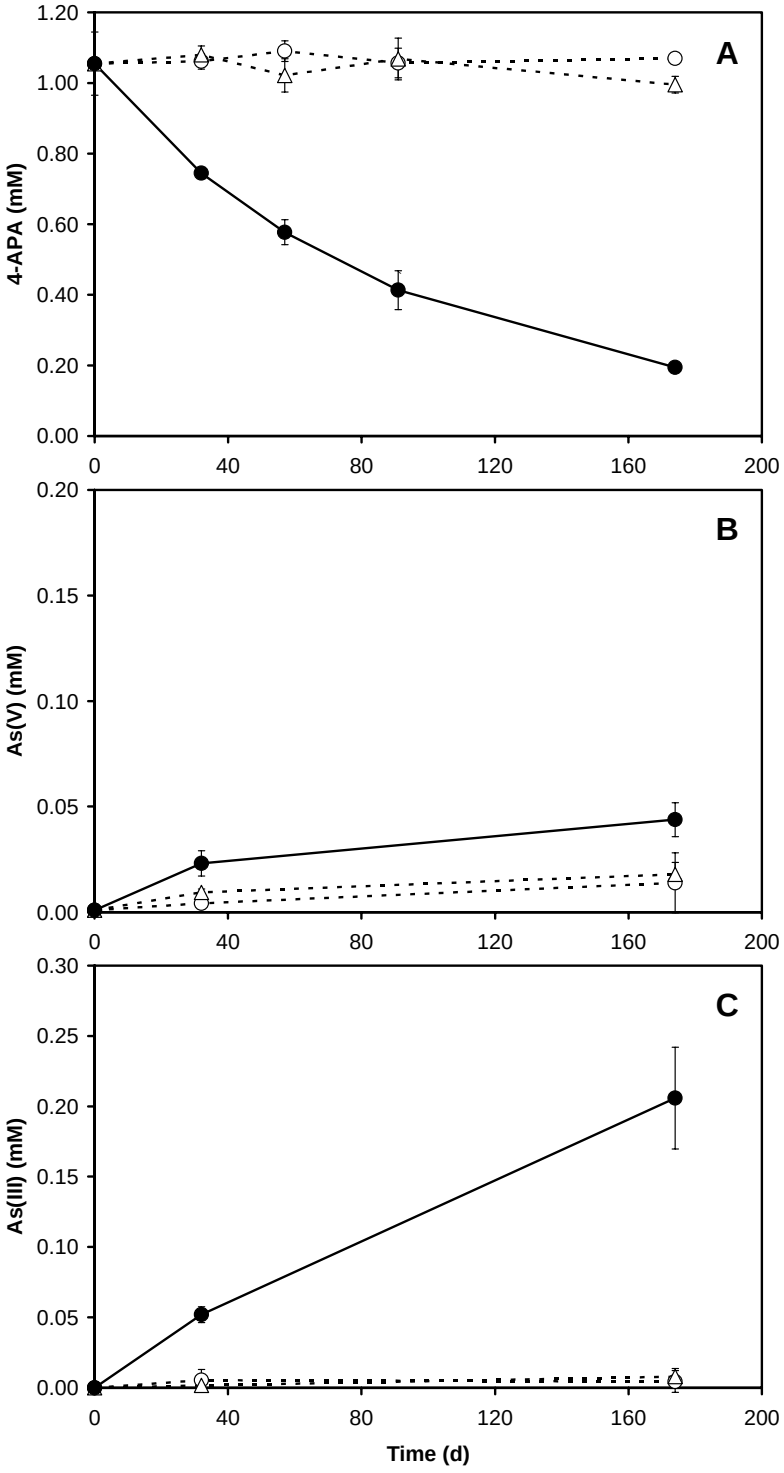



**Figure 10.** Anaerobic biotransformation of 4-APA during a long-term incubation with methanogenic sludge .**A.** 4-APA concentrations determined by HPLC-DAD. **B.** As(V) concentrations determined by HPLC-ICP-MS. **C.** As(III) concentrations determined by HPLC-ICP-MS. **Legend**: abiotic control ( - - O - - ); heat killed sludge control ( - - Δ - - ); and methanogenic condition ( —— ● —— ).

Discussion on biodegradation of N-substituted phenylarsonic compounds**.** The results from this project demonstrate that the commonly utilized feed additive of broiler chickens, roxarsone, is rapidly transformed under anaerobic conditions to the corresponding aromatic amine, HAPA. The formation of HAPA is attributable to the facile reduction of the nitro-group and occurred only under methanogenic or sulfate reducing conditions. During the period of HAPA formation, no significant release of inorganic arsenicals from the phenylarsonic acid structure was observed. However, HAPA and the closely related 4-APA were slowly degraded under anaerobic conditions. This degradation was associated with the partial release of freely soluble inorganic arsenicals in molar yields ranging from 19 to 28% of the amino-substituted phenylarsonic acids removed. The predominant inorganic arsenic species was arsenite, most likely due to reduction of released arsenate.

The facile reduction of the nitro group in roxarsone to an aromatic amine is in accordance with the rapid reduction of nitroaromatics in anaerobic mixed cultures from bioreactor sludge (22) and aquatic sediments (17). Many anaerobic microorganisms are also known to readily reduce a variety of nitroaromatic compounds (6, 13). The best studied are *Clostridia*, sulfate reducing bacteria and methanogens. The reduction of a nitroaromatic group requires the donation of six-electrons. Anaerobic microorganisms typically catalyze the reactions with nonspecific nitroreductases converting the nitro group via nitroso- and hydroxylamine intermediates to aromatic amines by successive addition of electron pairs from the metabolism of an electron donating substrate. The electrons to support the biological nitro-group reduction are released during the anaerobic degradation of substrates. Remarkably, substantial rates of roxarsone reduction to HAPA were evident in treatments without any added electron donor indicating that endogenous decay of sludge biomass was probably supplying the electrons. The endogenous substrate level in the sludge was estimated from the methane production after incubating the sludge with inorganic basal medium for 30 days. The methane yield corresponds in value to 18.8 *e* meq $l^{-1}$. This concentration of endogenous substrate was in excess of the 6 meq *e* $l^{-1}$ required to reduce 1 mM roxarsone to HAPA. Addition of the electron donating substrates increased the rates of roxarsone reduction beyond the endogenous rate. The substrates hydrogen, glucose, lactate and a mixture of volatile fatty acids, significantly increased rates. The best results were obtained with hydrogen. On the other hand, acetate had no effect on improving the rate. These observations are consistent with studies evaluating the effects of electron donors on nitrophenol reduction (8, 13). Either hydrogen, or substrates supplying interspecies hydrogen are required for rate enhancement; whereas, acetate had no effect because it is not commonly a source of interspecies hydrogen.

In this project, heat-killed sludge was also found to reduce roxarsone to HAPA at rates that were 5-fold slower than the biologically active treatments. Nitroaromatics are known to be reduced by chemical reducing agents such as ferrous iron (30) and sulfides (13) that are potentially present in anaerobic environments. The measured freely dissolved $Fe^{2+}$ in the assays ranged from 0.018 to 0.072 mM, which would only be sufficient to reduce 1.2% of roxarsone added., Significant chemical reduction of roxarsone to HAPA was observed in this study when $FeSO_4$ was supplied at a stoichiometric concentration. The total Fe and total S content of the sludge utilized in the experiments of this study was 56.6 and 46.6 mg $g^{-1}$ VSS, respectively (28). The assay

sludge concentration of 1.5 g VSS $l^{-1}$ could thus potentially introduce up to 1.5 *e* meq $l^{-1}$ of Fe(II) and 4.4 *e* meq $l^{-1}$ of $S^{2-}$, sufficient to reduce about 1 mM of roxarsone to HAPA.

The aromatic amines, HAPA and 4-APA, were slowly biologically eliminated under anaerobic conditions. The metabolism of these two aminophenylarsonic acids was associated with the partial release of inorganic arsenic. This constitutes the first report of a biologically catalyzed rupture of the phenylarsonic group under anaerobic conditions. If the phenylarsonic group were hydrolyzed, arsenate would be the most likely species of inorganic arsenic released and arsenate was indeed detected in this study. However, arsenite was the predominant inorganic species recovered most likely due to subsequent biological reduction of arsenate to arsenite in methanogenic sludge (10). There is ample evidence that aminophenols, aminobenzoates and aminosalicylates are mineralized under methanogenic conditions to $CH_4$, and $NH_3$ (8, 23, 24).

Implications. Nitrogen substituted phenylarsonic compounds utilized as poultry food additives can be transformed under anaerobic conditions to eventually produce the toxic arsenic species, arsenite (25). Thus in anaerobic environments such as sediments and subsurafce soil such compounds if present would lead to potential damage to the ecosystem.

**Acute Toxicity of Organoarsenicals**

Toxicity of arsenic species. Initially toxicity testing was based on using the AMES test and mitochondrial toxicity testing (MTT). Parent compounds and incubates from the biodegradation of DMA(V) and MMA(V) were found to be non-toxic in both the AMES and MTT. Incubates from the biotransformation of roxarsone gave weak toxic responses in the AMES test. In project meetings, a large number of questions regarding AMES and MTT testing were raised. On the one hand, incubates need to be diluted, and on the other hand the tests have very specific targets. In order to address these concerns, we decided to use ecotoxicity testing. Toxicity to methanogens was evaluated by incubating arsenicals with anaerobic sludge and monitoring impacts on the methane production rate. Table 5 summarizes the results. Pentavalent compounds, such as As(V), MMA(V) and DMA(V) were basically non-toxic. Trivalent compounds, As(III) and MMA(III) were very toxic. Roxarsone displayed toxicity which was not as severe as As(III). The biotransfomation product of roxarsone, 4-hydroxy-3-aminophenylarsonic acid (HAPA), was non-toxic. However, HAPA is susceptible to autoxidation and it was only non-toxic if autoxidation was prevented by preparing stock solutions with 200 mg/l ascorbic acid.

Toxicity of oxidized HAPA products. During the research of this project, we have noticed that HAPA is very susceptible to autooxidation in air. The products of HAPA oxidation were observed to be toxic to microbial activities in the anaerobic sludge. The phenomenon was first noted when studying the toxicity of HAPA to methanogens. If the HAPA stock solution was prepared without the antioxidant (ascorbic acid), HAPA was observed to be significantly more toxic compared to HAPA prepared with ascorbic acid. To evaluate the toxicity of oxidized HAPA, stock solutions of HAPA were adjusted to

pH 9 and aerated for different periods of time: 4 minutes, 1 hour, 16 h, 4 days and 16 days. These oxidized HAPA solutions were incubated at different dilutions to determine the 50% inhibiting concentrations (expressed in terms of the original HAPA concentration in the stock solution). Toxicity was assayed by measuring the methane production in anaerobic sludge with fed with acetate as substrate. To illustrate the protocol, the graph in Figure 11 shows the time course of methane production in assay bottles exposed to different levels of 16h oxidized HAPA. The slope of each line (the activity) is compared to the slope of the uninhibited control (0 mM HAPA) and the relative activity is plotted as a function of the HAPA concentration. The activity versus HAPA concentration graph is shown in Figure 12. The concentration that corresponds to the point where the relative activity line crosses 50% is referred to as the 50% inhibiting concentration (50%IC). The assignment of a 50%IC of 1.16 mM and 0.15 mM for HAPA oxidized for 4 minutes and 16 hours, respectively, is shown in Figure 11. This procedure was repeated for each of the oxidation times. The net result is a graph, which illustrates the change in the 50%IC of HAPA versus oxidation time as shown in Figure 13. The first few minutes of the oxidation dramatically increases the toxicity of HAPA (lowers the 50%IC). As the oxidation continues for the next 16 h, the toxicity continues to increase reaching a maximum around 16 h. Thereafter, continued oxidation up to 16 days results in only a small decline in the toxicity of the oxidized HAPA solutions. Preliminary measurements with a mass spectrometer indicate the formation of oligomers as oxidation products of HAPA. These oxidation products, apparently exhibit a high level of microbial toxicity.

To test whether the toxicity is universal and not just restricted to prokaryotes, a mitochondrial toxicity test (MTT) was conducted. The MTT test revealed that HAPA incubated without ascorbic acid was significantly more toxic than HAPA incubated with ascorbic acid (Figure 14). Clearly, the difference can be attributed to the formation of oxidized products from HAPA during the aerobic assay. In addition, 240 μM of HAPA previously oxidized for 16h was also more toxic than HAPA protected from oxidation by ascorbic acid. The early oxidation products formed from HAPA during the MTT were apparently more toxic than HAPA oxidized for 16 h prior to the test. Also it was observed that HAPA was more toxic to mitochondria compared to roxarsone. Roxarsone displayed the same weak toxicity in the presence and absence of the antioxidant. This observation is consistent with the fact that roxarsone is not susceptible to autooxidation.

Implications. The results taken as a whole suggest that certain products from organarsenical biotransformation can be toxic. MMA(III), a biotransformation product of MMA(V) displayed high toxicity. As(III) a biotransformation product from the long term incubation of roxarsone with anaerobic sludge was also toxic. HAPA a biotransformation product of roxarsone was itself non-toxic; however the compound upon exposure to air generates toxic autoxidation products.

A rapid initial biotransformation of roxarsone to HAPA can be expected in anaerobic environments, such as those occurring during storage of poultry manure. The HAPA formed is unstable in air and is rapidly autooxidized to very toxic intermediates. Therefore land application of poultry manure may have serious ecological consequences due to the formation of toxic oxidized HAPA species. The sequence of events required for the toxic products to accumulate would be an initial anaerobic conversion followed by

aerobic conversions. This sequence is consistent with initial composting of manure followed by land application.

**TABLE 5.** Concentrations of arsenical compounds causing a 20, 50 and 80% inhibition of the methanogenic activity of anaerobic sludge with acetate as substrate

| Compound | Molecular Weight | 20% IC (µM) | 50%IC (µM) | 80%IC (µM) |
|---|---|---|---|---|
| **Inorganic species** | | | | |
| As(III) | 122.9 | 9.1 | 15.5 | 23.5 |
| As(V) | 138.9 | >500 | > 500 | > 500 |
| **Methylated organoarsenic compounds** | | | | |
| MMA(V) | 140.0 | > 5,000 | > 5,000 | > 5,000 |
| DMA(V) | 138.0 | > 5,000 | > 5,000 | > 5,000 |
| MMA(III) | 123.9 | 2.2 | 9.1 | 17.9 |
| **N-substituted Phenylarsonates** | | | | |
| Roxarsone | 263.0 | 251 | 425 | 780 |
| HAPA | 233.1 | > 600 | > 600 | > 600 |
| *p*-arsanilic acid | 217.1 | > 2,300 | > 2,300 | > 2,300 |

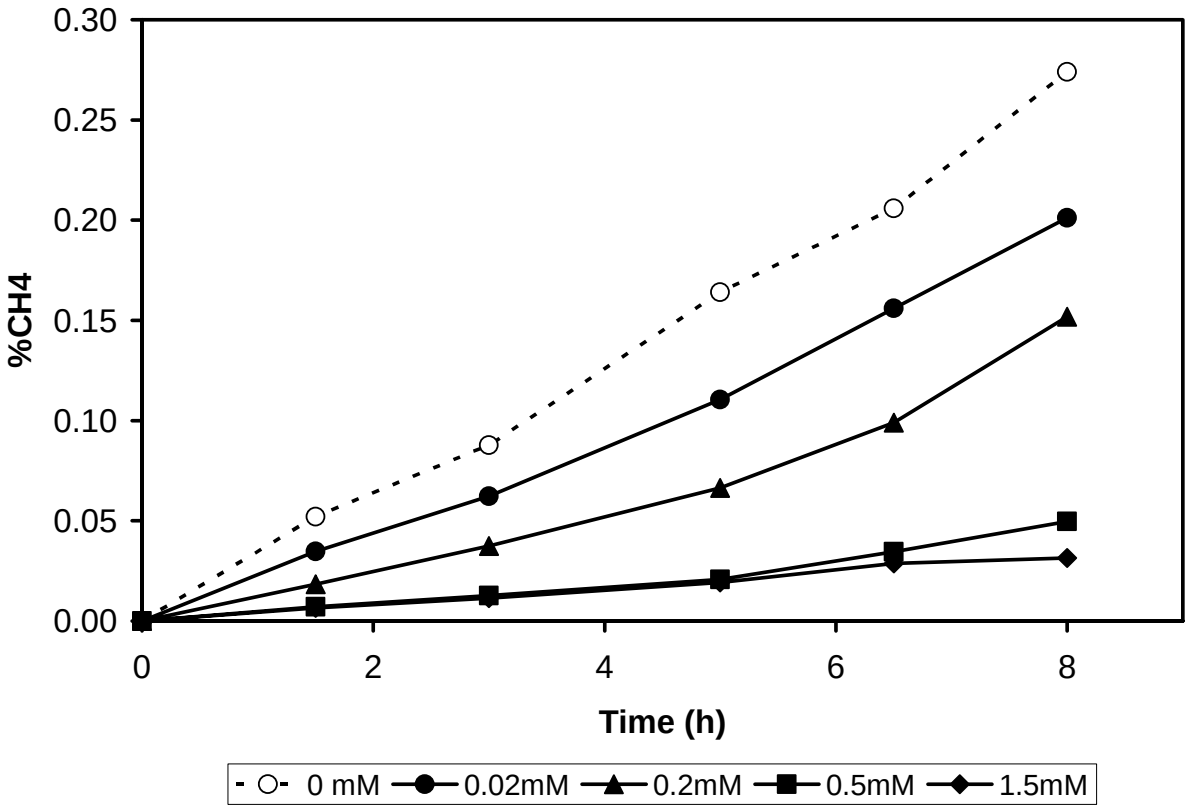



**Figure 11**. The time course of methane production during the acetoclastic methanogenic toxicity assay supplied with different concentrations of 16-h oxidized HAPA. The concentrations correspond to HAPA in the original unoxidized stock solution.

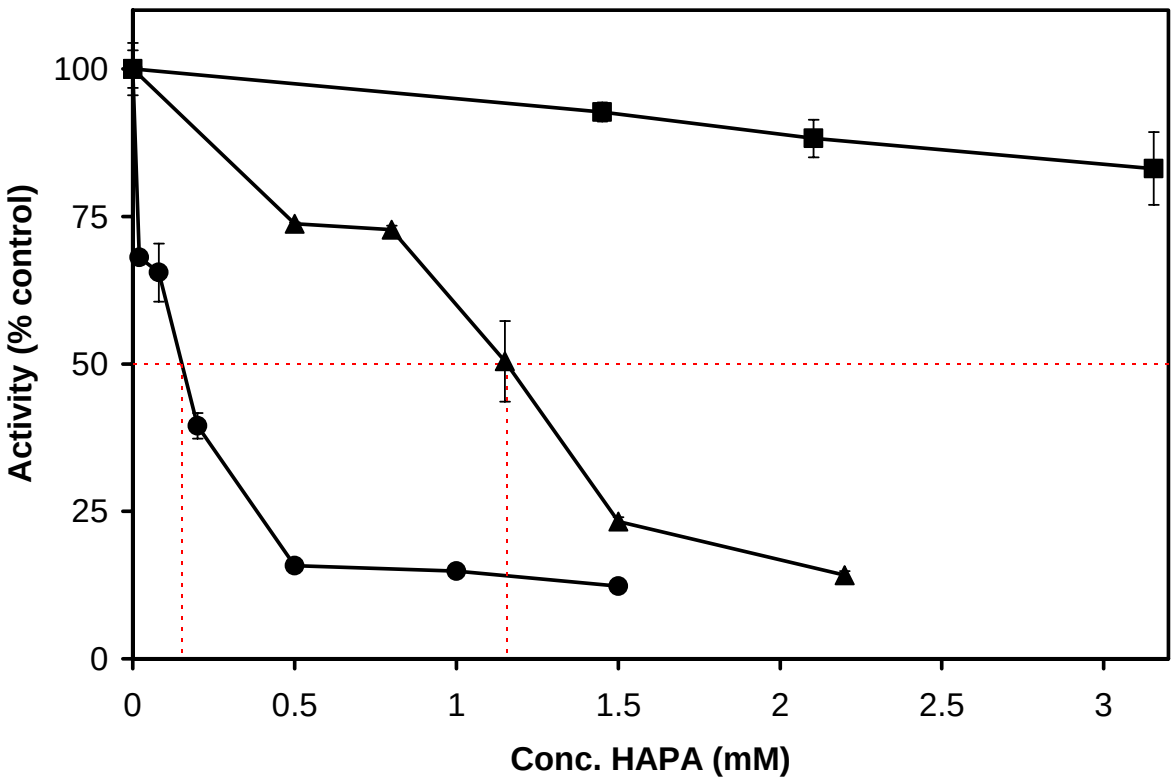



**Figure 12.** The relative activity of HAPA oxidized for 16 hours (closed circles), 4 minutes (closed triangles) and unoxidized HAPA (closed squares) as a function of the HAPA concentration. The concentrations correspond to HAPA in the original unoxidized stock solution.

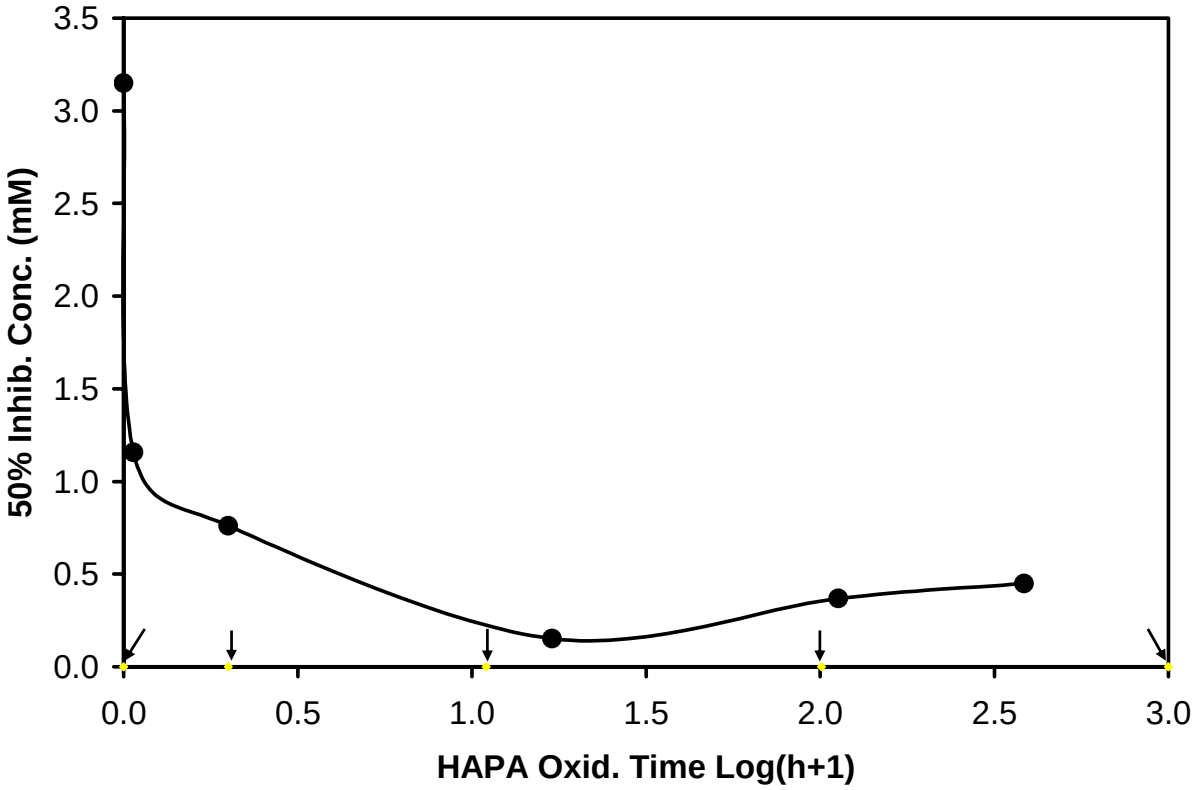



Figure 13. The 50% inhibiting concentration (50%IC) of HAPA to acetoclastic methanogenesis as a function of HAPA oxidation time. The graph is plotted on a logarithmic scale. From left to right the arrows indicate oxidation times corresponding to 0, 1, 10, 100 and 1000 h, respectively. The inhibitory concentration plotted at time 0 corresponds to the highest concentration tested of unoxidized HAPA, which correspond to 17% inhibition.

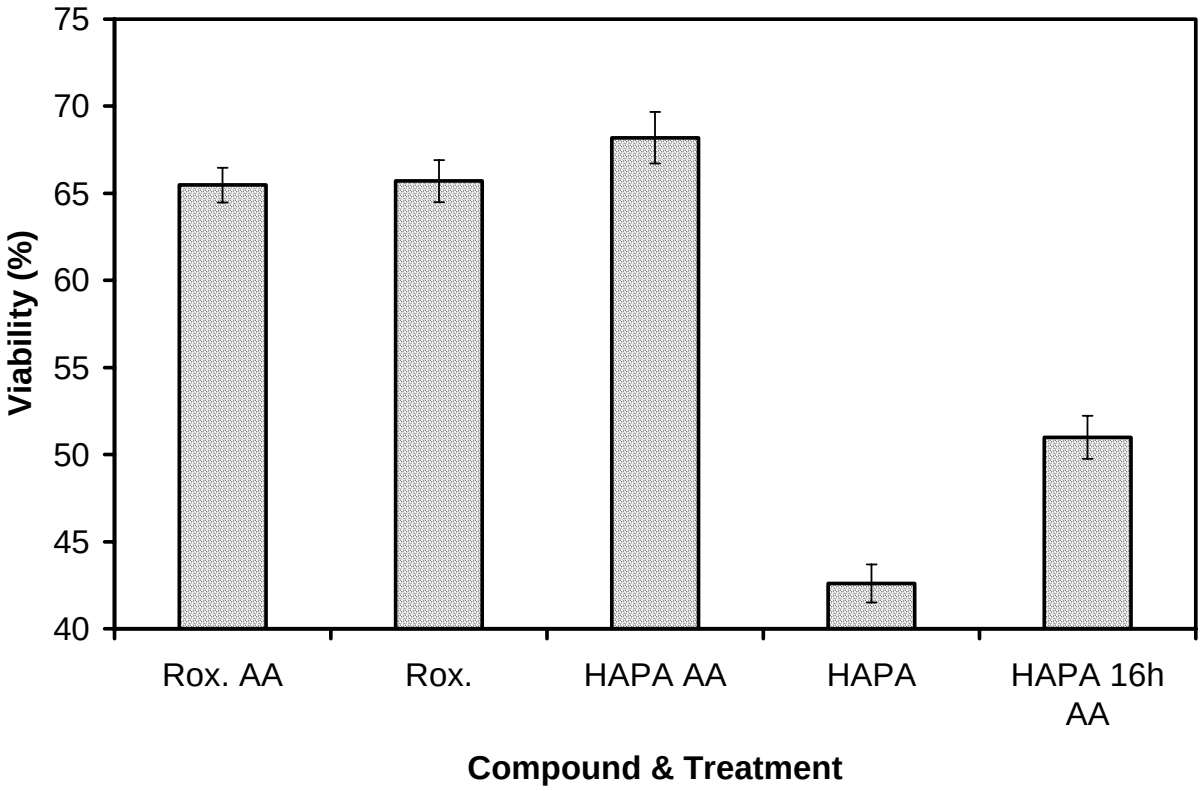



**Figure 14.** The toxicity of roxarsone and HAPA to the mitochondrial toxicity text (MTT) when supplied at a final assay concentration of 240 μM. Several assays conducted with ascorbic acid (AA) to prevent further oxidation of the compounds. The viability data of compounds incubated with AA is expressed as a percent of control viability in an assay the same level of AA.

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# Information Transfer Program

# Information Transfer

## Basic Information

| | |
|---|---|
| **Title:** | Information Transfer |
| **Project Number:** | 2005AZ109B |
| **Start Date:** | 3/1/2005 |
| **End Date:** | 2/28/2006 |
| **Funding Source:** | 104B |
| **Congressional District:** | 5 |
| **Research Category:** | Not Applicable |
| **Focus Category:** | Education, Management and Planning, Water Supply |
| **Descriptors:** | EDU, WS, M&I, LIP |
| **Principal Investigators:** | Sharon Megdal, Susanna Eden, Joe Gelt, Kathy Jacobs, Jackie Moxley, Kerry Schwartz, Terry Wayne Sprouse |

## Publication


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## INTRODUCTION

The University of Arizona's Water Resources Research Center (WRRC) promotes understanding of critical state and regional water management and policy issues through research, community outreach and public education.

A research and extension unit of the College of Agriculture and Life Sciences, the UA's WRRC is the designated state water resources research institute established under the 1964 Federal Water Resources Research Act. The WRRC conducts water policy research and analysis, and its information transfer activities include publications, conferences, lectures, seminars and workshops. Water news and information are provided to the academic community, water professionals, elected and appointed officials, students and the public. The WRRC is one of four UA water centers responsible for implementing the Water Sustainability Program, which receives funding from the UA's Technology and Research Initiative Fund.

## STAFF OUTREACH AND EDUCATION

The WRRC has established itself as a primary link between water-related personnel in the academic community; local, state, and federal government; and the private sector. The WRRC, with 40 years of experience addressing water resource problems and issues, places great importance on utilizing its experience and expertise address statewide water issues. WRRC staff reaches out to the community through presentations and lectures, service on boards, committees and panels, written articles and research activities.

WRRC Director, Dr. Sharon Megdal, has made numerous presentations on topics such as water management, drought planning, environmental restoration, climate and rural water resources issues. She has made presentations to variety of audiences ranging from undergraduate classes to keynote addresses at conferences. Dr. Megdal also made several conference speeches and presentations during the past year at locations throughout Arizona, as well in places outside the state, such as Washington, DC. In collaboration with Katharine Jacobs, Dr. Megdal teaches an annual graduate-level Arizona water policy seminar. Dr. Megdal has worked on four federal grant projects. Two of the projects dealt with the evaluation of environmental restoration and enhancement projects in Arizona. She completed a study for the Metropolitan Pima Alliance to determine water availability for the Tucson Metropolitan Area. Dr. Megdal also worked with an interdisciplinary team of researchers, funded by the UA Law School, WSP, and the National Science Foundation (NSF), to study irrigation districts and their incentives to transfer water. She has authored papers on a range of water-related issues, including Arizona recharge and recovery policies, the Central Arizona Groundwater Replenishment District, effectiveness of sustainable riparian systems, and the Arizona water/growth dilemma. On May 10, 2006, Dr. Megdal presented testimony before the United States House of Representatives, Committee on Resources, Subcommittee on Water and Power, with respect to S. 214 and H.R. 469, the "United States-Mexico Transboundary Aquifer Assessment Act."

Katharine Jacobs, Professor and Specialist with the Department of Soil, Water and Environmental Sciences, and the WRRC, is the new Executive Director of the Arizona Water Institute, a consortium of the three Arizona state universities. She was a lead staff person in developing Arizona's first drought management plan in cooperation with the Arizona Department of Water Resources and the Climate Assessment for the Southwest. Ms. Jacobs is project manager on a major grant with the U.S. Bureau of Reclamation on incorporation of climate information into modeling activities associated with managing the Colorado River. She is the Deputy Director of the NSF Center for Sustainability of Arid Region Hydrology and Riparian Areas at the UA. Ms. Jacobs has been involved in three National Academy panels over the past two years. She is currently chairing the committee reviewing the GAPP (GEWEX [Global Energy and Water Cycle Experiment] America Prediction Project) Water Cycle Research Program and is preparing to address a committee reviewing the GAPP Water Communication Uncertainty in Weather and Climate Forecasts.

Jackie Moxley, Program Coordinator for the Water Sustainability Program, is co-author of the publication, "Arizona Know Your Water: A Consumer's Guide to Water Sources, Quality Regulations, and Home Water Treatment Options." This handy guide will be reprinted in English and translated into Spanish. Over 5000 copies of the original version were distributed to County Extension offices, public libraries, state agencies and through individual requests. A Spanish translation of this popular booklet will provide much needed information on home water treatment alternatives to the Spanish-speaking population of Arizona. A new printing of the English version will further enhance the distribution of the water information booklet to the public. A downloadable web-based version is available on the WSP web site www.uawater.arizona.edu.

WRRC researcher, Terry Sprouse continues his U.S.-Mexico border research with a WSP grant to study the economic value to the area of Mexican effluent that flows into southern Arizona. He is working on local rainwater harvesting projects with public schools and non-profit organizations. Dr. Sprouse also serves as a board member on the International Boundary and Water Commission's Southeastern Arizona Citizens' Forum.

New Additions to the WRRC Staff

Susanna Eden re-joined the WRRC on November 14, 2005, when she assumed the position of Coordinator for Applied Research. From 1988 to 1993, she worked as a Research Specialist at the WRRC. She worked at the Minnesota Water Resources Center from 1994 to 1996 and more recently, for the U.S. Climate Change Science Program's Global Water Cycle Program in Washington, D.C. Since returning to Arizona, she has and Dr. Megdal provided a chapter on water and growth to the background report for the Spring 2006 Arizona Town Hall. She is heading the WRRC collaborative effort with the Water Education Foundation to develop an Arizona volume for the WEF's "Layperson's Guide" series. An outline has been developed, and the *Layperson's Guide to Arizona Water* is expected to be completed by early Spring 2007. She is working on several other projects, including working with Dr. Megdal on a volume on recharge for our *Arroyo* series.

Kristine Uhlman is the Arizona NEMO (Non-point Education for Municipal Officials) Program Coordinator will join the WRRC this summer. She is responsible for educational outreach to land-use decision makers on non-point source pollution issues. In fall 2006, as she becomes an Area Assistant Agent with Extension, she will assume faculty status and become affiliated both with the Pima County Cooperative Extension and the Water Resources Research Center. Kristine's work focuses on watershed characterization and mapping.

New WRRC Associate Director

Carl Bauer will be the new Associate Direct of the WRRC. He comes to the WRRC from Resources for the Future. His research is in comparative water law, policy, and management in Latin America and the Western United States. He has focused on issues of water rights; water markets; privatization and regulation; hydroelectric power; and governance of river basins. Dr. Bauer's work is interdisciplinary as well as comparative, reflecting his background in law, geography, geology, political economy, and history. He has long had a special interest in property rights, since property is where law, society, economy, and nature come together. He has written two books about water rights and water markets in Chile, which is the world's leading example of free-market water policies and institutions (*Siren Song* and *Against the Current*). Both books have also been published in Spanish.

ARIZONA WATER RESOURCE NEWSLETTER

The *Arizona Water Resource*, a 12-page newsletter that focuses on state and regional water issues, is published six times per year and is sent free of charge to over 2600 people. The newsletter has wide distribution; the majority of its readers are from Arizona, but the newsletter also is mailed to 42 states and 14 foreign nations. The publication regularly includes a main feature, guest view, special project, news briefs, legislation and law section and public policy column written by the WRRC Director, as well as announcement and publication notices. Feature articles during the past year addressed issues of special concern to the state including the upgrading of the Nogales Wastewater Treatment Plant, the Yuma Desalter controversy, need for water information in rural areas of the state, and the reliability in Central Arizona Project water deliveries. (The feature on the Nogales Treatment Plant has been reprinted in May, 2006, *The Water Report*.)

Each newsletter includes a four-page special supplement inserted as a center fold. By sponsoring a supplement and having it "piggy-back" on the newsletter, an agency or organization is distributing information about its activities and also helping to pay newsletter costs. The organizations sponsoring a supplement this year were U.S. Geological Survey, Bureau of Reclamation and UA's Water Quality Center and UA Water Sustainability Program. A special issue of the newsletter was published this year to honor the life and work of WRRC Director Emeritus, Sol Resnick who passed away on Dec. 11, 2005.

2005 Feature Articles

- Biomonitoring Checks Bodily Tissues, Fluids for Exposure to Chemicals: Universities and Private Sector Find Common Interests
- Interbasin Groundwater Transfers Revisited
- Opposing Sides Find Common Ground in Yuma Desalter Controversy
- Researchers Study DNA to Determine Human Response to Contaminants
- Rural Water Info is Key to New Laws

2006 Feature Articles to Date

- Need Grows for CAP Tucson Reliability
- Long Delayed, Nogales Wastewater Treatment Plant Now On Track
- Interconnected Energy/Water Savings and Uses Worked Into Conservation Planning

The WRRC web page provides access to the newsletter and other WRRC papers. It includes presentations made by WRRC faculty and presentations by selected guest speakers at WRRC seminars. Annual reports from 104B funded research projects and from Water Sustainability Program grants are posted on the web page. The web site, which is presently being upgraded to improve public access to WRRC materials, includes links to many state and national water related web sites, including the National Institutes for Water Resources (NIWR) homepage.

Another component of WRRC information transfer is keeping researchers of the three Arizona universities apprized of upcoming conferences and other special events. The WRRC receives many announcements for conferences and special events of interest to researchers. The staff reviews the announcements and determines the most appropriate audience for each announcement. The announcement is then sent out through one of several email lists that the WRRC maintain.

WRRC SEMINAR PROGRAM

The WRRC hosts a Brown Bag Luncheon Seminar Series that provides a forum for university personnel and other experts from around the state. The WRRC also sponsors special seminars to host renowned experts from outside the state. One of the special seminars was by former-Governor (1978-1987) and past-Secretary of the Interior (1993-2001), Bruce Babbitt, entitled, “At Water’s Edge.” Babbitt, speaking to an audience of 75 persons, identified the need to advocate for "smart growth" to maintain the natural space that supports wildlife, to promote clean streams, and to retain the ecological functioning of the land.

2005 Seminars

- Eugenia McNaughton, EPA Region 9 Border Team, Water Division, “Bloom and Boom in the Desert: The Water Quality Challenge on the Arizona-Sonora Border.”
- Gregg Garfin, UA Climate Assessment for the Southwest (CLIMAS) Program Manager, Institute for the Study of Planet Earth, “Is the Drought over? and CLIMAS Update.”
- Ralph Marra and Tim Thomur, Tucson Water, “Tucson Water Plan, 2000-2050.”
- James Hogan, UA Sustainability of semi-Arid Hydrology and Riparian Areas (SAHRA), “SAHRA Research Activities: Overview of Current Results and Planned Activities.”
- Sid Wilson, General Manager of the Central Arizona Project, “Balancing Water Needs on the Lower Colorado River: Recommendations of the Yuma Desalting Plant/Cienega de Santa Clara Workgroup.”
- Bonnie Colby, UA Department of Agricultural and Resource Economics, Book reception hosted by SAHRA and WRRC, “Negotiating Tribal Water Rights, Fulfilling Promises in the Arid West.”
- Steve Rossi, Principal Water Resource Planner, City of Phoenix, “Phoenix Water Planning and Salinity Issues.”
- Ian Pepper and Chris Choi, UA Environmental Research Laboratory, NSF Water Quality Center, “Water Village/Water Security Project.”
- Placido Dos Santos, Border Coordinator, Arizona Department of Environmental Quality (ADEQ), “The Environment and Water on the Border: Conflict, Cooperation and the Pursuit of Consensus.”
- Bruce Babbitt, former Governor of Arizona, former Sect. of the Interior, “At Water's Edge.”
- Karen Smith, Deputy Director, Arizona Department of Water Resources (ADWR), “Arizona Water Priorities for 2006.”
- Carl Bauer, Resources for the Future, “Free Markets and Water Reforms: The Chilean Experience and International Water Policy Debates.”

2006 Seminars to Date

- Brad Lancaster, author, permaculturalist and water harvester, “Turning Water Scarcity into Water Abundance with Water Harvesting: Guiding Principles to Welcome Rain Into Your Life and Landscape.”
- Margot Garcia, Professor Emeritus, and member of the Natural Resources Council Panel to restore Louisiana Wetlands, “Oops! There goes New Orleans.”
- Val Little, Director, Water Conservation Alliance of Southern Arizona (Water CASA), “The ECoBA Program: an evaluation and cost-benefit analysis of municipal water conservation programs.”
- Joe Abraham, PhD student, UA Department. of Geography and Regional Development, “Assessing Drought Vulnerability.”

- Eric Betterton, Professor, UA Atmospheric Sciences, "Cloud Seeding in Arizona – Theory and Practice."
- Bradley Udall, Director, Western Water Assessment Center, "The Colorado River Compact: A Perspective from the State of Colorado."

## THE NEW DESERT LANDSCAPING CD

The newly up-dated WRRC Desert Landscaping CD was released in 2005. It was designed as a project to encourage appropriate desert plant selection. Desert Landscaping is a valuable tool for desert gardening needs, whether identifying the right plant for a container or choosing low water-use vegetation to landscape yard or patio. The CD has become a standard Southwest resource, used by thousands of home and professional gardeners. Over 10,000 copies of the first version were sold. The Desert Landscaping CD-ROM is available at nurseries and bookshops.

The CD is also being utilized and promoted by the UA Master Gardeners program in Maricopa, Pima and Cochise counties. The WRRC hosted displays of the new CD at Water and Plant Expos in Tucson, Phoenix and Sierra Vista.

## THE TRIF WATER SUSTAINABILITY PROGRAM

Part of the recent growth of WRRC was made possible through funding for the UA's Water Sustainability Program (WSP). It is a campus-wide collaboration of scientists and educators and is coordinated by the WRRC and three National Science Foundation funded water centers. Funds to create WSP originated from a November 2000 voter approved proposition to increase sales taxes to support education. Ten percent of the new sales tax money was allocated to the three state universities in Arizona. Funds derived from this source were used to establish the WSP and to conduct six other UA initiatives. The new funding source was designated as the Technology and Research Initiative Fund (TRIF).

WSP, now in the fifth year of a five-year program, consists of various components, each with a different strategy to promote water knowledge and understanding. WRRC has played a central role in implementing, developing, and managing program components that include a competitive grants program, a student fellowship awards program, a recruitment and research initiative, and center-directed initiatives. In this role, WRRC personnel interact with faculty and staff throughout the UA, including Cooperative Extension offices. The second five-year period of funding, beginning July 1, 2006, has been approved by the Arizona Board of Regents. This will allow the four centers and the large UA water community to continue to expand their water resources research, education and outreach.

A key component of the WSP is the competitive grants program. Each year about $1million is allocated to UA faculty and staff to fund projects relevant to Arizona water issues in research, education and outreach. Thirty-nine projects have been selected in three cycles of the competitive grants program through an expert review process and have

received a total of $3.5 million. Over 100 faculty and staff from 23 departments/schools/units, across six colleges are working on these projects in cross-disciplinary collaborations. More than 120 student opportunities through paid positions or research assistantships have been created through these projects and at least 80 new partnerships with city, county, state and federal agencies, the private sector, schools, and NGOs, providing direct dollar matches, in-kind contributions, and consultative input, have been formed. These projects have attracted about $850,000 in direct dollar support from off-campus sponsors.

In 2006, 20 new projects were selected and four projects were granted continuation of funding for the next cycle beginning July 1, 2006. Each project funded through the TRIF WSP competitive grant process is associated with one of the four UA water centers. More information on the grants can be found at www.uawater.arizona.edu.

For the 2005-06 grant cycle, the following TRIF WSP projects were hosted by the WRRC.

1. ***Arizona Project WET Evaluation: Examining Impact and Developing Water Education Assessment Tools for Students***. **$49,351 - 1 year**. Dr. Jerome D'Agostino, Department of Educational Psychology, College of Education, Kerry Schwartz, Water Resources Research Center, College of Agriculture and Life Sciences.

2. ***The Value of Binational Effluent and Sustainable Watershed Management in the Upper Santa Cruz Basin.*** **$62,879 - 1 year.** Terry Sprouse, The Water Resources Research Center and George Frisvold, Department of Agricultural and Resource Economics, College of Agriculture, and Life Sciences.

3. ***Promoting The Adoption Of Subsurface Drip Irrigation By Arizona's Farmers.*** **$20,094 - 3 years.** Thomas L. Thompson, Department of Soil, Water, and Environmental Science, Edward Martin, Department of Agricultural and Biosystems Engineering, Patrick Clay, Maricopa County Cooperative Extension, Mary Olsen, Department of Plant Pathology, Russell Tronstad, Department of Agricultural and Resource Economics, James Walworth, Department of Soil, Water, and Environmental Science. All the units are within the College of Agriculture and Life Sciences.

In the most recent 2006-07 grant cycle, the following TRIF WSP projects will be hosted by the WRRC.

1. **Spanish Translation and Reprints of the Booklet,** ***Arizona: Know your Water*** **(2004**). **$23,100 – 1 year.** Janick Artiola, Department of Soil & Water Science, Katherine Farrell-Poe, Department of Agricultural and Biosystems Engineering.

2. **On-line Access to Distance-Learning Tools for Watershed Stewardship in Rural Arizona. $41,469** - **1 year.** Robert Emanuel, Arizona Cooperative Extension, Garry Forger, Learning Technologies Center, George Zaimes, School of Natural Resources, Michael Crimmins, Department of Soil, Water, & Environmental Science.

3. **Valuation of Binational Effluent in the Upper Santa Cruz Basin: Estimating Willingness to Pay**. **$39,552- 1 year.** George Frisvold, Department of Agricultural & Resource Economics, Terry Sprouse, Water Resources Research Center.

4. **Watershed Rainfall, Ground Water Usage, Riparian Stream Flow and Vegetation Monitoring, Middle San Pedro River Basin, Cochise County, AZ. $2,500 - 1 year.** Phil Guertin, Kristine Uhlman, School of Natural Resources.

5. **Predicting Groundwater Vulnerability to Nitrate in Arizona. $69,992 - 1 year.** Tauhidur Rahman, Department of Agricultural and Resource Economics, Kristine Uhlman, School of Natural Resources.

6. **Mapping Accumulation of Soil Salinity in Landscapes Irrigated with Reclaimed Water**. **$31,154- 1 year.** Ursula Schuch, Department of Plant Sciences, James Walworth, Department of Soil, Water and Environmental Sciences.

7. **Implementation of Efficient Surface Irrigation Practices in the Lower Colorado River Region. $40,000 - 1 year.** Dawit Zerihun, Charles A. Sanchez, Yuma Agricultural Center.

WRRC – TRIF DIRECTED INITIATIVES

In addition to the TRIF grants projects conducted in-house or hosted by the WRRC, the new Directed Initiatives component of WSP has provided opportunities for the WRRC to interact with other departments and colleges and to strengthen WRRC educational programming. The WRRC provided start-up funds to a Maricopa County Cooperative Extension project to launch a home water quality and conservation hotline for the public. The project was done in collaboration with state agency and city personnel and included training a volunteer corps of Master Consumer Advisors.

Two funded projects address important policy issues in the state. The first is a two year joint study by faculty from the WRRC, the Law College, the Department of Agricultural and Resource Economics and Eller College of Management to analyze opportunities and barriers for voluntary, temporary water transfers and exchanges. The project will also promote public understanding of the issues and to propose policy options to facilitate water transfers. The second undertaking, "Water Management Innovations for Arid Regions: Arizona Policy and Practice," provided support for a book (soon to be released) and public forum intended to offer multiple perspectives for arid regions facing competing water demands and periodic drought.

Recruitment of a new faculty member in the Department of Geography and Regional Planning who brings expertise in water policy to UA was supported by WRRC through Directed Initiative funds. The new faculty will have a joint appointment to the WRRC as Associate Director.

WRRC Director, Sharon Megdal, was instrumental in recruiting new faculty as part of the broader WSP Recruitment and Research Initiative for FY07 for water-related positions in law and political science.

## ARIZONA PROJECT WET

The goal of the WRRC's Arizona Project WET program is to educate people about Arizona's water resources in an engaging and understandable way. To accomplish this goal the Center is utilizing the nationally recognized Project WET (Water Education for Teachers) program, in which solid pedagogy has been used in developing workshops for teachers and resource specialists. The lessons are interactive, multidisciplinary, research-based, constructivist and promote critical thinking.

The Director of Arizona Project WET is supported by a full time and part time staff position and five UA students working together keep this program effective and thriving. Facilitators are trained in water content and teaching/facilitation methods in intensive workshops. At present the Program has 100 volunteer facilitators in three areas of the state available to conduct workshops for K-12 teachers and other educators. Coordinators and facilitators trained 891 teacher/educators in 53 workshops, educating a reported 62,530 students in 2005.

### Arizona Makes a Splash with Project WET Water Festivals Program

The comprehensive Arizona Project WET program is the foundation for the *Arizona Make a Splash with Project WET Water Festivals*. Water Festivals are intensive and interactive learning experiences for 4$^{th}$ grade students and their teachers. Content is in accordance with the state mandated learning objectives for the students and includes the water cycle, watersheds and water supply, riparian systems, groundwater and water conservation. At the festival, structured hands-on lessons are used to engage students in understanding natural systems and water resources while having fun.

Arizona water festivals were held at six locations reaching 4,338 students, 190 teachers, 63 schools and 423 volunteers in 2005. The addition of a Program Coordinator in 2004 has enabled the water Festival program to expand while maintaining effective education standards.

These Arizona Water Festivals were supported by sponsors, school administrators, teachers, students and communities. The ongoing commitment of the U.S. Bureau of Reclamation, Arizona Department of Water Resources, Arizona Department of Environmental Quality, Arizona Game and Fish, Salt River Project and Central Arizona Project as well as other local sponsors resulted in a budget of $51,549 for 2005 Water Festivals. In-kind donations for 2005 from over 40 organizations totaled over $80,000.

Kerry Schwartz, who has guided Arizona Project WET through much expansion, has been appointed by Arizona Cooperative Extension and the Department of Agricultural Education to Associate Specialist. While Kerry and her programs will remain housed at

the WRRC, this new faculty position will provide expanded opportunities for professional and programmatic growth.

## WATER EXPO AT THE ARIZONA STATE CAPITOL

*Water Expo 2006*, a January 23 event cosponsored by the UA's Water Sustainability Program was an opportunity to acquaint Arizona lawmakers with water projects throughout the state concerned with water sustainability. Conducted on the Senate lawn of the Arizona State Capitol, Water Expo included 46 exhibitors from 9 municipalities, the 3 state universities, multiple state and federal agencies, private water companies and numerous other organizations.

## PROFESSOR SCHLAGER'S SABBATICAL HOSTED BY WRRC

The WRRC was please to host Professor Edella Schlager's sabbatical in spring 2006. Dr. Schlager is an associate professor in the Department of Public Administration and Policy. Her research focuses on environmental policy and water resource management.

## THE ARIZONA WATER INSTITUTE

The Arizona Water Institute is a collaboration of the state's three universities focused on supporting community efforts to resolve water problems, promoting economic development through technology transfer, and expanding educational opportunities. Partners in this effort include state agencies, water stakeholders, the governor's office and private sector interests. The AWI began functioning formally in January with the appointment of the WRRC's Kathy Jacobs as the executive director.

The opportunities that are expected to result from the Institute are important from both a strategic and an economic perspective. This entity is expected to be largely self-supporting, and result in significant influx of federal funding, grants and contracts, and private and foundation support. The business plan shows that the AWI is expected to generate $7.5 million in new annual revenues within 5 years. The key foci for the AWI are: 1) research, community assistance and analytical support; 2) education, training and professional capacity building; and 3) technology and economic development.

Four initial projects are underway. Each project is collaborative, involving two or more universities, as well as governmental agencies and public and private sector participants:

1) Arizona Hydrologic Information System: This project will develop the information infrastructure of the AWI and provide access to data relevant to water-related research, technology, planning, education, and outreach from multiple sources within the Southwest.

2) AWI Water Quality Priority Projects: Two water quality research themes are under way: arsenic and other inorganic contaminants in drinking water and source waters, and emerging contaminants in wastewater.

3) Water Conservation Technology Exchange: Intel held a very successful initial forum on Dec. 9 to enhance water conservation technology exchanges between industrial water users, water providers, policy makers, research and educational institutions, and other community groups in Chandler. Additional forums will be held.

4) Meeting Water Management and Planning Needs within Watersheds: This prototype project is intended to bring the water talent of Arizona's three universities together to address rural and community watershed issues.

## ANNUAL WRRC CONFERENCES

### 2005 Conference

The 2005 WRRC Conference, entitled "Water and the Environment: The Role of Ecosystem Restoration," was held in Tucson, Arizona on April 6th. The conference was an opportunity for various agencies or organizations to showcase ecosystem restoration projects in which they are involved. Maricopa County, Pima County, City of Phoenix, Salt River Project, U.S. Geological Survey and researchers at Northern Arizona University, Arizona State University and the UA described their ecosystem restoration work. Conference presentations are posted on the Water Resources Research Center's web site: www.cals.arizona.edu/AZWATER.

The conference underscored the importance of early development of partnerships and the need to involve all interested parties. These partnerships can take many different forms and often involve the private and public sectors. The needs for monitoring and multidisciplinary research was clear, both at the front-end of projects as well as after projects are completed. The need for communication at many levels was apparent. Whether relating the reasons for spending millions of dollars on the Lower Colorado River Multispecies Conservation Program or for putting up elk fencing, communicating with the general public and stakeholders is always important.

The need for funding was also emphasized. Restoration efforts often take many years and involve significant investments, especially when land acquisition is involved. Partnerships are needed to get things done. The last session of the day, which focused on funding, made it clear that parties will have to be more resourceful to assemble the necessary financial, water and other resources.

### 2006 Conference

The 2006 WRRC Conference, held in Phoenix on June 20-21, titled "Providing Water to Arizona's Growing Population: How Will We Meet the Obligation?" provided a timely dialogue on water and growth. Day one of the conference featured a session on the different ways city, town and rural area managers addressed the issue "water planning for growth," followed by a panel of diverse water professionals who considered "where is the water coming from?" Afternoon sessions included perspectives from developers, home builders, and realtors; the role of the Central Arizona Groundwater Replenishment Dis-

trict; and views from public officials.

Day 2, an optional half-day workshop, hosted by Arizona State University's Global Institute of Sustainability, focused on in-depth discussions for meeting the long-term water needs of Central Arizona and implications for the rest of the state. Presentation of a background paper and a panel discussion on the political decisions, infrastructure investments and water management programs necessary were followed by a facilitated open discussion on key policy questions and issues that need to be addressed.

WRRC RESEARCH PROJECTS 2005 - 2006

Listed below are 2005-2006 WRRC research projects and funding sources.

- Enhancing Water Supply Reliability through Improved Predictive Capacity and Response. Funding: U.S. Bureau of Reclamation
- Environmental Restoration, Preservation and Enhancement Projects
  Funding: U.S. Bureau of Reclamation
- Arizona Water Resources Cooperative Agreement
  Funding: U.S. Bureau of Reclamation
- Examination of Enhancement Projects In Arizona's Environment
  Funding: U.S. Bureau of Reclamation
- Supplement to: Examination of Enhancement Projects In Arizona's Environment
  Funding: Vice President for Research, UA
- Harnessing Science and Technology for Sustainability
  Funding: Harvard University
- Report on Water Resources Availability for the Tucson Metropolitan Area
  Funding: Metropolitan Pima Alliance
- The Value of Binational Effluent and Sustainable Watershed Management in the Upper Santa Cruz Basin.
  Funding: Water Sustainability Program
- Binational and Collaborative Watershed Management In The Santa Cruz River
  Funding: Sonoran Institute/Altria Group
- Valuation of Binational Effluent in the Upper Santa Cruz Basin: Estimating Willingness to Pay
  Funding: Water Sustainability Program
- Arizona Project Wet Evaluation: Examining Impact and Developing Water Education Assessment Tools for Students
  Funding: Water Sustainability Program
- Environmental Restoration, Preservation, & Enhancement Projects in Arizona
  Funding: Army Corps of Engineers

# Student Support

| Student Support | | | | | |
|---|---|---|---|---|---|
| **Category** | **Section 104 Base Grant** | **Section 104 NCGP Award** | **NIWR-USGS Internship** | **Supplemental Awards** | **Total** |
| **Undergraduate** | 15 | 5 | 0 | 0 | 20 |
| **Masters** | 6 | 0 | 0 | 0 | 6 |
| **Ph.D.** | 4 | 4 | 0 | 0 | 8 |
| **Post-Doc.** | 0 | 0 | 0 | 0 | 0 |
| **Total** | 25 | 9 | 0 | 0 | 34 |

# Notable Awards and Achievements

On May 10, 2006, WRRC Director, Dr. Sharon Megdal, presented testimony before the United States House of Representatives, Committee on Resources, Subcommittee on Water and Power, with respect to S. 214 and H.R. 469, the United States-Mexico Transboundary Aquifer Assessment Act.

Kathy Jacobs was appointed in January 2006, by Governor Janet Napolitano, to be the first executive director of the Arizona Water Institute (formerly called the Arizona Virtual Water University).

Kerry Schwartz was appointed to be a Board Member of the Arizona Association for Environmental Education.

Kerry Schwartz was asked to serve on the Project WET USA Council.

Kerry Schwartz was appointed by Arizona Cooperative Extension and the Department of Agricultural Education to Associate Specialist on January 1, 2006.

Susanna Eden was a participant in the 4th World Water Forum, held in Mexico City, March 16 - 22, 2006. She served on a panel of experts in the session on Space-Based Water Observations, and presented the results of three virtual workshops conducted as part of the preparations for the Forum.

Joe Gelt had an Arizona Water Resource newsletter article re-published in The Water Report, a regional journal that reports on water rights, water quality, and water solutions in the West.

Jackie Moxley completed a three-month course to become an Arizona Master Watershed Steward.

Terry Sprouse serves as a Board Member on the International Boundary and Water Commissions, Southeast Arizona Citizens Forum.

Michael Bellefeuille (104B research project 2005AZ90B) participated in an international competition sponsored by GE, DowJones ECOnomics for a research fellowship ($50,000) to study an environment-related subject. He was among the 20 candidates selected for interview. The title of his

research proposal was Using Microalgae to Convert Septic Tank Effluent into Biodiesel.

Michael Bellefeuille (104B research project 2005AZ90B) won the Best Paper Presentation Award in Biology" for his presentation "Biological Carbon Dioxide Sequestration Using a Recently Isolated Microalgal Strain" at the 50th annual Arizona-Nevada Academy of Science meeting, Tucson, AZ (April 8, 2006).

Findings from 104B research project 2005AZ 81B have facilitated the acquisition of additional research funds to continue work in the area of nitrate bioremediation. A two-year project entitled Autotrophic Denitrification for the Treatment of Drinking Water, which is funded by TRIF and by the engineering company, Hydro Geo Chem Inc., is currently ongoing (June, 2005-June 2007).

Research collaboration, under 104B project 2004AZ81B, was established with the Department of Microbiology, Autonomous University of Madrid, Spain. The purpose of the joint effort is to characterize the sulfoxidizing, nitrate-reducing microorganisms in the reactor biofilms utilizing molecular ecology techniques.

The article below, produced under 104G project 202AZ9G, was selected by the editorial board as the featured news article in Environmental Science and Technology.

Cortinas, I., J. A. Field, M. Kopplin, J. R. Garbarino, A. J. Gandolfi and R. Sierra-Alvarez. 2006. Anaerobic biotransformation of roxarsone and related N-substituted phenylarsonic acids. Environ. Sci. Technol. (in press).

# Publications from Prior Projects

1. 2004AZ50B ("Impact of drought on management of salt sensitive plants with reclaimed water") - Articles in Refereed Scientific Journals - Schuch, U.K., 2005. "Effects of reclaimed water and drought on salt-sensitive Perennials," HortScience 40(4):1095.
2. 2004AZ42B ("Permeable Reactive Biobarriers for the Containment and Remediation of Acid Mine Drainage") - Articles in Refereed Scientific Journals - Sierra-Alvarez, R., S. Karri, S. Freeman, J.A. Field. 2006. Biological Treatment of Heavy Metals in acid mine drainage using sulfate reducing bioreactors. Water Sci. Technol. (In press).
3. 2004AZ42B ("Permeable Reactive Biobarriers for the Containment and Remediation of Acid Mine Drainage") - Conference Proceedings - R. Sierra-Alvarez, S. Karri, S. Freeman, J.A. Field. 2005. Biological Treatment of Heavy Metals in Acid Mine Drainage Using Sulfate Reducing Bioreactors. VIII Latin American Workshop and Symposium on Anaerobic Digestion. October 2-5, 2005. Punta del Este, Uruguay. Pp. 298-303
4. 2004AZ42B ("Permeable Reactive Biobarriers for the Containment and Remediation of Acid Mine Drainage") - Dissertations - Karri, Sri Lakshmi, 2004, Bioremediation of Heavy metals Using Sulfate Reducing Bacteria, MS Thesis University of Arizona, Dept Chemical and Environmental Engineering.
5. 2004AZ42B ("Permeable Reactive Biobarriers for the Containment and Remediation of Acid Mine Drainage") - Articles in Refereed Scientific Journals - Karri S, Sierra-Alvarez R, Field JA, Zero valent iron as an electron-donor for methanogenesis and sulfate reduction in anaerobic sludge, Biotechnology And Bioengineering 92 (7): 810-819 Dec 30 2005.