Water Resources Research Center
Annual Technical Report
FY 2004

Introduction

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

Research Program

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

Basic Information

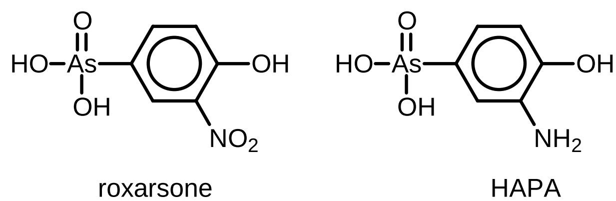
Title:	Agricultural Chemicals as a Major Non-Point Source of Arsenic: Microbial Transformation of Organic Arsenicals
Project Number:	2002AZ9G
Start Date:	9/1/2002
End Date:	8/31/2005
Funding Source:	104G
Congressional District:	AZ05
Research Category:	Water Quality
Focus Category:	Toxic Substances, Agriculture, 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

Overview of Third Year's Activities

During the last year, research has focused on the biodegradation and toxicity of two nitrogen-substituted phenylarsenates. One of the compounds studied was roxarsone and the other compound was 4-hydroxy-3-aminophenylarsonic acid (HAPA), a biotransformation product of roxarsone. The structure of the two compounds are illustrated in Figure 1. Roxarsone is used extensively in the poultry industry and finds it way into the environment through land application of poultry manure. Additionally, the long term aerobic biodegradability of the herbicide, cacodylic acid (dimethylarsinic acid, DMA(V)), was evaluated in incubations with activated sludge.

Figure 1. Chemical structure of nitrogen substituted phenylarsenates: roxarsone and 4-hydroxy-3-aminophenylarsonic acid (HAPA).



Biodegradation of Nitrogen-Substituted Phenylarsenates.

<u>Biotransformation of roxarsone to HAPA in anaerobic sludge.</u> We previously reported the rapid reductive biotransformation of roxarsone to HAPA in microcosms inoculated with anaerobic sludge (2nd Annual Report). This year the research explored the environmental and physiological conditions for rapid bioconversion. Since the reaction requires the input of electrons, the role of different electron donating substrates on the rate of the bioconversion was examined as shown in Figure 2. Hydrogen, glucose and lactate supported the first, second and third fastest rates of biotransformation; respectively. Biotransformation also occurred at a modest rate in treatments not receiving any electron-donating substrate. Most likely due to the slow hydrolysis and degradation of biomass in the sludge, referred to as endogenous substrate. Acetate was a poor electron donating substrate, since it did not significantly increase the endogenous rate of roxarsone reduction. HAPA was shown to be formed stoichiometrically from the reduction of roxarsone. In the killed sludge control, a small fraction of roxarsone was converted to HAPA, most likely due to chemical reduction by reducing agents in the heat-killed sludge.

Reductive biotransformation reactions are known to be accelerated by extracellular redox shuttles. The impact of two extracellular shuttles, riboflavin and anthraquinone-2,6-disulfonate (AQDS) was evaluated. The electron shuttles were supplied at 0.05 mM to stimulate the reduction of 1 mM roxarsone by anaerobic sludge with hydrogen gas as the electron donor. The results of the experiment (Figure 3), demonstrate that AQDS and riboflavin significantly increase rates of roxarsone reduction. AQDS is often used as a model of quinone moieties in humus. Thus the results implicate humus as a possible electron shuttle that could dramatically increase the rates of roxarsone biotransformation.

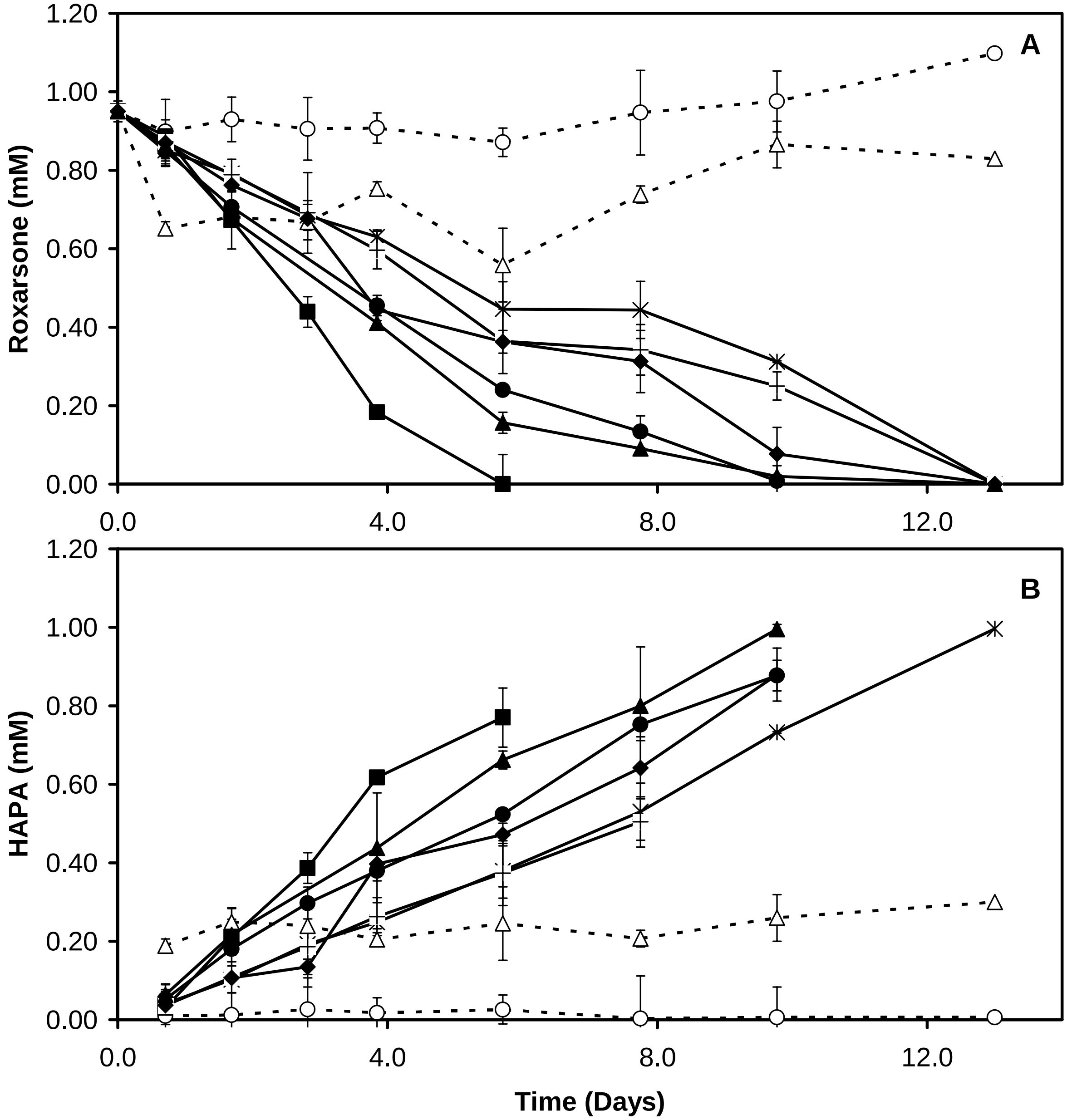


Figure 2. The role of electron-donating substrates on the time course of roxarsone biotransformation to HAPA in anaerobic sludge. The roxarsone and HAPA concentrations are shown in panels A and B; respectively. Legend: medium only control, open circles; killed sludge control, open triangles; no added electron donor, asterisk; acetate, plus sign; mixture of volatile fatty acids, closed diamonds; lactate, closed circles; glucose, closed triangles; hydrogen gas, closed squares

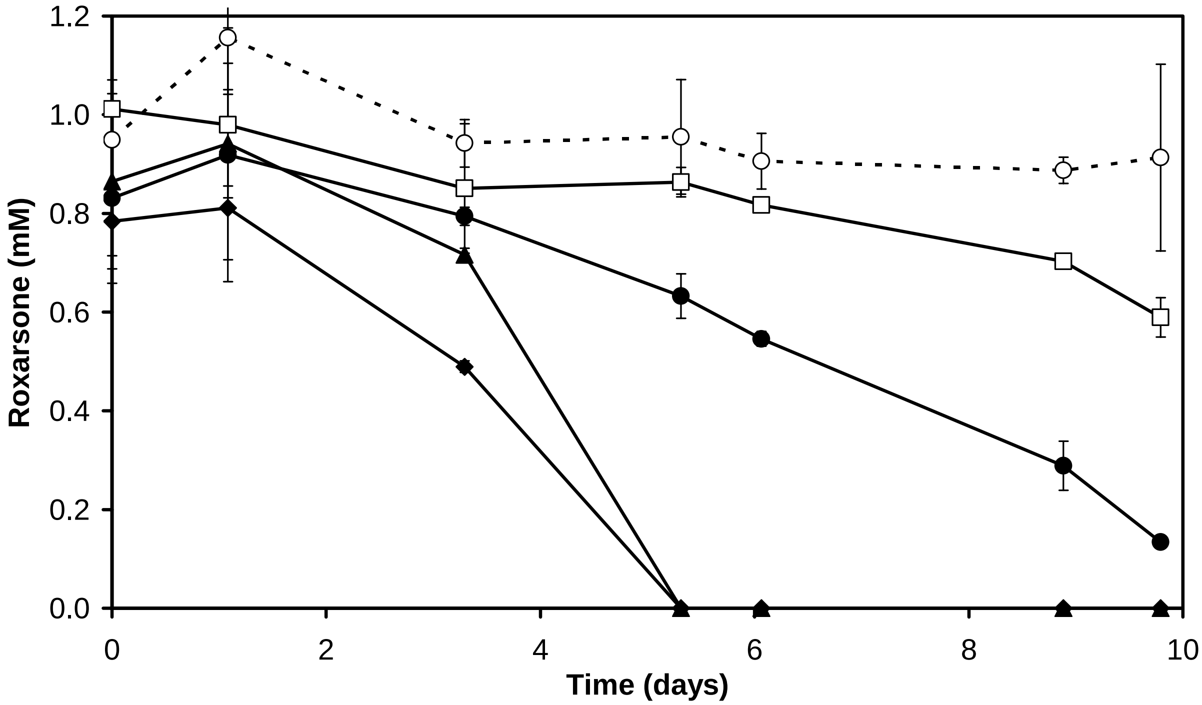


Figure 3. Effect of extracellular electron shuttles on the rate of roxarsone reduction in anaerobic sludge. The shuttles, riboflavin or anthraquinone-2,6-disulfonate (AQDS) were each supplied at 0.05 mM. Legend: medium only, open circles; no electron donor added control, open squares; hydrogen, closed circles; hydrogen and riboflavin, closed triangles; hydrogen and AQDS, closed diamonds.

Biotransformation of roxarsone to HAPA in aerobic sludge. The biodegradation of roxarsone was also evaluated under various redox conditions with aerobic activated sludge, obtained from a secondary treatment unit at Ina Road municipal wastewater treatment plant, Tucson. Biodegradation was compared under aerobic (O_2 added), denitrifying conditions (NO_3^- added) sulfate reducing (SO_4^{2-} added) and under methanogenic (no electron acceptor added) conditions as well as under methanogenic conditions with added lactate (as an electron donating substrate). The results (Figure 4) indicate that there was no bioconversion of roxarsone under aerobic and denitrifying conditions. Biotransformation was only evident under methanogenic and sulfate reducing conditions, with the highest level of biotransformation occurring in the methanogenic treatment with added lactate. HAPA was observed again as a biotransformation product, but it was not recovered stoichiometrically as was observed in the experiments with anaerobic sludge. Instead, HAPA only accounted for about 37 to 56% of the roxarsone eliminated. The lower than stoichiometric yield is not due to adsorption of arsenic on sludge or volatilization because total non-speciated arsenic concentrations were constant at 1 mM (which indicates that all arsenic was still present in solution). The missing arsenic was due to unidentified species (note that arsenate, arsenite and methylated species were monitored and not found). The results taken as a whole indicate that degradation does not occur readily under aerobic or denitrifying conditions. However an aerobic inoculum, can cause the reductive biotransformation of roxarsone to HAPA and other unidentified products when incubated anaerobically.

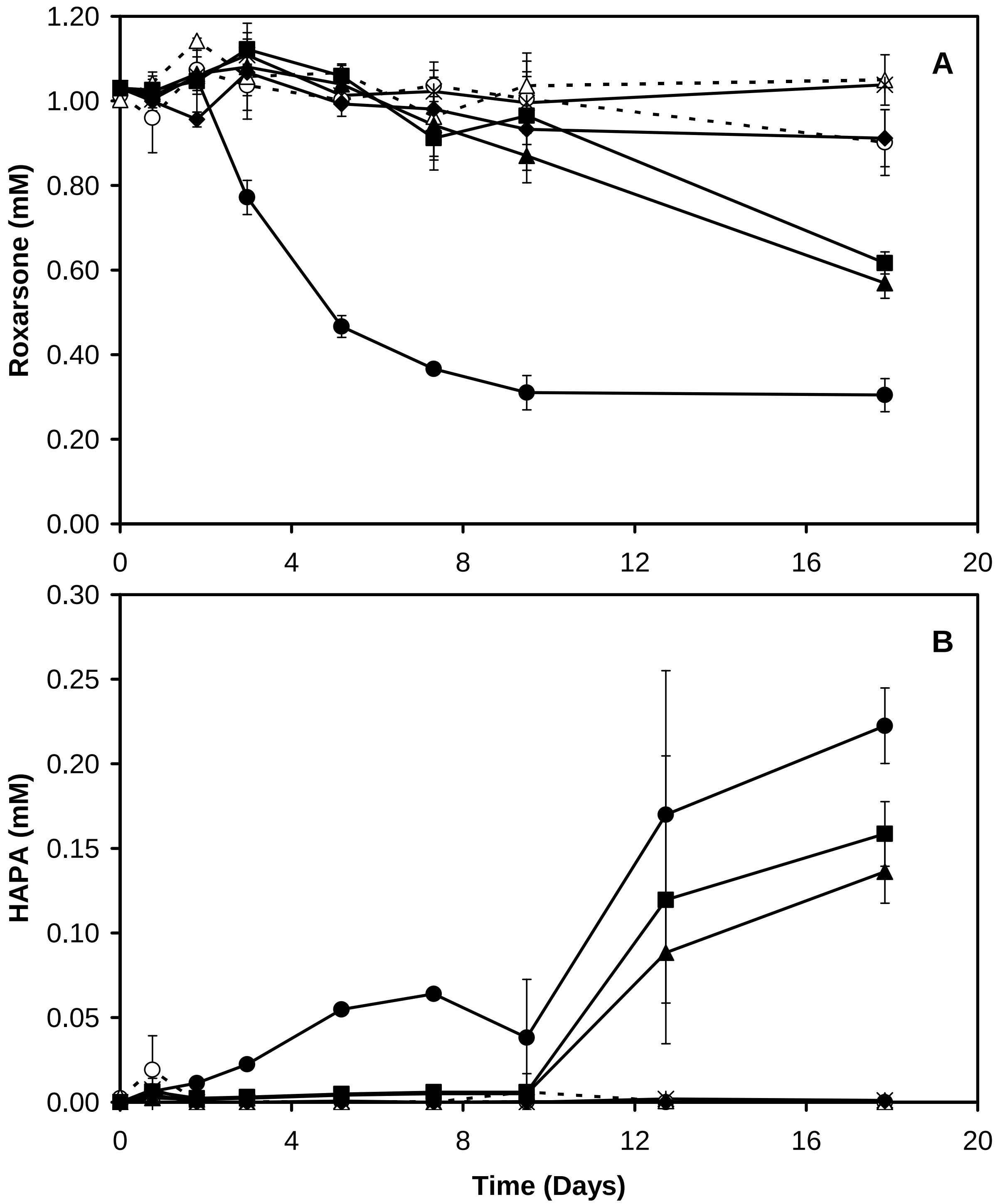


Figure 4. Biotransformation of roxarsone in aerobic activated sludge incubated under variable redox conditions. Roxarsone and HAPA concentrations are given in panels A and B; respectively. Legend: medium only control, open circles; killed sludge control, open triangles; aerobic, astericks; denitrifying, closed diamonds; sulfate reducing, closed triangle; methanogenic, closed square; methanogenic+lactate, closed circle.

Long-term incubation of roxarsone with anaerobic sludge. In various experiments with anaerobic sludge, roxarsone was rapidly converted to HAPA in stoichiometric amounts. However after long incubation periods (*eg* 120 days), HAPA that had previously accumulated was found to be largely eliminated (by 95.3%) as is shown in Figure 5. Two inorganic arsenicals, arsenate and arsenite were found to be formed. The combined recovery of arsenate and arsenite accounted for about 20.5% of the arsenic added initially to the system as roxarsone. The lower than stoichiometric yield was not due to adsorption of arsenic on the sludge or volatilization because total non-speciated arsenic concentrations were constant at 1 mM (which indicates that all arsenic was still present in solution). The remaining arsenic is therefore attributed to unidentified arsenic species in solution.

Long-term incubation of HAPA with anaerobic sludge. A similar experiment was conducted as that described in the paragraph above, except that HAPA was incubated for a long incubation period (of 113 days). The results of this experiment are shown in Figure 6 for incubations of 29 and 83 days, and in Figure 7 for the incubation of 113 days. After 29 days, HAPA was largely recovered. In the biologically active methanogenic and sulfate reducing treatments small eliminations were evident but these were of questionable significance. On days 83 and 113, HAPA levels dropped in all treatments and controls, but the decreases were significantly greater in the biologically active methanogenic and sulfate reducing treatments. The HAPA levels in the denitrifying treatment were similar to the medium only and killed sludge control on day 83, whereas the values declined a little more than the controls on day 113. Inorganic arsenicals (arsenate and arsenite) were shown to accumulate as a result of the decline in HAPA concentration. The increases were significantly greater in the biologically active treatments compared to the medium only and killed sludge control. However, as was indicated above, the recovery of inorganic arsenicals was only partial. The best recovery of inorganic arsenicals was 16.9% on day 83 and 24.7% on day 113 compared to the initial HAPA used at the beginning of the experiment. As was noted before, the remaining arsenic can be attributed to unidentified arsenic species in solution based on total non-speciated arsenic measurements. The loss of HAPA in the medium only and killed sludge controls would suggest that chemical mechanisms of HAPA transformation were occurring. The bottles were wrapped with aluminium foil, so photolysis reactions can be excluded. Nonetheless, a greater removal of HAPA and greater release of inorganic arsenicals in the biologically active treatments would indicate the involvement of biologically catalyzed mechanisms as well.

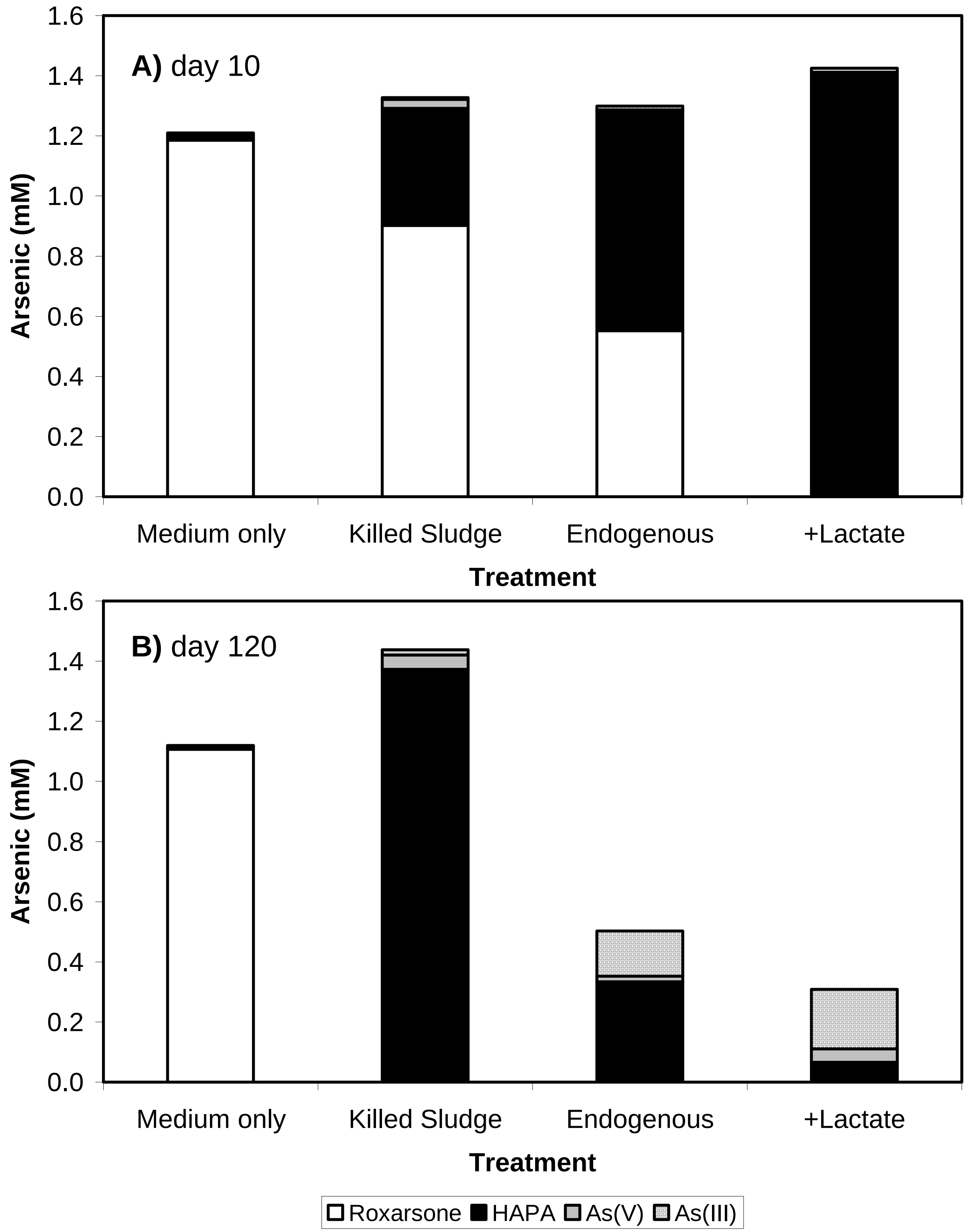


Figure 5. Long term incubation of roxarsone and recovery of products in anaerobic sludge under methanogenic conditions. The incubation was either supplemented with electron donating substrate (+lactate) or not (endogenous). The products recovered are shown after 10 and 120 days of incubation in panel A and B; respectively.

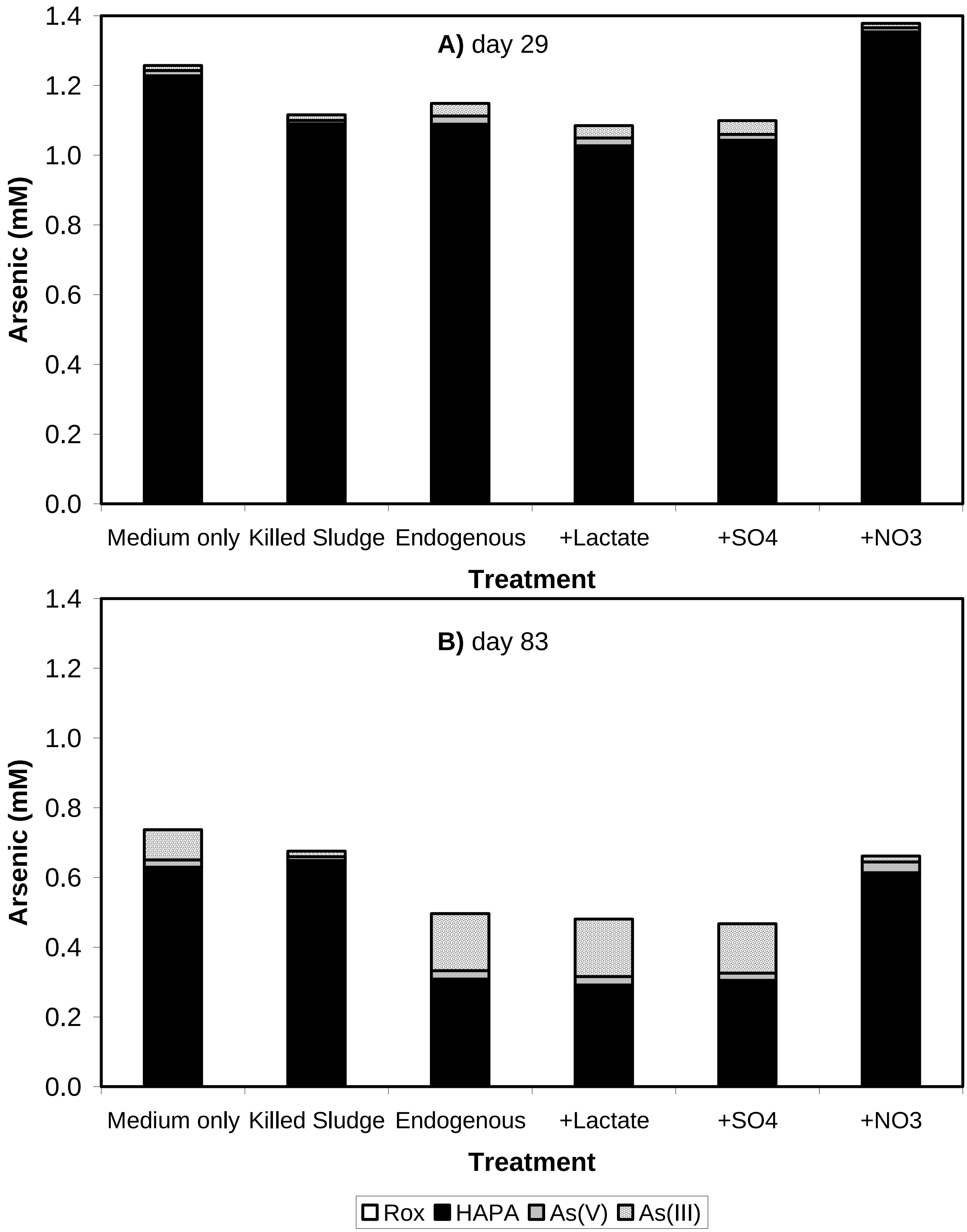


Figure 6. Long term incubation of HAPA and recovery of products in anaerobic sludge under methanogenic conditions, sulfate reducing and denitrifying conditions. The methanogenic incubation was either supplemented with electron donating substrate (+lactate) or not (endogenous). The products recovered are shown after 29 and 83 days of incubation in panel A and B; respectively.

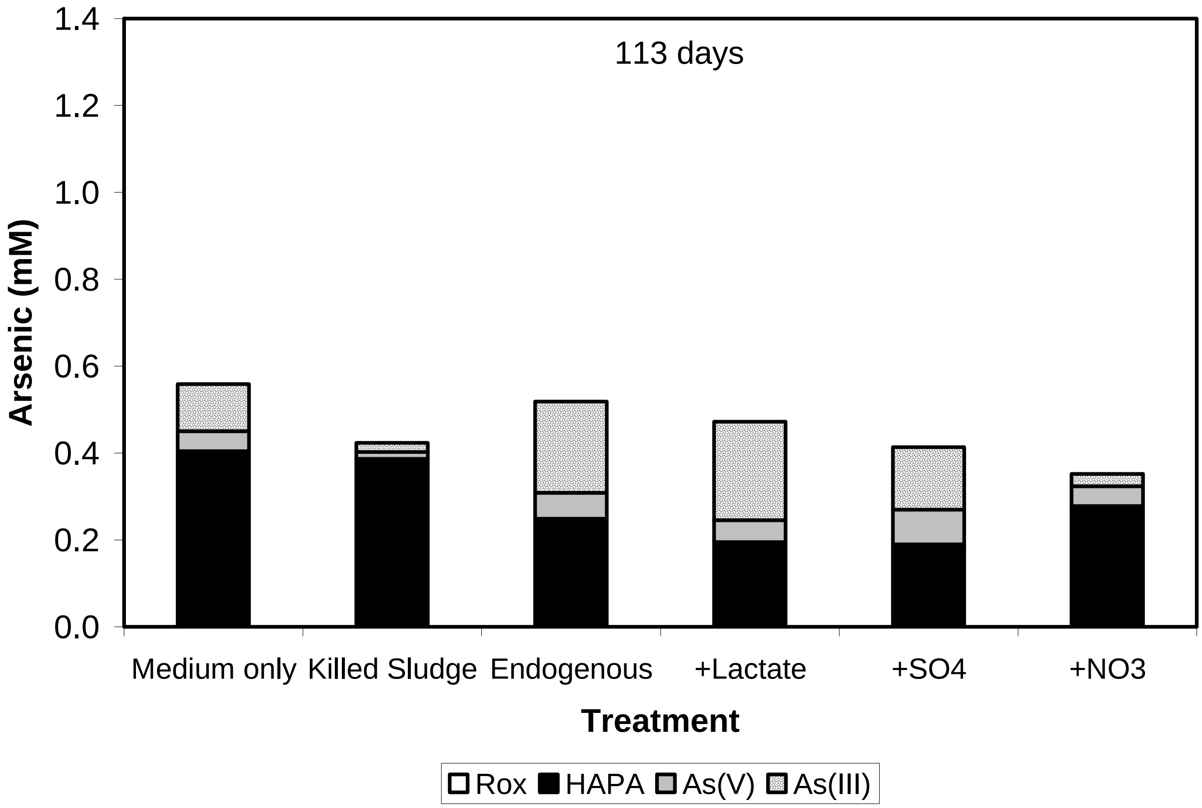


Figure 7. Long term incubation of HAPA and recovery of products in anaerobic sludge under methanogenic conditions, sulfate reducing and denitrifying conditions. The methanogenic incubation was either supplemented with electron donating substrate (+lactate) or not (endogenous). The products recovered are shown after 113days of incubation.

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 8 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 9. 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 9. 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 10. 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. Therafter, 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 11). 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.

The results taken as a whole indicate a rapid initial biotransformation of roxarsone to HAPA. 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.

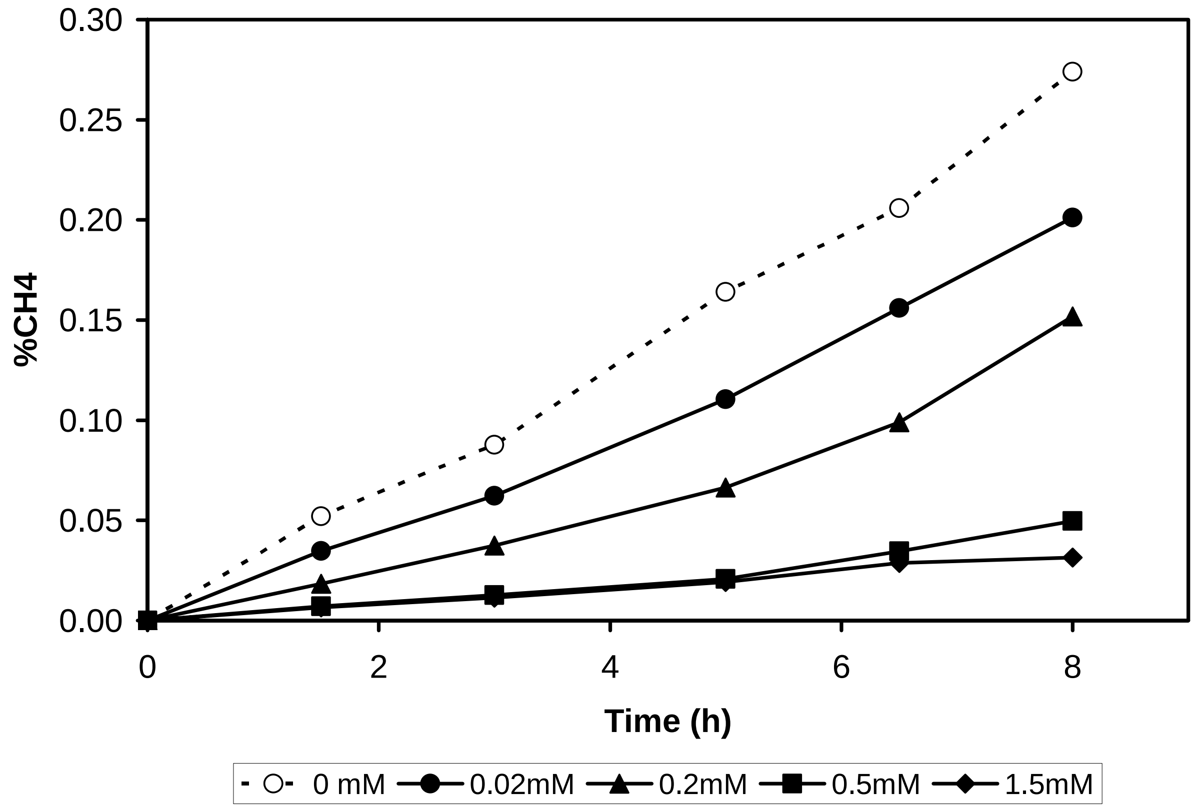


Figure 8. The time course of methane production during the acetoclastic methanogenic toxicity assay supplied with different concentrations of 16-h oxidized HAPA. The concentrations correspond to HAPA in the original unoxidized stock solution.

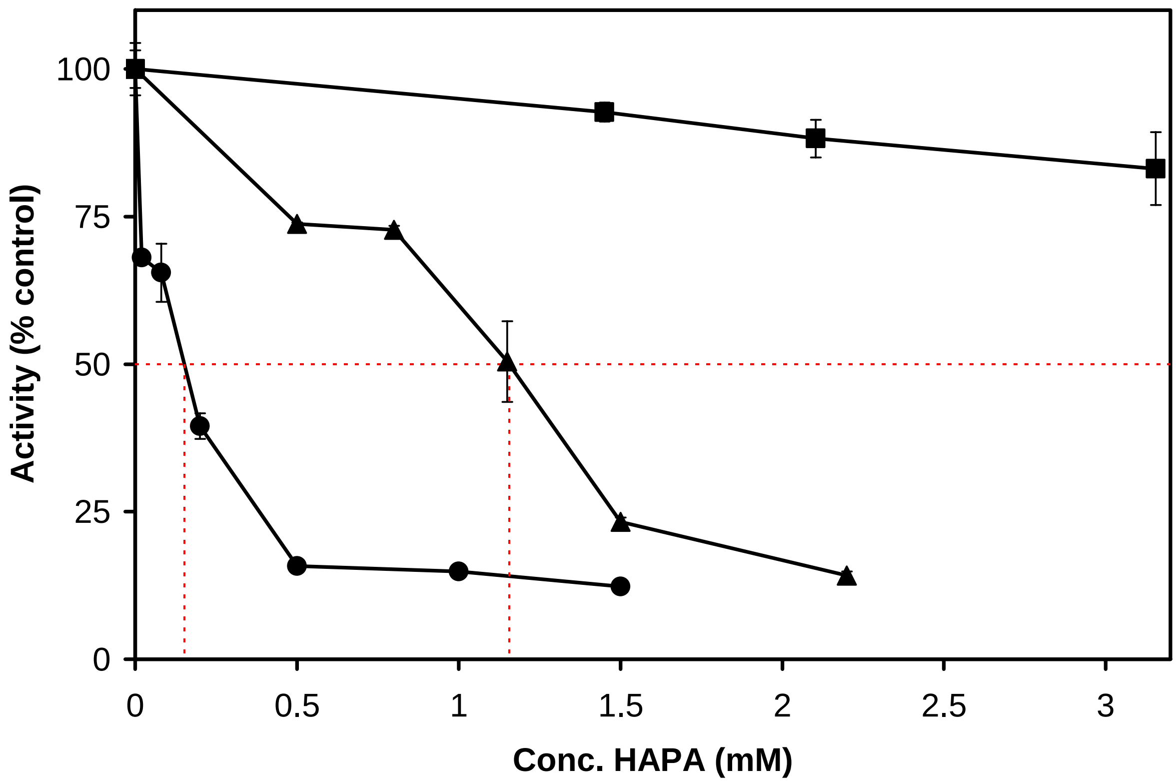


Figure 9. The relative activity of HAPA oxidized for 16 hours (closed circles), 4 minutes (closed triangles) and unoxidized HAPA (closed squares) as a function of the HAPA concentration. The concentrations correspond to HAPA in the original unoxidized stock solution.

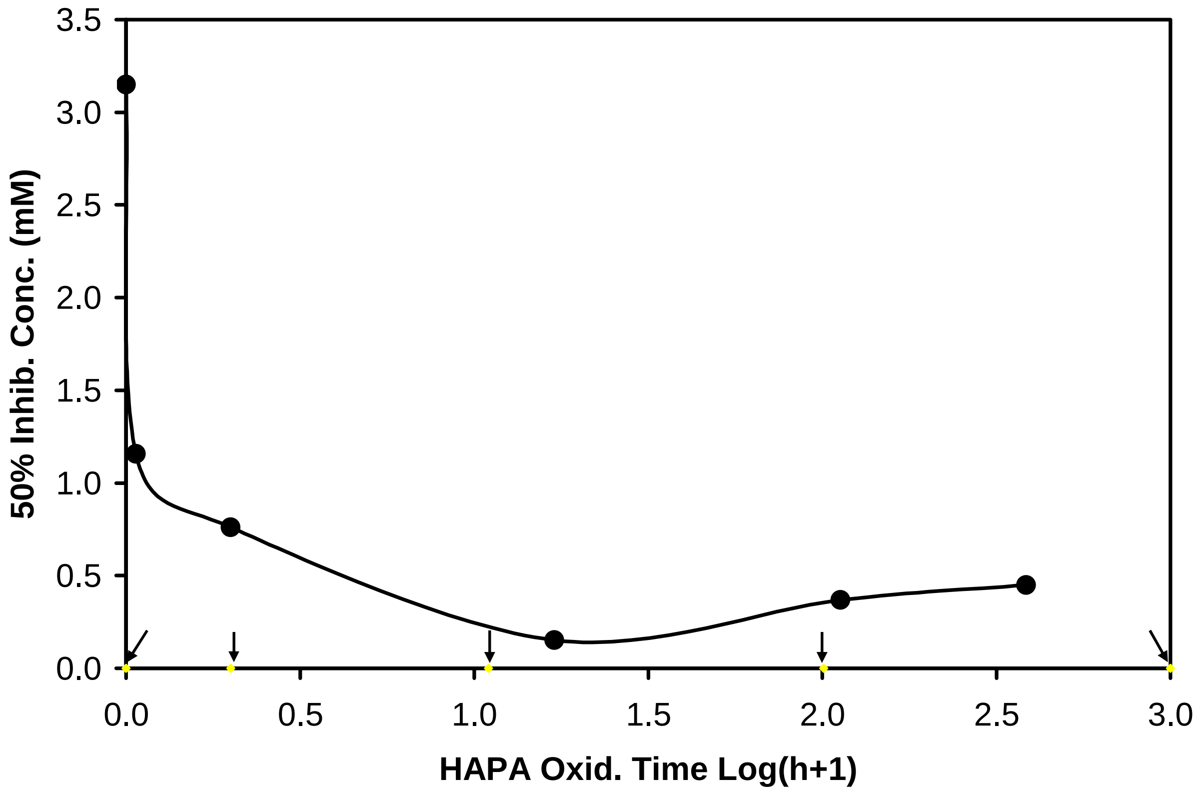


Figure 10. The 50% inhibiting concentration (50%IC) of HAPA to acetoclastic methanogenesis as a function of HAPA oxidation time. The graph is plotted on a logarithmic scale. From left to right the arrows indicate oxidation times corresponding to 0, 1, 10, 100 and 1000 h, respectively. The inhibitory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

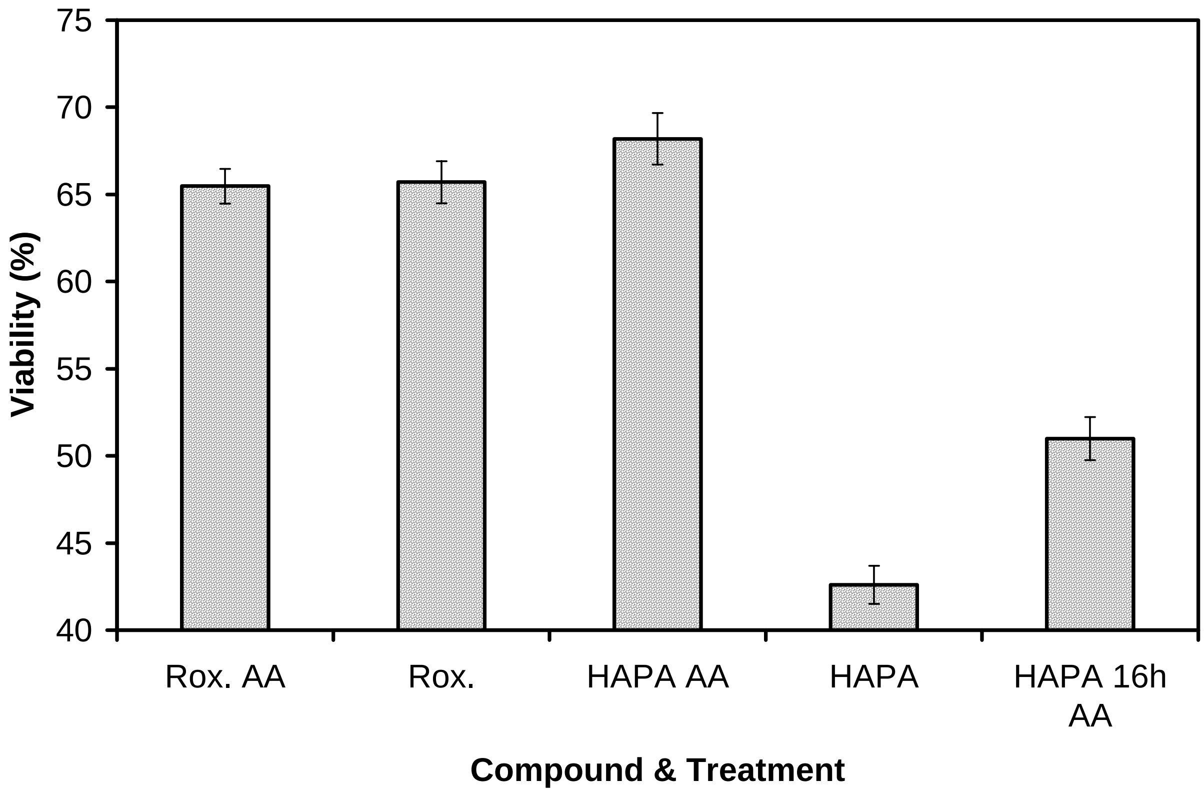


Figure 11. The toxicity of roxarsone and HAPA to the mitochondrial toxicity text (MTT) when supplied at a final assay concentration of 240 µM. Several assays conducted with ascorbic acid (AA) to prevent further oxidation of the compounds. The viability data of compounds incubated with AA is expressed as a percent of control viability in an assay the same level of AA.

Long-Term Aerobic Biodegradability of Dimethylarsinic acid.

The long term aerobic biodegradability of dimethylarsinic acid, DMA(V), was evaluated in incubations with activated sludge. During the first 50 days no degradation was evident. However on the next sampling at 121 days, evidence for aerobic biodegradation was apparent. The evidence is three-fold. DMA(V) concentrations decreased 1.01 mM more in inoculated treatments compared to uninoculated controls (Figure 12A) and arsenate, As(V), increased to 0.4 mM as a mineralized product of DMA(V) conversion. (Figure 12B). Respiratory evidence is shown in Figure 13. The complete treatment containing sludge and DMA(V) consumed 4.72% more O_2 compared to the endogenous substrate control (sludge only). The theoretical oxygen demand of the 1.01 mM DMA(V) consumed was equivalent to 72.7% of the extra O_2 consumption due to the presence of DM(V) (that which is beyond the endogenous respiration). This O_2 balance is very satisfactory when considering experimental error in the measurements.

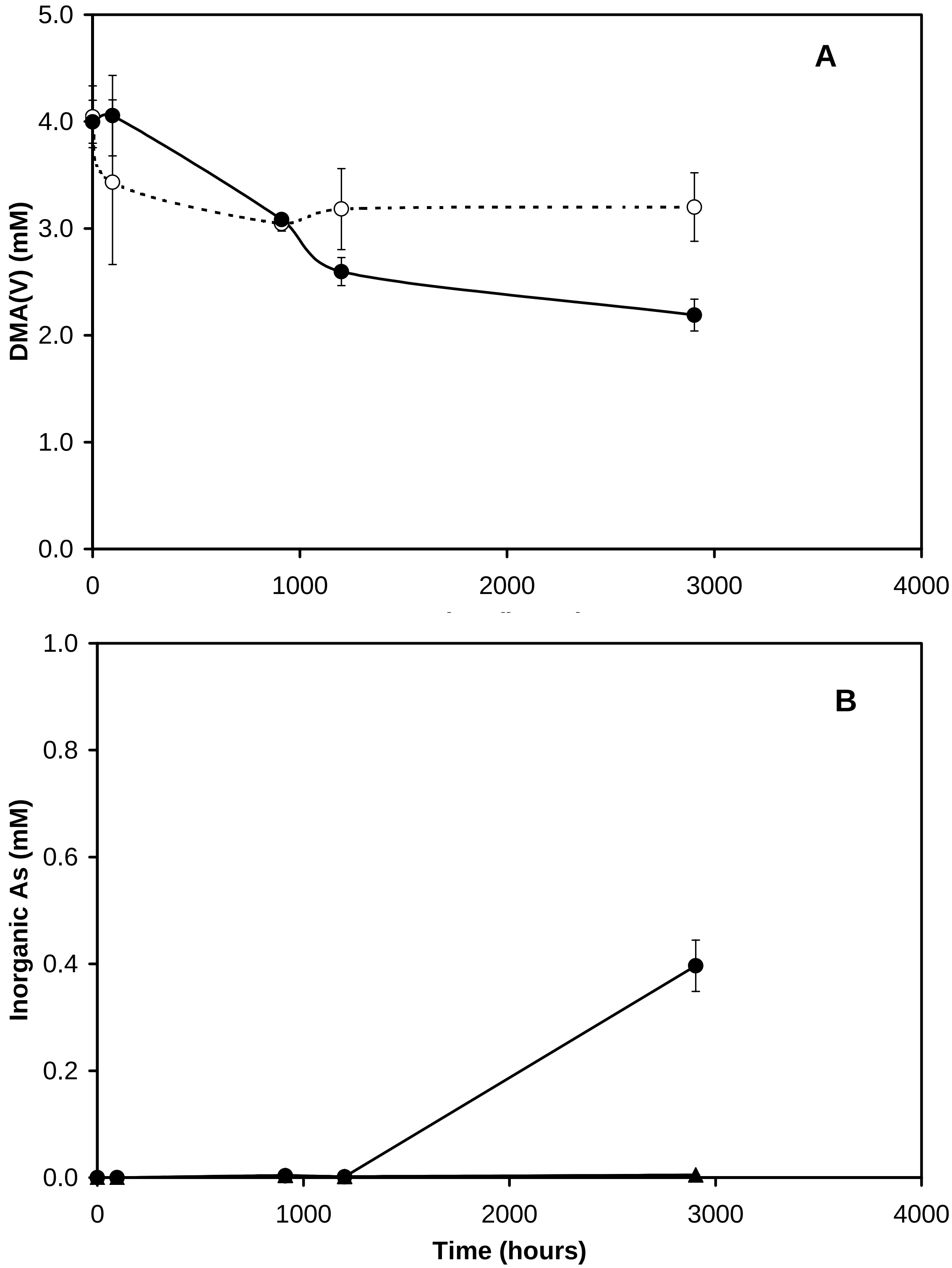


Figure 12. Aerobic biodegradability of DMA(V) by aerobic activated sludge. Panel A shows the consumption of DMA(V). Legend: DMA(V) in uninoculated control, open circles; DMA(V) incubated with active sludge, closed circles. Panel B shows the release of inorganic arsenicals. Legend: formation of As(V), closed circles; formation As(III), closed triangles.

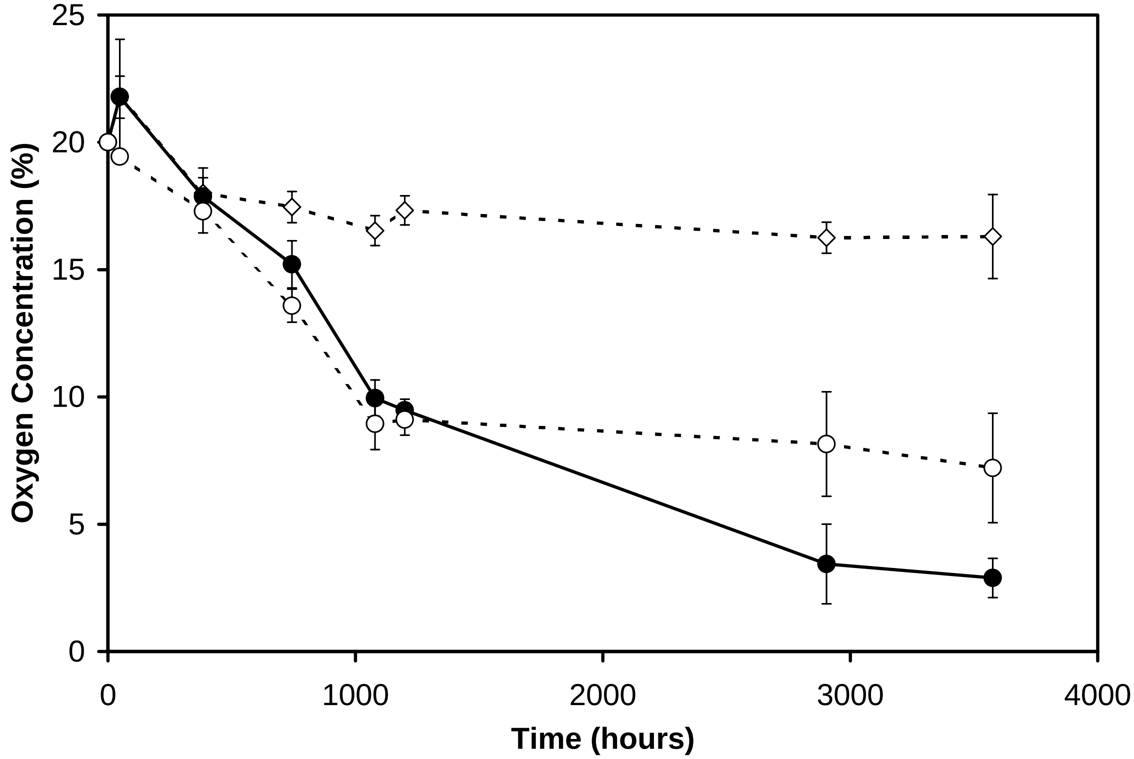


Figure 13. O_2-consumption during the experiment evaluating the aerobic biodegradability of DMA(V) with aerobic activated sludge. Legend: O_2 concentration in head space with blank medium, open diamonds; O_2 concentration in head space with medium and activated sludge, open circles; O_2 concentration in head space with medium, activated sludge and DMA(V), closed circles.

Permeable Reactive Biobarriers for the Containment and Remediation of Acid Mine Drainage

Basic Information

Title:	Permeable Reactive Biobarriers for the Containment and Remediation of Acid Mine Drainage
Project Number:	2004AZ42B
Start Date:	3/1/2004
End Date:	2/28/2005
Funding Source:	104B
Congressional District:	07
Research Category:	Water Quality
Focus Category:	Toxic Substances, Groundwater, Treatment
Descriptors:	Acid Mine Drainage, Biobarriers, Mine Remediation
Principal Investigators:	James Field, Reyes Sierra

Publication

1. Karri, S., Sierra-Alvarez R, Field JA. (2005). Toxicity of copper to methanogenic and sulfate reducing microorganisms. Chemosphere. (In press).
2. Karri, S., Sierra-Alvarez R, Field JA. (2005). Zero valent iron as an electron donor for methanogenesis and sulfate reduction in anaerobic sludge. (Under review).
3. Karri, S., Sierra-Alvarez R, Field JA. (2005). Treatment of acid mine drainage by sulfidogenic bioreactors. (In preparation).
4. Karri, S. (2004). Bioremediation of heavy metal using sulfate reducing bacteria. M.Sc. thesis. Department of Chemical and Environmental Engineering. The University of Arizona. Tucson, Arizona.

A. Problem and Research Objectives

Statement of Critical Regional or State Water Problems

Due to a long history of mining and ore smelting in Arizona, abandoned mines and tailing piles threaten the State's surface and groundwater quality. Over 40 mines or metal-processing sites in Arizona are registered as CERCLIS (*Comprehensive Environmental Response, Compensation, and Liability Information System*) hazardous waste sites and 24 uranium mill processing sites are designated for remediation by the U.S. Department of Energy. The uncontrolled release of acid mine drainage (AMD) at many of these sites introduces acidity and elevated concentrations of sulfates, ferrous iron (Fe(II)), heavy metals and radionuclides into our water resources. In the 2002 report on the Status of Water Quality in Arizona (Arizona Department of Water Quality), metals/metalloids were the most important pollutant category responsible for impaired or non-attaining streams. Resource extraction (mining) was identified as the number one source for stream impairment. Metals and radiochemicals are also problematic groundwater pollutants responsible for 19 and 14%, respectively, of all index wells and target monitoring wells exceeding drinking water standards.

The overwhelming majority of Arizona's mining or tailings impacted sites are no longer in industrial operation. Consequently, cleanup funds are limited, leaving only low-cost extensive treatment or containment as viable options. Arizona State agencies, county- city- or tribal governments will be the most likely candidates for coordinating clean-up, restoration or containment operations.

Related Research

The most common methods for AMD remediation involve physical-chemical methods with high operating costs, and generation of bulky volumes of toxic sludges. Environmental biotechnologies offer interesting potentials for metal removal and recovery. Microbial processes for the removal of metals from aqueous streams generally rely on biosorption, reduction of metals to less soluble forms or chemical precipitation with biogenic products, e.g., phosphates or sulfides (8,9,14). This project considers the application of sulfate reducing bacteria (SRB) for the bioremediation of AMD. SRB are a diverse group of anaerobic prokaryotes characterized by their capacity to use sulfate (SO_4^{2-}) as a terminal electron acceptor. SRB are able to precipitate a wide spectrum of heavy metals found in AMD as sulfides minerals (3,6,7,5,23). SRB can also reduce the acidity- and sulfate levels in AMD (3,10). A simplified stoichiometric equation involving reduction of sulfate with organic substrates and precipitation of metals with formed sulfide can be represented as follows:

$$2\ CH_3CH_2OH + SO_4^{2-} \longrightarrow 2\ CH_3COO^- + HS^- + H^+ + 2\ H_2O$$
$$CH_3COO^- + SO_4^{2-} \longrightarrow 2\ HCO_3^- + HS^-$$
$$2\ HS^- + M^{2+} \longrightarrow M_2S \text{ (highly insoluble precipitates)}$$
Where, M^{2+} is a divalent heavy metal cation

Removal of heavy metals by SRB has been applied for the removal of metals in AMD at pH values as low as 3 (13). Important examples include the mineralization of copper, zinc, cadmium, and arsenic as sulfides in SRB biofilms (23). The solubility product of most metal sulfides is extremely low enabling almost complete metal removal (eg., Cu, Zn, Cd) (12).

Bioreactor technology is well developed for the application SRB. High rate sulfidogenic bioreactors are already implemented at full-scale for the treatment of metals at a semiconductor plant (Phillips) and a zinc refinery (Budelco) in The Netherlands (1,22). However, for application in Arizona, the sulfate-reducing biotechnology should be adapted to applications in permeable reactive barriers, constructed wetlands or other extensive techniques that are low-cost and low-management.

PRBs provide an innovative, low-cost solution to prevent contaminant migration in groundwater. The technology is extremely simple involving trenches intercepting contaminated plumes. The trenches are filled with porous materials, nutrients and substrates to encourage the development of an active microbial population capable of metal removal (**Figure 1**). The reactive materials, which consist of organic substrates and/or zero valent iron, promote microbial-mediated sulfate reduction, the generation of hydrogen sulfide, and the subsequent precipitation of a wide spectrum of metal as well as metalloid contaminants as sulfide minerals. Several studies have previously considered reactive barriers that exploit the activity of sulfate-reducing microbial populations for the treatment of AMD or other heavy metal-laden leachates (2,7,15,19). In these studies, insoluble organic biomass was incorporated in the barrier for long-term release of electron donating substrate. When applied at field-scale to AMD from coal-mining, reduction in

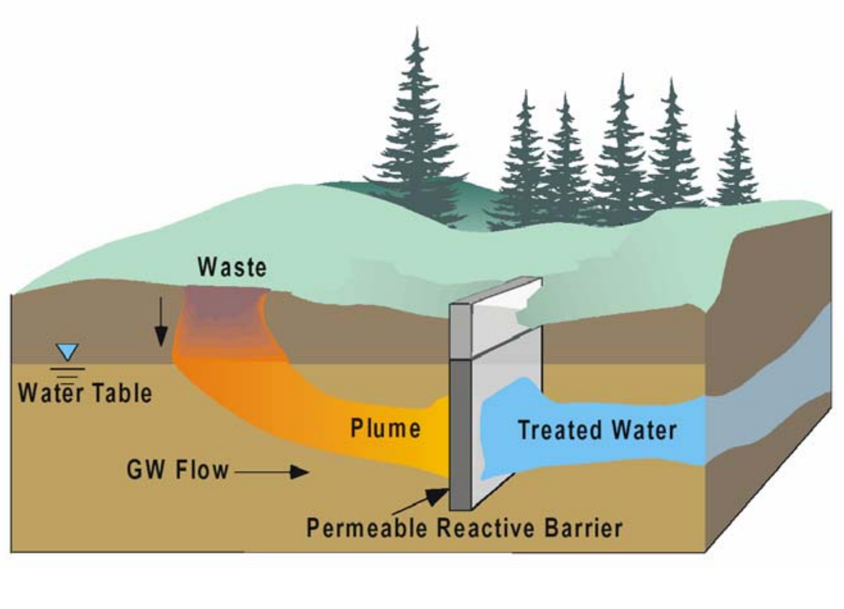


Figure 1. Schematic representation of a permeable reactive biobarrier. (source: http://207.86.51.66/download/rtdf/prb/reactbar.pdf).

alkalinity and soluble iron could be demonstrated over several years (2).

<u>Nature, Scope and Objectives:</u>

The aim of this research is to develop low-cost extensive remedial strategies aimed at preventing water contamination from Arizona's mine or tailings impacted sites. Specifically, the project will examine the potential of permeable reactive biobarriers (PRBs) to prevent the spread of acidity, sulfates, and metals from acid mine drainage (AMD) to surface or groundwater. The study will also assess the applicability of zero valent iron as slow-release electron donor to promote sulfate-reducing microbial activity in PRBs. ZVI has been shown to be a suitable e-donor for methanogenesis and reductive dehalogenation (4, 16, 20), and thus is expected to function under sulfate reducing conditions . Sulfate reducing bacteria (SRB) have been observed in PRB based on zero valent iron materials (11). The release of ferrous iron (Fe(II)) will have the additional benefit of immobilizing sulfides, and thus physically removing sulfur from the plume.

The main tasks of the project are as follows:

Task 1: Assessment of Inhibitory Effects of Heavy Metals to Anaerobic Microorganisms.
Toxicity batch bioassays were conducted to evaluate the effect of heavy metals on the specific activities of SRB and methanogens. Hydrogen and acetate were utilized as the electron donors for the process and copper was the model compound. Cu is the most frequent heavy metal found in the AMD. Inhibitory concentrations were determined from the bioassays.

Task 2: Suitability of Zero Valent Iron for Sulfate Reduction and Methanogenesis.
Zero valent iron (ZVI) was examined in long-term batch experiments, carried out over a 3-month period, to test the hypothesis that ZVI can serve as an electron donor for SRB and for methanogenic microorganisms. Series varying the grade and particle size of ZVI were compared with negative controls lacking electron donor and positive controls supplemented with a soluble electron donor. Methanogenesis or sulfate elimination and sulfide formation were measured as a function of incubation time, depending on the assay. The most effective slow-release electron donor was selected for application in future research.

Task 3: Remediation of AMD in Permeable Reactive Bio-barriers.
Continuous flow studies were conducted in ethanol/acetate-fed bench-scale columns simulating the operation of sulfate reducing PRBs. The study evaluated the immobilization of three predominant metals found in AMD-impacted streams (Cu, Zn, Ni), starting with addition of copper and, later on, shifting to a cocktail of the three metals. The operation of the bioreactor supplied with the simulated AMD was compared with that of a control bioreactor (no metals in the influent) operated under the same conditions.

B. Methodology

Microorganisms

A sulfate reducing anaerobic granular sludge was obtained from a full-scale upward sludge blanket (UASB) reactor treating rayon fiber manufacturing wastewater (Twaron, Twente, The Netherlands). The sludge had an initial content of volatile suspended solids (VSS) of 7.24%. The maximum methanogenic activity of the Twaron sludge in assays utilizing acetate and hydrogen, as substrate was 26.9 and 85.2 mg CH_4-COD g^{-1} VSS day^{-1}, respectively. The maximum sulfidogenic activity of the Twaron sludge in assays utilizing acetate and hydrogen, as substrate was 7.6 and 10.7 mg S-SO_4^{2-} reduced g^{-1} VSS day^{-1}, respectively. The microbial cultures were elutriated to remove the fines and stored under nitrogen gas at 4°C.

Media for Bioassays

The anaerobic basal mineral medium (pH 7.2) used in methanogenic bioassays (*M-1*) contained (in mg L^{-1}): NH_4Cl (280); $NaHCO_3$ (5,000); K_2HPO_4 (250); $CaCl_2$•2 H_2O (10), $MgCl_2$•6 H_2O (183), yeast extract (100), and 1 mL L^{-1} of trace element solution. The basal medium (pH 7.2) utilized in the sulfate reducing bioassays (*M-2*) consisted of (in mg L^{-1}): NH_4Cl (280); $NaHCO_3$ (5,000); K_2HPO_4 (600); NaH_2PO_4•2 H_2O (796), $CaCl_2$•2 H_2O (10), $MgCl_2$•6 H_2O (100), Na_2SO_4 (2960); the specific methanogenic inhibitior 2-bromoethane sulfonate (6,330), yeast extract (20), and 1 mL L^{-1} of trace element solution. The trace element solution contained (in mg L^{-1}): H_3BO_3 (50), $FeCl_2$•4 H_2O (2000), $ZnCl_2$ (50), $MnCl_2$•4H_2O (50), $(NH_4)_6Mo_7O_{24}$•4H_2O (50), $AlCl_3$•6 H_2O (90), $CoCl_2$•6 H_2O (2,000), $NiCl_2$•6 H_2O (50), $CuCl_2$•2 H_2O (30), $NaSeO_3$•5 H_2O (100), EDTA (1,000), resazurin (200) and 36% HCl (1 mL L^{-1}).

Batch Bioassays

Different grades of ZVI were utilized in the bioassays as electron donors to test the slow release electron donating capacity. The various types of ZVI utilized were: < 10 micron (0.010 mm diameter), 325 mesh (0.044 mm particle diameter), 100 mesh (0.149 mm particle diameter) and an industrial sample of sieve size –8+50 mesh (average particle diameter of 1.129 mm). Initial experiments of sulfate reduction and methanogenesis were conducted with 46.6 g L^{-1} of 325 mesh ZVI. Additional assays were later conducted to analyze the effect of particle diameter on the rate of electron releasing capability of ZVI for both sulfate reduction and methanogenesis. A ZVI concentration of 18.64 g L^{-1} was utilized for these tests. Hydrogen was used as the electron donor in positive controls and was supplied as H_2/CO_2 gas (80/20, v/v) at 1.5 atm.

Methanogenic Test with ZVI**.** Shaken batch bioassays to test the effect of particle diameter on the rate of production of methane were conducted in 165 mL serum flasks. Anaerobic sludge (3 g VSS L^{-1}) was transferred to serum flasks with 28 mL basal medium M-1. ZVI was added at 18.6 g L^{-1}. The flasks were incubated overnight at 30±2˚C to adapt the sludge to the medium conditions. On the following day, the flasks containing H_2 were reflushed with N_2/CO_2 (80/20, v/v), and then pressurized with H_2/CO_2 (80/20, v/v, 1.5 atm), while all the other flasks where flushed with N_2/CO_2 for 3 min. All the flasks were incubated for 2 h. Methane, total iron and soluble iron were monitored periodically for the subsequent 75 days. The controls containing H_2 as an electron donor were reflushed after 355 and 736 h, respectively, for 3 min (80/20, v/v, 1.5 atm), after flushing first with N_2/CO_2. At the same time periods, all the other flasks were reflushed with N_2/CO_2 to avoid build up of methane.

Sulfate Reduction Test with ZVI. Anaerobic sludge (1.5 g VSS L^{-1}) was transferred to 335-mL serum flasks containing 250 mL of medium M-2. In flasks containing H_2 as the electron-donor, 100 mL of basal medium was utilized instead. ZVI was added at 46.6 or 18.64 g L^{-1}, depending on the assay. The medium and the headspace were flushed with N_2/CO_2 gas (80:20, v/v) to exclude oxygen, and the bottles were sealed with butyl rubber septa. Flasks containing H_2 as electron donor were first flushed with N_2/CO_2 and then pressurized with H_2/CO_2 (80/20, v/v, 1.5 atm) for 3 min. The flasks were incubated overnight at 30±2˚C to adapt the sludge to the medium conditions. On the following day, the flasks containing H_2, were reflushed with N_2/CO_2 and then pressurized with H_2/CO_2 (80/20, v/v, 1.5 atm) for 3 min. Sulfate and sulfide were monitored over the course of the experiment of 109 day duration. The controls containing H_2 were reflushed after 902 or 1744 h for 3 min (80/20, v/v, 1.5 atm), depending on the assay, after flushing first with N_2/CO_2. Sample analysis for sulfate, sulfide, total and soluble iron were measured periodically.

Various controls (uninoculated controls, no-substrate controls, positive controls with H_2 as electron-donor) were included, for all the experiments. All flasks were sealed with butyl rubber stoppers and aluminum crimp seals, and they were incubated in a climate-controlled chamber at 30±2˚C in an orbital shaker (75 rpm). All assays were conducted in triplicate.

Bioreactors

Biological removal of heavy metals was investigated in two different sulfidogenic anaerobic bioreactors (each of volume 409 mL) continuously fed with a synthetic acid mine drainage, one reactor being the Control Reactor (CR) and the other being the Metal Reactor (MR). Both reactors were operated under similar influent conditions for a period of 73 days. Subsequently, the influent of MR was supplied with increasing concentrations of heavy metals. The reactors were placed in a climate controlled room at 30 ± 2°C. The reactors were inoculated with 10 g VSS L^{-1} of the anaerobic sludge. **Figure 2** presents a schematic drawing of the

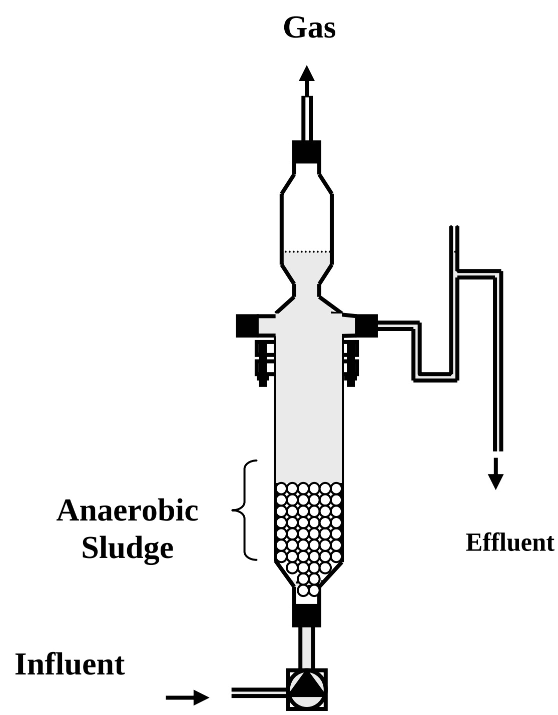


Figure 2. Schematic representation of the 0.5-L laboratory scale up-flow sludge bed reactors used in this study.

CR and MR systems. The reactors were maintained at an average hydraulic retention time (HRT) of 24 h.

The reactor medium was prepared using basal mineral medium M-1, Na_2SO_4 (2,660 mg L^{-1}) and ethanol (490 mg L^{-1}). After a period of 134 days, acetate also added at concentrations ranging 180 to 250 mg L^{-1}. The chemical oxygen demand (COD) factors for ethanol and acetate are 2.089 and 1.067, respectively. The pH value of the influent was decreased stepwise from 8.0 to only 4.5. Both reactors were operated with the metal-free influent for 73 days, at which point copper (II) (as $CuCl_2$) was added to MR at a concentration of 10 mg L^{-1}. The concentration of copper was increased periodically to 20 mg L^{-1} (on Day 173); 50 mg L^{-1} (on Day 255); 100 mg L^{-1} (on Day 291). To simulate AMD conditions, the MR was supplied with a cocktail of heavy metals that contained: copper (100 mg L^{-1}), nickel (15 mg L^{-1}) and zinc (15 mg L^{-1}) (added as the respective chloride salts) from Day 343 to Day 393. The $NaHCO_3$ concentration in the influent medium during this period was increased to 1 g L^{-1} for providing an effective buffering of the system. The various periods of operations for the sulfidogenic reactors are presented in **Table 1.**

Both reactors were monitored daily for influent and effluent pH, liquid volumetric flow rate, and gaseous methane flow rate. Reactor influent and effluent were sampled daily or every other day and analyzed for sulfate, ethanol, acetate and metal concentration (*i.e.*, Cu or Cu, Ni and Zn, depending on the experimental period). Effluent samples were also analyzed for sulfide concentration.

The methane production in the reactors was measured by liquid displacement using inverted 1-L serum flasks filled with 1 M NaOH solution to scrub H_2S and CO_2 from the biogas. The H_2S concentration in the biogas stream was calculated from the H_2S concentration in the liquid assuming equilibrium between the gas and liquid phases. CO_2 concentrations in the biogas were assumed to be 30% of the total methane flow rate.

For analyzing the changes in the microbial communities established in the bioreactors, samples for cloning and fluorescence *in situ* hybridization (FISH) analysis were also taken every time the influent metal concentration was changed. The results from these analyses are part of separate study and hence will not be reported here.

Analytical Methods

The acetate concentration in liquid samples from both the reactors, as well as the methane content in the headspace of the activity assay 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). The GC was fitted with a Nukol fused silica capillary column (30 m length x 0.53 mm ID, Supelco, St. Louis, MO). The temperature of the column, the injector port and the detector was 140, 180 and 275°C, respectively. The carrier gas was helium at a flow rate of 9.3 mL min^{-1} and a split flow of 32.4 mL min^{-1}. Formic acid (22.5 µL per mL of sample) was added prior to volatile fatty acid (VFA) analysis. Samples for measuring methane content (100 µL) in the headspace were collected using a pressure-lock gas syringe. Ethanol was analyzed by GC-FID using a DB-FFAP column (J&W Scientific, Palo Alto, CA). The temperature of the column, the injector port and the detector was 70, 180 and 275°C, respectively. The carrier gas was helium at a flow rate of 9.3 mL min^{-1} and a split flow of 32.4 mL min^{-1}.

Sulfide was analyzed colorimetrically by the methylene blue method (17). 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. The mobile phase was 15 mM KOH at a flow rate of 1.2 mL min^{-1}. The column temperature was maintained at room temperature. The injection volume was 25 µL. Total Cu and soluble Cu in liquid samples were quantified with atomic absorption spectrometry**.** The total copper content in sludge sample of the metal reactor was measured following extraction of the samples with 10 mL of HCL (6.75 N) in a microwave digestion system (MDS2100, CEM Corporation, Matthews, NC) for 35 min. For analyzing soluble copper, all liquid samples were membrane filtered (0.40 µm) immediately after sampling. The samples were acidified with 2-3 drops of (5%, v/v) nitric acid to pH< 2 to prevent metal precipitation and adsorption to surfaces and stored in plastic vials for analysis. The copper, nickel and zinc concentration in liquid samples was analyzed by ICP-MS (Agilent 7500a system). The analytical system was operated at a Rf power of 1500 watts, a plasma gas flow of 15 L min^{-1} and a carrier gas flow of 1.2 L min^{-1}. The pH was determined immediately after sampling with a Orion model 310 PerpHecT pH-meter with a PerpHecT ROSS glass combination electrode. Volatile suspended solids (VSS) were determined according to *Standard Methods for Examination of Water and Wastewater* (1998. Clesceri et al. (eds.). 20th Ed. Washington D.C., American Public Health Association).

Chemicals

Iron powder, (-325 mesh; 97% purity) and iron powder, (<10 µm, 99.9+%) was obtained from Sigma Aldrich (St. Louis, MO); Iron powder (100 mesh; 99.9%) was obtained from Mallinckrodt (Hazelwood, MO) and the industrial iron sample (-8+50 mesh; 98%) from Conelly GPM Inc, (Chicago). Specialty gases N_2/CO_2 and H_2/CO_2 (80/20, v/v) were delivered from US Air weld (Phoenix, AZ). Cupric chloride dihydrate (Cu(II); 100.2%) was obtained from Mallinckrodt (Hazelwood, MO); nickel chloride (99.3%) from Alfa Aesar (Ward Hill, MA); and zinc chloride ($ZnCl_2$) and sodium sulfate (99%+) from Sigma-Aldrich (St. Louis, MO). Ethanol (100%) was purchased from Aaper alcohol (Shelbyville, KY).

C. Principal Findings and Significance

Task 1 - Toxicity of copper to acetoclastic and hydrogenotrophic activities of methanogens and sulfate reducers in anaerobic sludge

Heavy metals could negatively impact anaerobic microorganisms in anaerobic sulfate reducing bioreactors utilized for metal removal. The objective of this study was to evaluate the inhibitory effect of copper to acetoclastic and hydrogenotrophic activities of methanogens and sulfate reducers in sludge obtained from a full-scale sulfate reducing bioreactor. The 50% inhibiting concentration (50%IC) of Cu(II) to acetoclastic and hydrogenotrophic methanogens was 20.7 and 8.9 mg L^{-1}, respectively (**Figure 3**). The 50%IC of Cu(II) to acetoclastic sulfate reduction was 32.3 mg L^{-1}. The hydrogenotrophic sulfate reducers were only inhibited by 27% at the highest concentration of Cu(II) tested, 200 mg L^{-1}, indicating a high level of tolerance. The soluble Cu(II) was observed to decrease rapidly in both the methanogenic and sulfate reducing assays. The highest level of decrease was observed in the hydrogenotrophic sulfate-reducing assay which was over 99% in 5 h. Thus, the production of sulfides during the sulfate reducing assays may have accounted in part for the higher tolerance of sulfate reducers to Cu^{2+} toxicity compared to methanogens in this study. The results of this study indicate that sulfate reducing biotechnologies would be robust at relatively high inlet concentrations of Cu(II).

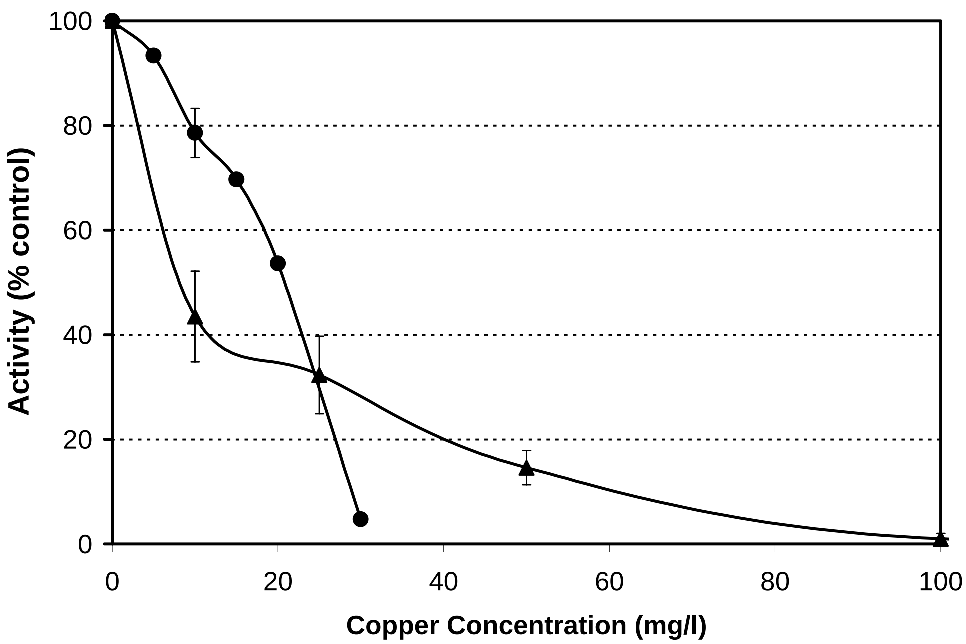


Figure 3. The role of the initial Cu(II) concentration on the methanogenic activity normalized with respect to the control in assays with either acetate (●) or hydrogen (▲) as the assay substrate.

Task 2 - Zero valent iron as an electron-donor for methanogenesis and sulfate reduction in anaerobic sludge

Zero valent iron (ZVI) is a reactive media commonly utilized in permeable reactive barriers. Sulfate reducing bacteria are being considered for the immobilization of heavy metals in PRBs. The purpose of this study was to evaluate the potential of ZVI as an electron donor for sulfate reduction in natural mixed anaerobic cultures. The ability of methanogens to utilize ZVI as an electron donor was also explored since these microorganisms often compete with sulfate reducers for common substrates.

Figure 4 illustrates the time course of the sulfate concentration in an uninoculated control; a control containing inoculum but no ZVI, and the complete treatment containing ZVI (325 mesh) and inoculum. Some sulfate was eliminated slowly from the two controls; however, the loss in sulfate concentration was distinctly greater and more rapid in the complete treatment. The results clearly indicate that ZVI was utilized by sulfate reducing bacteria.

Four grades of ZVI of different particle sizes (1.120, 0.149, 0.044 and 0.010 mm diameter) were compared as electron donor in batch bioassays inoculated with anaerobic bioreactor sludge.

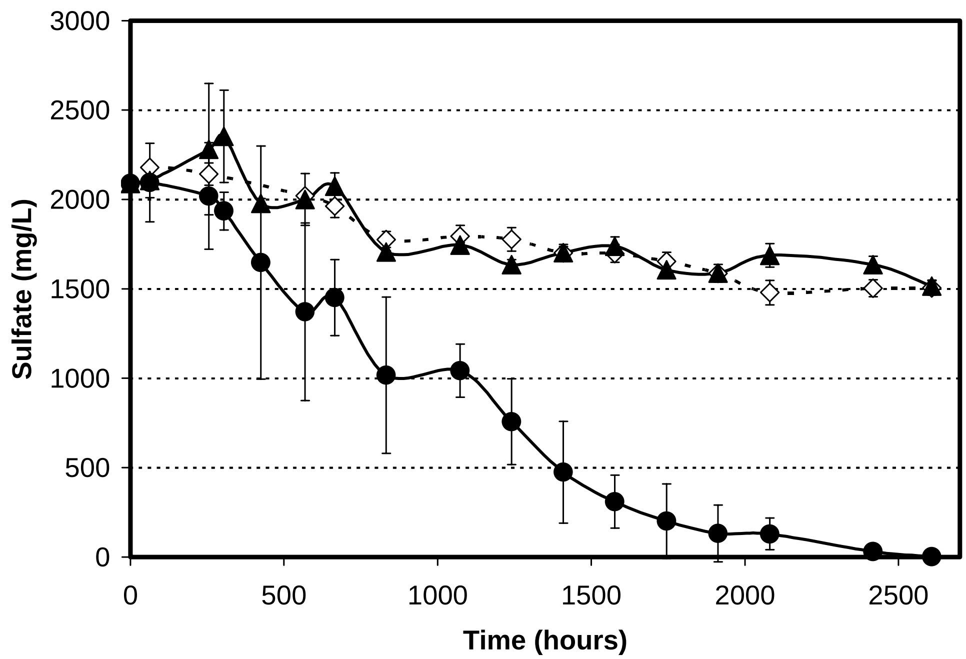


Figure @@. The time course of the sulfate concentration with 46.6 g L^{-1} of ZVI (325 mesh) and 1.5 g VSS L^{-1} of anaerobic sludge. Legend: (●), complete treatment with sludge and ZVI; (▲), endogenous sludge control; and (◊), uninoculated ZVI.

Methanogenesis was evaluated in mineral media lacking sulfate. Sulfate reduction was evaluated in mineral media containing sulfate and the specific methanogenic inhibitor, 2-bromoethane sulfonate. ZVI contributed to significant increases in methane production and sulfate reduction compared to endogenous substrate controls. The rates of methane formation or sulfate reduction were positively correlated with the surface area of ZVI. The highest rates of 0.310 mmol CH_4 formed (mol Fe^0)$^{-1}$ d^{-1} and 0.804 mmol SO_4^{2-} reduced (mol Fe^0)$^{-1}$ d^{-1} were obtained with the finest grade of ZVI (0.01 mm). The results demonstrate that ZVI is readily utilized as a slow-release electron donor for methanogenesis and sulfate reduction in anaerobic sludge; and therefore, has a promising potential in bioremediation applications.

Task 3 - Treatment of acid mine drainage by sulfidogenic bioreactors

High concentrations of heavy metals, sulfate and acidity are a frequent problem associated with AMD. Biogenic production of sulfide from sulfate reducing biosystems can be successfully utilized to remediate these acidic streams. Continuous-flow anaerobic reactors were designed to demonstrate the removal of heavy metals from a synthetic AMD with defined concentrations of sulfate and metals. A mixture of ethanol and acetate was used as the electron donating substrate for the system. Copper (II) concentrations from 10 to 100 mg L^{-1} were tested. After successful removal of copper, biological removal of simulate AMD containing a heavy metal cocktail consisting of copper (100 mg L^{-1}), zinc (15 mg L^{-1}) and nickel (15 mg L^{-1}) was evaluated. Sulfate reducers were able to precipitate these heavy metals with efficiencies greater than 99.5% (**Figures 5 and 6, Tables 2 and 3**). The overall acidity of the system was reduced effectively from pH values as low as 4.5 in the influent to near neutral pH values in the treated effluent (**Figure 7**). During the final operation periods, about 1.42 and 1.03 g SO_4^{2-} L^{-1} reactor day^{-1} was removed in the control and metal bioreactors. Anaerobic sulfate reducing bioreactor systems have great potential to remediate high influent pH, sulfate and metal concentrations with high efficiencies.

Table 1. Periods of reactor operation[a] .

Period	Days of Operation	Operational Conditions
Reactor 1 (Control Reactor)		
I	Day 0 - Day 73	Influent pH: 8.0; Steady state, sulfidogenic conditions (ethanol)
II	Day 74 - Day 133	Influent pH: 6.5
III	Day 134 - Day 221	Influent pH: 5.0; Addition of acetate: 197 mg COD L^{-1}
IV	Day 222 - Day 393	Influent pH: 4.5; Addition of acetate: 267 mg COD L^{-1}
Reactor 2 (Metal Reactor)		
I	Day 0 - Day 73	Influent pH: 8.0; Steady state, sulfidogenic conditions (ethanol)
II	Day 74 - Day 133	Influent pH: 6.5; Addition of 10 mg L^{-1} Cu (II)
III	Day 134 - Day 173	Influent pH: 5.0; Addition of acetate: 197 mg COD L^{-1} and 10 mg L^{-1} Cu (II)
IV	Day 174 - Day 221	Influent pH: 5.0; Addition of acetate: 197 mg COD L^{-1} and 20 mg L^{-1} Cu (II)
V	Day 222 - Day 255	Influent pH: 4.5; Addition of acetate: 267 mg COD L^{-1} and 20 mg L^{-1} Cu (II)
VI	Day 256 - Day 291	Influent pH: 4.5; Addition of acetate: 267 mg COD L^{-1} and 50 mg L^{-1} Cu (II)
VII	Day 292 - Day 342	Influent pH: 4.5; Addition of acetate: 267 mg COD L^{-1} and 100 mg L^{-1} Cu (II)
VIII	Day 343 - Day 393	Influent pH: 4.5; Addition of acetate: 267 mg COD L^{-1} and addition of 100 mg L^{-1} Cu (II); 15 mg L^{-1} of Ni (II) and Zn (II)

[a] Ethanol Concentration: 0.9 ± 0.2 g COD L^{-1}; Sulfate Concentration: 0.6 ± 0.1 g S-SO_4 L^{-1}

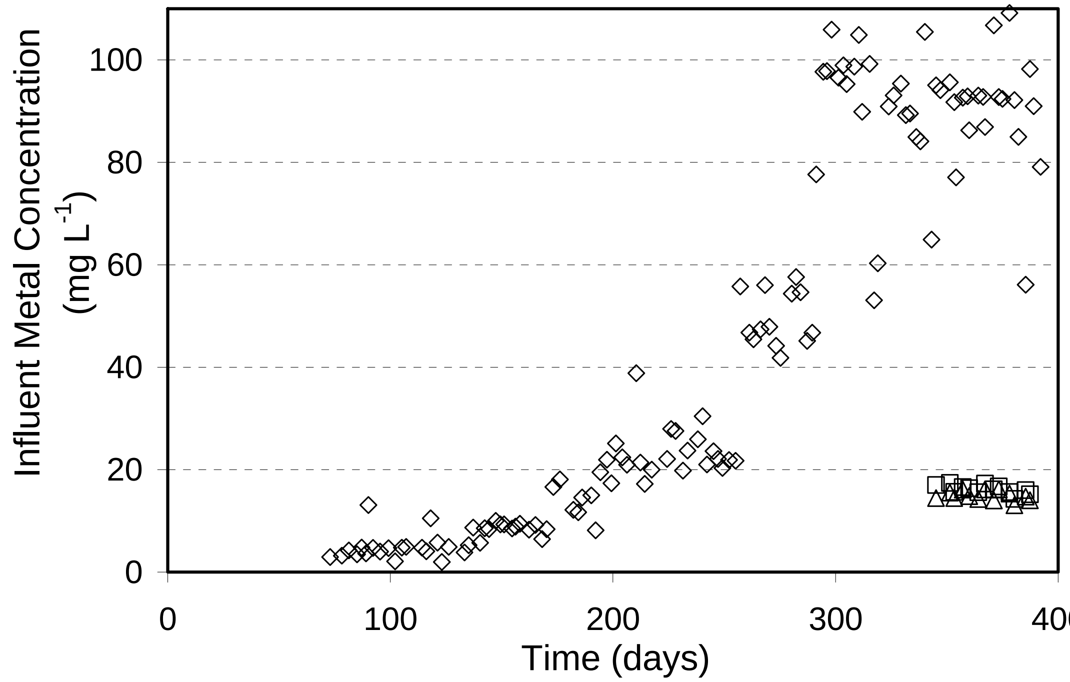


Figure 5. Concentration of heavy metals in the bioreactor influent: (◊) Cu, (□) Zn; (Δ) Ni.

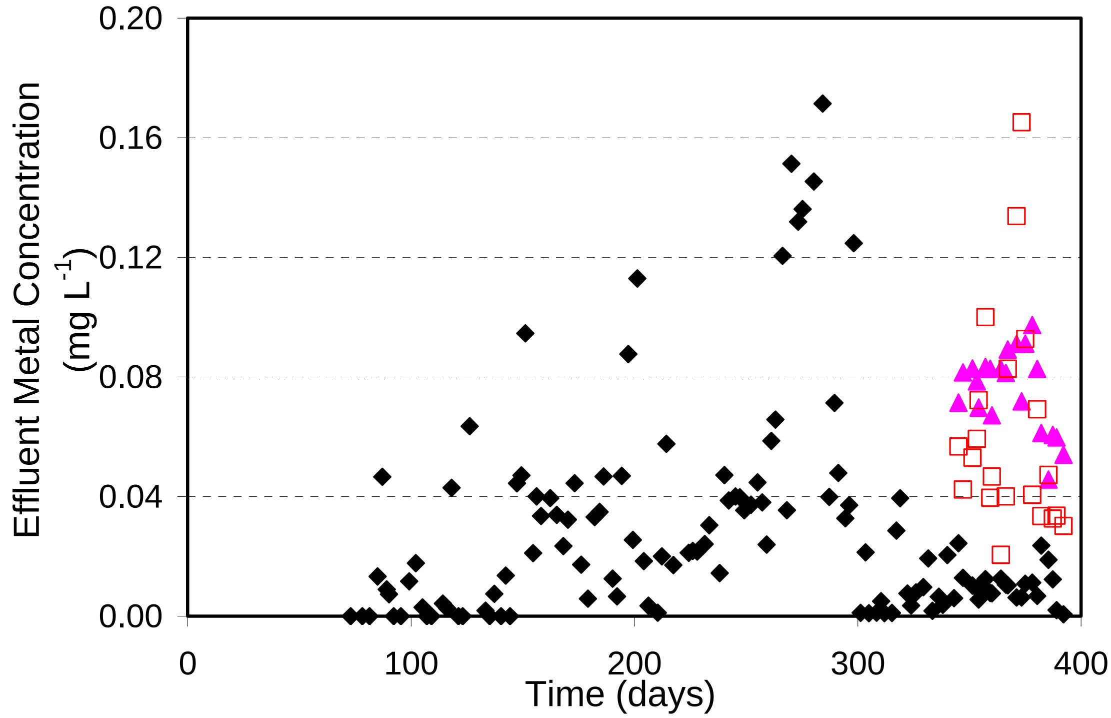


Figure 6. Concentration of heavy metals in the bioreactor effluent. (♦) Cu, (□) Zn; (▲) Ni.

Table 2. Average performance of the metal reactor (MR) during the various operational periods - Nickel and zinc data

Parameter	
Influent Zinc (mg L^{-1})	15.8 ± 0.8
Zinc Loading Rate (mg Zn L^{-1} d^{-1})	15.5 ± 1.9
Soluble Zinc Removal Efficiency (%)	99.2 ± 0.5
Total Zinc Removal Efficiency (%)	99.6 ± 0.2
Influent Nickel (mg L^{-1})	14.6 ± 0.6
Nickel Loading Rate (mg Ni L^{-1} d^{-1})	14.3 ± 1.8
Soluble Nickel Removal Efficiency (%)	99.3 ± 0.2
Total Nickel Removal Efficiency (%)	99.5 ± 0.1

Table 3. Average performance of the metal reactor (MR) during the various operational periods - Copper data.

	Period						
Parameter	II	III	IV	V	VI	VII	VIII
Influent Copper (mg L^{-1})	4.3±2.2	9.1±2.9	18.1±5.8	20.9±2.4	47.9±10.9	85.9±12.5	92.5±6.4
Copper Loading Rate (mg Cu L^{-1} d^{-1})	4.9±2.7	8.5±2.7	19.0±6.9	23.7±3.3	51.5±9.1	91.2±14.6	90.5±10.8
Soluble Copper Removal Efficiency (%)	N/A	N/A	N/A	N/A	N/A	N/A	100.0±0.0
Total Copper Removal Efficiency (%)	99.7±0.4	99.7±0.3	99.8±0.1	99.9±0.0	99.8±0.1	100.0±0.0	100.0±0.0

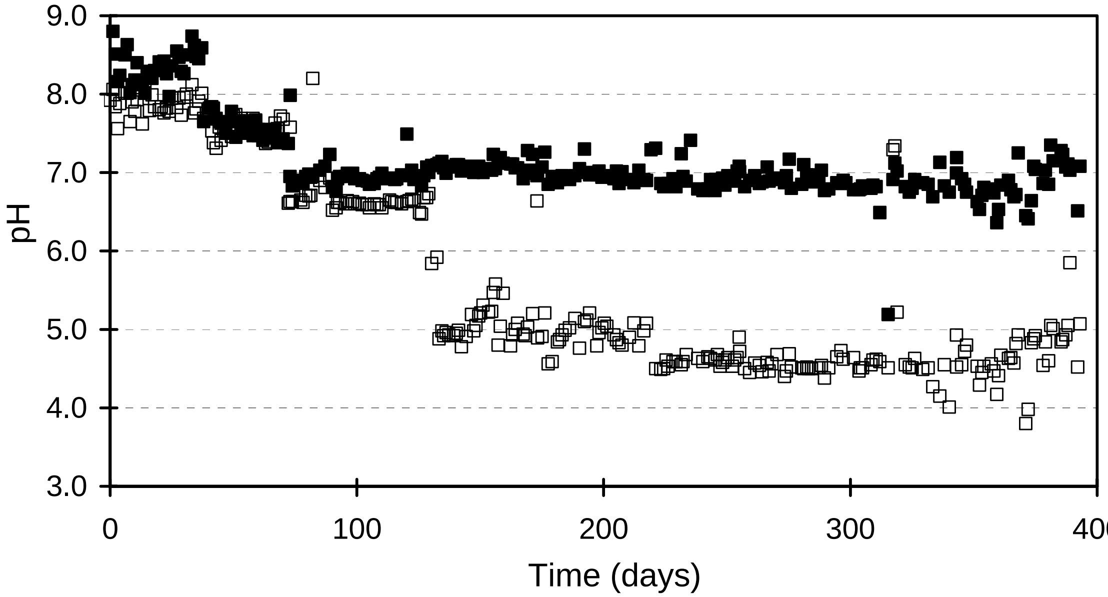


Figure 7. Influent (□) and effluent (■) pH values in the Metal Reactor as a function of time.

The results of this study indicate that there is a great potential for anaerobic sulfate-reducing bioreactor systems to remediate high influent pH, sulfate and metal concentrations with high efficiencies. Sulfate reducing biotechnologies proved robust at relatively high inlet concentrations of heavy metals. Furthermore, the results obtained confirm that sulfate reducing bacteria can utilize zero valent iron as an electron donating substrate. Methanogenic microorganisms, which often coexist and compete with SRB for available substrates were also able to use ZVI as electron donor. ZVI is an interesting slow-release electron donor to support sulfate-reducing activity in permeable reactive biobarrier ystems

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Impact of drought on management of salt sensitive plants with reclaimed water

Basic Information

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Project Number:	2004AZ50B
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End Date:	2/28/2005
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Principal Investigators:	Ursula K. Schuch

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1. Schuch, U.K. 2005. Effect of reclaimed water and drought on salt-sensitive perennials. HortScience (abstract in press).
2. Schuch, Ursula K., (2005), Salinity, What's Tolerant, What's Not?" Southwest Horticulture, March/April, Vol. 22 (2), 11 and 20.

A. Problem and Research Objectives

Growing plants under saline conditions may restrict growth because of drought stress through low water potential of the rooting media, ion toxicity because of excessive uptake of chloride and sodium, and imbalance of mineral nutrients, particularly of calcium (Marschner, 1995). Salt tolerance of plants is affected by an interaction of plant, soil, water, and environmental conditions and plant responses are best qualified on a relative rather than an absolute basis (Maas, 1986). Most crops are more sensitive to salinity in hot, dry climates compared to humid, cool ones. Plants are sensitive to salinity during all stages of growth, and sensitivity may change during different growth stages (Maas, 1986).

Salinity of water is an ever increasing issue in the arid Southwest. With water demand for municipal and industrial usage predicted to almost double from the 1990 levels by 2030, the question arises how to manage the existing water resources. The population of Arizona is expected to double in the next 40 years and is estimated to increase to 6.1 million by 2010 and to over 7.5 million by 2020. Residential and commercial landscapes will increase proportionally and require additional sources of irrigation. A compounding factor on water demand is that Arizona has been in a drought for several years. The Governor's drought task force plan has been initiated in March 2003 because precipitation throughout the State of Arizona for six of the last seven years has been significantly below normal and this lack of precipitation has reduced stream flows, surface and ground water supplied in the state, resulting in drought conditions throughout the state. Current suppliers of potable water will have increasing difficulties of meeting the needs of a rapidly expanding population in a state where droughts occur periodically. Finite ground water and surface water resources can be stretched by reclaiming water. Reclaimed water is the only water source that will increase proportional to water use and is expected to become increasingly vital to sustain the quality of life for desert communities.

Increasing salinity of soils and water supplies are a constant challenge in arid areas. Phoenix area water officials contend that about one million tons a year of salt are coming into the area and will contribute to deteriorating water quality. Groundwater supply in the southwest area of Phoenix has already reached salt levels of 2,500 mg/L. Tucson water supply generally does not exceed salinity of 650 mg/L, probably because water supplies are pumped from deeper and deeper wells. With desalination of brackish water being explored to augment the drinking water supply in the state, it is likely that in the future more potable water will be allocated for municipal and industrial use and restrictions for using potable water for landscape irrigation may be imposed. Drought conditions in the state exacerbate the salinity problem as less natural leaching occurs because of decreased precipitation and a greater amount of higher salinity water is used for irrigation. Reclaimed water is already supplied to Phoenix and Tucson area golf courses, schools, and parks, and is also used for recharging aquifers and wetlands in both metropolitan areas. The amount of reclaimed water produced will increase significantly in the future, and guidelines for the safe use of reclaimed water for landscape irrigation will benefit the landscape industry and residents alike.

Plants add an important component to the quality of life and fulfill many functional needs such as trees and shrubs that provide shade, filtering air, buffering noise, and serving as visual screens. Landscapes add significant value to properties and contribute to the aesthetics of both residential and commercial properties. Loss of plants in landscapes due to saline water and soil conditions can become very costly and result in tremendous input requirements for replacement of plants, labor for installation, and the loss of functional and aesthetic value of plants in the interim, which can be several years. Previous research has shown that several of the species widely used in Southwestern landscapes are not tolerant to higher salinity and will likely be damaged if irrigated with higher salinity water.

Citrus trees in orchards have been irrigated successfully with reclaimed municipal wastewater in Florida (Koo and Zekri, 1989). Trees under wastewater irrigation appear to benefit from the higher mineral content in the water, but juice quality was adversely affected by the wastewater. Different application rates of reclaimed water to ornamental plants in Florida resulted in three response groups, with the higher irrigation rate resulting in sufficient leaching to prevent damage to salt sensitive plants (Parnell, 1989). When 31 cultivars of ornamental plants were watered with a blend of half recycled and half fresh water, relative growth of those plants averaged 106% compared to those irrigated with fresh water. Relative growth rates ranged from 73% to 171% for the different species (Skimina, 1986).

Reclaimed water with higher salinity for irrigation of ornamental plants brings potential problems of increasing soil salinity and possibly causing reduced growth, foliar injury, or death of plants over time. An estimated 200 to 400 mg/L increase in salt content are expected from water that has been reclaimed one time from a treatment plant. Further treatments of effluent from reclaimed water will incrementally increase salinity of the water. Irrigation water with TDS (total dissolved solids) of up to 1,280 mg/L or an EC (electrical conductivity) of up to 2.0 dS/m is considered acceptable in terms of salinity (Marschner, 1995). However, evaporation without leaching will lead to salt accumulation in the soil and therefore much higher concentrations of salts in the plant root zone (Maas, 1986). Current drought conditions, now entering the 6^{th} year have set the stage for such a scenario in the State of Arizona.

The first objective of this study was to determine the performance of salt sensitive plants when irrigated with reclaimed or potable water. A second objective determined how those plants performed under drought stress.

B. Methodology

On May 24, 2004 liners were obtained from local nurseries and were planted in 5-gal. containers in media consisting of 80% compost and 20% sand. Species used in the experiment were *Chilopsis linearis* 'Warren Jones' (desert willow), *Tecoma stans (yellow bells), Salvia greggi* 'Cherry' (Chihuahuan sage), and *Verbena pulchella gracilior (moss verbena)*. These species were identified as salt sensitive in previous experiments. Each container was amended with Micromax (Scotts Co., Marysville, Ohio) at the recommended rate and Osmocote 18-6-12 (9 month release) at 62 g per container for *C.*

linearis and *T. stans*, and at 35 g per container for *S. greggii* and *V. pulchella gracilior*. Fertilizers were topdressed and incorporated into the top 4 cm of the media immediately after transplanting.

Half of the plants were irrigated with reclaimed water and half of the plants were irrigated with potable water. Irrigation was applied to achieve an average of 20% leaching and application rates and runoff were measured several times during the experiment to maintain the desired leaching. Starting 5 weeks after transplanting, irrigation was switched from a timer to a system activated by tensiometers (Model LT, Irrometer Co., Riverside, Calif.) when soil moisture dropped below -6 kPa.

EC of water was determined regularly during the experiment. EC and nitrate-N of runoff were measured three times during the experiment. Total mineral content of runoff from *C. linearis* treated with either potable or reclaimed water was measured on July 22, 2004. After plants had received their regular irrigation and were drained, 1 L of distilled water was applied to the top of the substrate and runoff was captured.

Plant growth data was recorded 4, 8, and 12 weeks after transplanting and included plant height, two canopy widths, number of flowers, and number of buds. Growth index was calculated by adding plant height and the two canopy widths and dividing it by three. The percent increase in growth index was calculated between measurement dates for each plant. Dieback was rated as percentage of canopy area affected in 10% increments. One flower at anthesis was considered a terminal raceme for *C. linearis*, a panicle for *T. stans*, a racemose inflorescence for *S. greggii* and a dense spike appearing head like for *V. pulchella gracilior*. After 12 weeks of treatment with potable or reclaimed water, plants were harvested and number of flowers, buds, and percentage of dieback was recorded. Shoots and roots were separated and dry weights were determined.

On September 13, 2005 plants were moved from outdoors into a retractable roof structure and were watered to saturation. When the substrate had drained and container capacity was reached, plants were weighed and the weight was recorded. Plant water potential was recorded with a pressure chamber. Shoot tips of approximately 10 cm length were cut and were immediately measured in the pressure chamber. Stomatal conductance, transpiration, and leaf temperature of desert willow and yellow bells were measured with a porometer immediately following the water potential readings. Plants were allowed to dry out in full sun conditions (roof and side walls fully retracted) or with the side walls of the building closed to slow down wind speed during measurements. Plant weight and the accompanying physiological measurements were taken once or twice during the following days, depending on the speed of drying out. The experiment was concluded when plants started to wilt and irrigation was applied again. At the conclusion of the experiment plants were well irrigated and damage from the drought treatment as expressed in leaf abscission or branch dieback was recorded after two weeks. The same procedure was repeated with another set of previously well watered plants.

The experiment was arranged in a completely randomized block design. Data for each species were analyzed separately and analysis of variance and mean separation was used to determine treatment effects. The statistical program SAS was used for the analysis.

C. Principal Findings and Significance

Water quality and substrate characteristics

Chemical composition of potable and reclaimed water sources used in the experiment are shown in Table 1. The potable water mineral concentration and chemical characteristics indicate a high quality that is very well suited for irrigation of even salt sensitive plants. No problems should result from using this water source (Southern Nurserymen's Assoc., 1997). Reclaimed water is still of good quality, however, increasing SAR, bicarbonates and chloride over time might result in some problems in salt sensitive plants.

Table 1. Chemical composition of reclaimed and potable water used for irrigating plants.

	Potable water	Reclaimed water	% increase reclaimed versus potable
N (ppm)	2.50	3.65	46
P (ppm)	0.0034	0.72	21,000
K (ppm)	1.0	6.0	500
Ca (ppm)	40.5	67.5	67
Mg (ppm)	2.0	13.0	550
S (ppm)	5.6	45.5	712
Na (ppm)	25.5	132	417
Cl (ppm)	15.5	102	558
B (ppm)	0.03	0.25	733
Bicarbonate(ppm)	140	241	72
EC (dS/m)	0.3	1.0	233
pH	7.9	7.7	-2
SAR	1.1	3.8	245

Table 2. Composition of leachate collected from desert willow 8 weeks after irrigation with reclaimed or potable water had started.

	Potable water	Reclaimed water
N (ppm)	82	151
P (ppm)	0.8	1.3
K (ppm)	36	74
Ca (ppm)	157	245
Mg (ppm)	30	58
S (ppm)	43	166
Na (ppm)	76	409
Cl (ppm)	52	336
B (ppm)	0.15	0.55
Bicarbonate(ppm)	266	351
EC (dS/m)	1.3	3.6
pH	7.7	7.8
SAR	1.4	6.1

A pour-through test conducted after plants were irrigated with the two water sources for 8 weeks indicated that all variables measured in the root zone solution of desert willow irrigated with potable water were within recommended limits thus allowing maximum growth. The root zone solution of plants under the reclaimed water regime started to accumulate Na and Cl, and bicarbonate and EC started to reach levels where problems in salt sensitive plants might be expected. SAR also increased to levels that suggest possible Na problems.

Pour-through tests conducted on all species after 12 weeks of treatment indicated that final EC in the root zone differed significantly between water treatments. Potable water resulted in root zone EC of 0.6 dS/m and reclaimed water resulted in an EC of 1.7 dS/m.

Plant growth

Canopy size, number of flowers or flower buds of desert willow and yellow bells were not affected by water quality during the 12 weeks of the experiment (Table 3). No dieback was observed for the two species and growth rate was not affected either. Sage plants irrigated with reclaimed water started to show symptoms of dieback, slower growth rate and had smaller canopies than plants irrigated with potable water within 8 weeks after treatments started (Table 3). This trend continued for the next 4 weeks, however, plants under reclaimed water treatment still maintained growth, though not as fast as those under the potable water treatment. Verbena plants had a larger growth index and more flowers and buds when irrigated with reclaimed water for four weeks (Table 3). After 8 weeks of treatments, reclaimed water still stimulated more flowers, but resulted in a slower rate of canopy growth. After 12 weeks, canopy size was smaller for those plants treated with reclaimed versus potable water. After 12 weeks, sage plants irrigated with reclaimed water were more attractive because of their compact canopy compared to plants irrigated with potable water.

Reclaimed water reduced one or more dry weight component of each species (Table 4). Leaf dry weight of desert willow was reduced by 15% for plants irrigated with reclaimed versus potable water, which was probably due to abscission of leaves that started to be damaged by the increase in salinity in the root zone. In yellow bells, reclaimed water significantly reduced root dry weight by 40% and reduced root:shoot ratio of plants. Reclaimed water applied to sage resulted in 50% to 61% reduction of leaf, stem, root, and total dry weight, while root:shoot ratios remained unchanged by the different water qualities. Verbena plants were not affected by potable and reclaimed irrigation, although there was a trend, especially for shoot dry weights, to decrease under the reclaimed irrigation regime.

First damage on some plants was observed on July 27, 2004 at the time when the two different sources of water were applied. Symptoms on sage included leaves abscising or leaves with leaf edge burn. On verbena damage was expressed as leaves turning yellow or brown. High temperatures seemed to exacerbate visual symptoms of salt stress which became more severe during August. However, when maximum daytime temperatures started to decrease, visual quality of plants improved and leaf edge burn was not much of a problem any more.

<u>Plant response to drought</u>

Plants irrigated with potable water and exposed to drought showed symptoms of wilting earlier and for some species showed more damage than plants irrigated with reclaimed water. No damage was observed on yellow bells exposed to drought in both drought cycles. Although plants wilted, all leaves fully recovered after rehydration. Desert willow irrigated with reclaimed water showed no damage from the first drying cycle when up to 28% of fully saturated soil moisture was depleted and showed only a few desiccated leaves on two plants when 32% of the fully saturated soil moisture was depleted. However, plants irrigated with potable water sustained between 10% to 90% leaf dieback in both drying cycles when moisture depletion reached up to 35% of fully saturated media. Sage plants defoliated in response to drought, and percent defoliation of potable and reclaimed water treated plants was 60% and 23%, respectively. Verbena irrigated with potable water sustained 83% mortality when fully saturated soil moisture was depleted by 27% or more. Plants irrigated with reclaimed water sustained up to 22%

Table 3. Effect of reclaimed and potable water on plant growth 4, 8, and 12 weeks after treatments began.

Weeks after treatments began	Water	Height (cm)	Growth index	Flowers (No.)	Buds (No.)	Dieback (%)	Growth increase (%)[z]
		C. linearis 'Warren Jones'					
4	Potable	66.3 a[y]	38.3 a	0.2 a	1.2 a	0	-
	Reclaimed	64.5 a	40.0 a	0 a	0.4 a	0	-
8	Potable	89.2 a	63.4 a	2.0 a	0.8 a	0	65 a
	Reclaimed	89.2 a	62.7 a	1.8 a	1.1 a	0	57 a
12	Potable	108.6 a	72.1 a	5.3 a	5.2 a	0	90 a
	Reclaimed	111.3 a	75.9 a	2.1 a	3.3 a	0	89 a
		T. stans					
4	Potable	31.6 a	24.2 a	0.1 a	3.9 a	0	-
	Reclaimed	34.7 a	25.3 a	0.5 a	4.6 a	0	-
8	Potable	34.7 a	38.5 a	5.8 a	3.2 a	0	61 a
	Reclaimed	35.2 a	38.3 a	4.3 a	3.0 a	0	52 a
12	Potable	48.1 a	45.3 a	6.3 a	1.8 a	0	87 a
	Reclaimed	43.6 a	42.9 a	4.7 a	1.4 a	0	70 a
		S. greggii 'Cherry'					
4	Potable	22.8 a	15.3 a	1.4 a	2.1 a	0	-
	Reclaimed	19.8 a	13.8 a	2.1 a	2.3 a	0	-
8	Potable	27.6 a	19.7 a	3.8 a	7.5 a	0 b	30 a
	Reclaimed	21.8 b	15.4 b	0.6 b	5.9 b	12 a	15 b
12	Potable	31.0 a	25.9 a	12.8 a	8.4 a	0 b	71 a
	Reclaimed	21.7 b	19.1 b	7.2 b	6.5 a	3 a	39 b
		V. pulchella gracilior					
4	Potable	19.1 a	20.9 b	2.5 b	1.0 b	0	-
	Reclaimed	22.5 a	27.8 a	5.9 a	3.6 a	0	-
8	Potable	24.5 a	44.7 a	27.4 b	4.3 a	7 a	120 a
	Reclaimed	26.1 a	44.6 a	37.1 a	2.4 b	14 a	63 b
12	Potable	22.5 a	49.9 a	66.9 a	13.3 a	2 a	148 a
	Reclaimed	25.1 a	45.4 b	59.0 a	7.3 b	4 a	66 b

[z] Growth increase is calculated as the difference between growth index from the current sampling and the previous sampling.
[Y] Means within a column and the same sampling date are significantly different at $p<0.05$ if followed by a different letter.

Table 4. Dry weight components of four species irrigated with potable or reclaimed water for 12 weeks.

Species	Water	Leaf	Stem	Root	Total	Root:Shoot ratio
		Dry weight (g)				
C. linearis ' Warren Jones'	Potable	52.7 a	93.4 a	40.3 a	186.5 a	0.27 a
	Reclaimed	45.0 b	77.4 a	38.2 a	160.7 a	0.31 a
T. stans	Potable	27.4 a	18.7 a	16.8 a	62.9 a	0.37 a
	Reclaimed	22.9 a	12.3 a	10.0 b	45.2 a	0.29 b
S. greggii 'Cherry'	Potable	7.9 a	6.5 a	1.9 a	16.4 a	0.14 a
	Reclaimed	3.9 b	2.5 b	0.8 b	7.2 a	0.12 a
V. pulchella gracilior	Potable	49.5 a	44.2 a	6.6 a	100.2 a	0.08 a
	Reclaimed	40.8 a	28.7 a	5.9 a	75.5 a	0.09 a

‡ ***, **, * or ns indicates significance of p<0.0001, p<0.01, p<0.05, or p>0.05.

leaf browning with subsequent dieback. Differences between individual plants varied greatly, and some plants sustained no damage, while others had up to 50% leaf damage. This suggests a potential for selecting cultivars of verbena that are more drought tolerant and possibly more salt tolerant.

A less negative water potential was maintained by yellow bells, sage, and desert willow up to about 20% media moisture loss for plants previously irrigated with potable versus reclaimed water (Fig. 1). With greater media moisture depletion water potential of plants treated with either water source decreased rapidly, indicating loss of turgor. Verbena irrigated with either water source maintained water potential around -0.13 MPa up to 27% media moisture loss, but with greater moisture loss started to loose turgor quickly (Fig. 1). All species were able to maintain functional water uptake and transpiration up to a media moisture reduction of 20% (Fig 1, 2). However, as less soil water was available, transpiration decreased sharply for yellow bells and for desert willow, regardless of the water source that had been used to irrigate plants before.

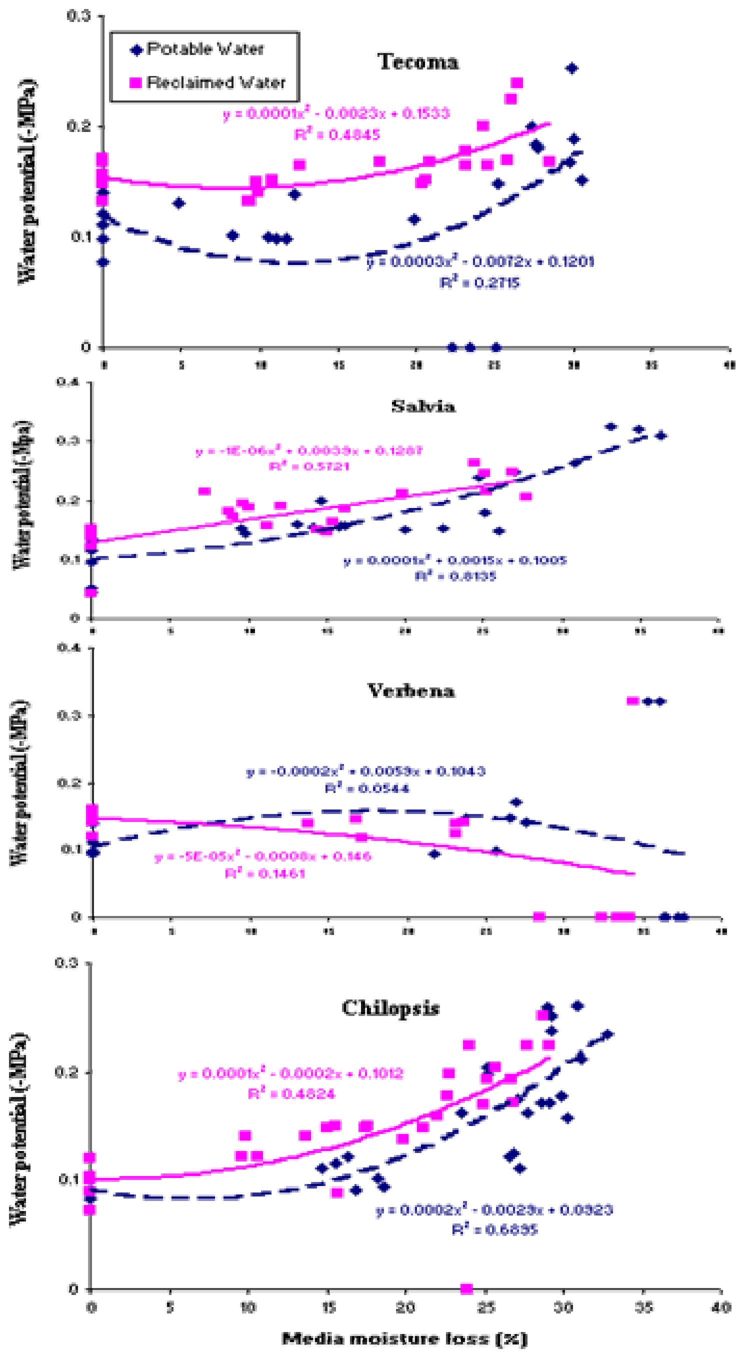

Fig. 1. Water potential of plants irrigated with reclaimed or potable water and exposed to drought conditions.

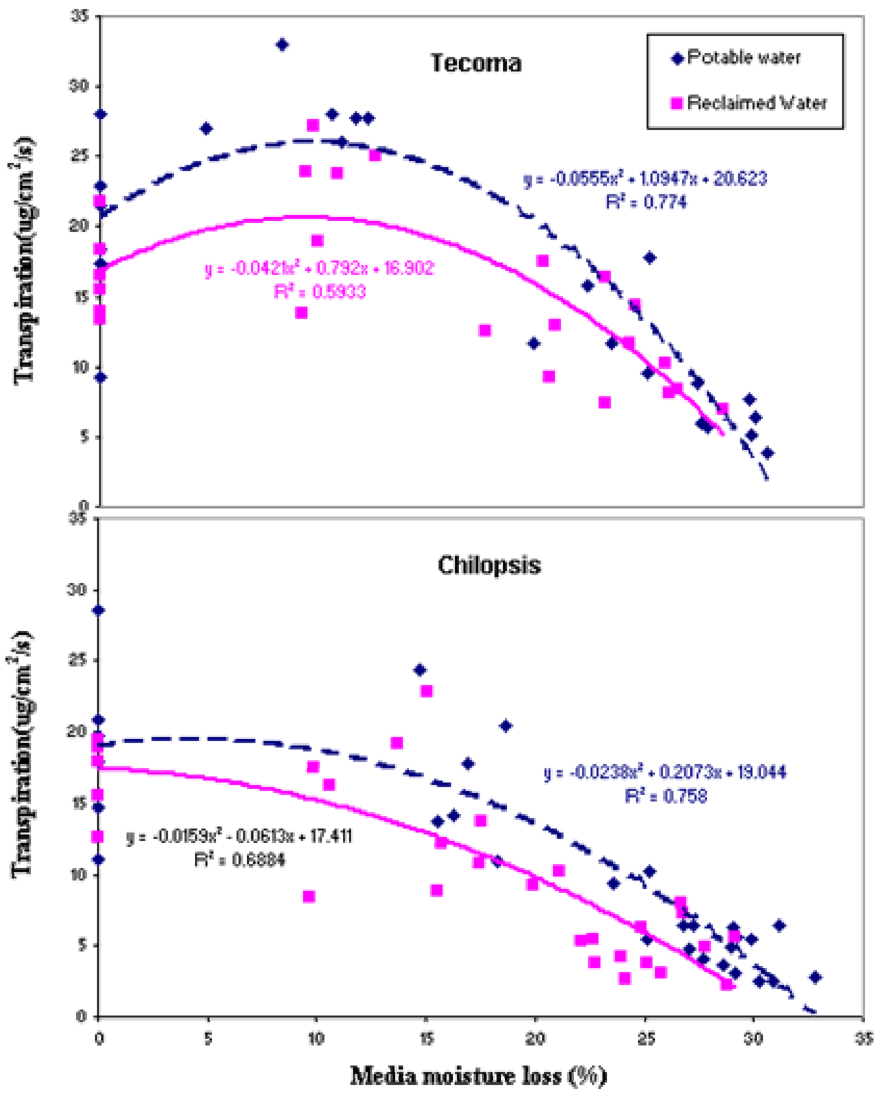

Fig. 2. Transpiration of plants irrigated with potable or reclaimed water and exposed to water stress conditions.

This response is not surprising since most plants under the potable irrigation regime had a greater leaf area as shown by greater leaf dry mass. Transpiration rates measured in yellow bells and desert willow were greater at mild water stress for plants irrigated with potable versus reclaimed water until about 25% of the fully saturated soil moisture was lost. These factors would result in a faster depletion of the available water in the media and result in faster wilting of plants treated with potable water.

The extensive damage of plants irrigated with potable water after the drought are likely a result of these plants being continuously well watered, while the plants irrigated with reclaimed water were gradually adjusted to increasing salinity and therefore were conditioned to physiological drought throughout the 12 weeks of treatments. Saturated paste tests of the dry media at the end of the drought cycle indicated that final EC in the root zone differed significantly between species. Root zone EC of desert willow was 0.8 dS/m (potable) and 2.05 dS/m (reclaimed), of yellow bells EC was 1.2 dS/m (potable and 2.8 dS/m (reclaimed), in sage EC was 0.9 dS/ m (potable) and 3.4 dS/m (reclaimed) and in verbena EC was 1.1 dS/m (potable) and 6.4 dS/m (reclaimed). These results suggest that verbena and sage may be salt excluders, while desert willow appears to include the salts. Yellow bells may incorporate both mechanisms to cope with the additional salts in the root zone. In conclusion, the increased salinity in the root zone conditioned plants irrigated with reclaimed water and thus enabled them to resist drought damage compared to the plants irrigated with potable water which were not acclimated to drought conditions and depleted the water through higher transpiration rates faster.

Salt sensitive plants can be produced with reclaimed water especially if they are well irrigated. While some component of plant dry weight will likely be affected and decrease when irrigating plants with reclaimed versus potable water, overall aesthetic appeal of ornamental plants is most important and small decreases in biomass are acceptable. However, during prolonged exposure to water with higher salinity, visual quality and parameters such as root weight may decrease below acceptable levels and impair the function of plants. This will depend on the species and the chemical composition of the irrigation water. Plants in this experiment likely would have benefited from some shade during the hottest part of the day in July and August when temperatures exceeded 40°C on a daily basis. Visual symptoms of salt injury were greatest during that time period and almost vanished when maximum daytime temperatures decreased. After 12 weeks, sage plants irrigated with reclaimed water were more attractive because of their compact canopy compared to plants irrigated with potable water.

Plants irrigated with reclaimed water and exposed to drought sustained less damage in terms of defoliation and plant dieback compared to plants irrigated with potable water. This is most likely due to the reclaimed water resulting in a mild physiological drought, conditioning plants to tolerate water deficits. Salts accumulated in the root zone of plants irrigated with reclaimed water and long-term studies of plants in the landscape irrigated with potable or reclaimed water are required to determine how much salt accumulation and how much water deficit different species will be able to tolerate.

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Measurement of estrogenic activity in sludges and biosolids

Basic Information

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Principal Investigators:	David Quanrud, Robert Arnold, Jon D Chorover, Wendell Ela

Publication

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5. Zhang, J., Dong, H., Arnold, R. Ela, W., Quanrud, D., and Lansey, K., 2004. The fate of nonylphenol and total estrogenic activity during sludge treatment processes. In: Proceedings, Seventeenth Annual Symposium of the Arizona Hydrological Society. Tucson, AZ. September 15-18, 2004.

A. Problem and Research Objectives

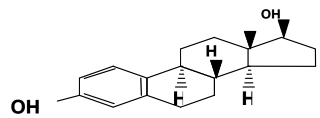
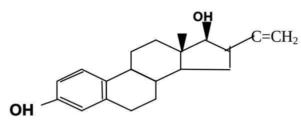
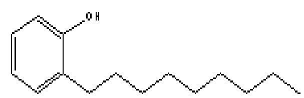
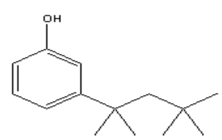
There is widespread speculation that exposure to endocrine disrupting compounds (EDCs) in the environment is responsible for recently observed increases in several types of human cancers and worldwide declining sperm levels in men. In fact, the effect of exposure to EDCs on human health is not known with certainty, and relevant epidemiological data is not likely to arise in the near future. A number of organic compounds that are responsible for estrogenic activity in municipal wastewater readily survive conventional wastewater treatment processes (Huang and Sedlak, 2001). They are either discharged to surface waters that serve as effluent receiving waters or they are separated from the aqueous phase onto solid materials that are captured as primary or waste activated sludge. Nonylphenol and several other compounds thought to be responsible for estrogenic activity in wastewater effluent are moderately hydrophobic. Consequently, these compounds should partition, in some measure at least, with sludges derived from wastewater treatment. The extent to which these compounds survive sludge stabilization and dewatering processes is not known. Their fate in biosolids that are used as soil amendments (as in Arizona) is of environmental relevance.

The hydrophobic nature of compounds reputed to contribute to total estrogenic activity in wastewater and wastewater effluent (Table 1) suggests that estrogenic activity is strongly associated with sludges produced during wastewater treatment. That is, the through-plant reduction in total estrogenic activity that typically accompanies the treatment of municipal wastewater (Ternes *et al*., 1999a,b; Holbrook *et al*., 2002) is due to not only biochemical destruction of responsible organic compounds but also transfer of the same chemicals to sludges and biosolids. Anaerobic digestion is a widely used sludge treatment process in municipal wastewater treatment plants because of advantages such as low energy consumption, possible production of energy, and reduced sludge volume. However, due to the persistence of estrogenic compounds under anaerobic condition (Ying *et al*., 2003, 2004; Lee and Liu, 2002), the fate of estrogenic activity during anaerobic sludge digestion is of environmental interest.

Several relatively recent investigations suggest that levels of estrogenic contaminants and estrogenic activity decrease significantly during conventional wastewater treatment. Due to the hydrophobic nature of these compounds, however, it seems likely that most of the difference in estrogenic activity between wastewater treatment plant influent and effluent can be accounted for in biosolids produced via wastewater treatment. Until recently, however, meaningful investigation of mechanisms for estrogen removal during wastewater treatment was impeded by a lack of reliable methods for extracting hydrophobic organics from sludges and biosolids. That is, it was not possible to extract estrogens from biosolids with confidence, making it difficult to assign mechanisms (biodegradation versus phase transfer, etc.) for treatment-related improvements in water quality.

The two goals of this project were to develop methods to extract estrogenic compounds from sewage sludges/biosolids and to then perform a preliminary assessment of the fate of estrogenic activity and nonylphenol content in sludges/biosolids during sludge digestion processes at a few selected full-scale wastewater treatment plants. Nonylphenol was chosen for analysis because it is an important estrogen mimic that is always present in municipal wastewater.

Table 1. Structures and properties of wastewater compounds with estrogenic behavior.

Chemical	Structure	Molecular Weight	Log K_{ow}	Relative Estrogenic Activity (YES bioassay)
17β-estradiol (E_2)		272	3.94	1.0
17α-ethinyl estradiol (EE_2)		296	4.15	1.4
Nonylphenol		220	4.48	$10^{-4} - 2\times10^{-4}$
Octylphenol		206	4.12	5×10^{-4}

A specific objective of this work was to measure total estrogenic activity and nonylphenol mass fluxes during wastewater treatment and solids handling operations at operational wastewater treatment plants. Those results would then be used to support analysis of removal mechanisms for estrogenic compounds during wastewater treatment operations.

The investigation at the wastewater treatment plants focused on the fate of nonylphenol and total estrogenic activity during anaerobic sludge digestion and subsequent dewatering processes. Sludge composting at one plant was also investigated. Total estrogenic activity and nonylphenol mass fluxes across each operation unit at treatment plants were determined.

There have been only a few successful efforts to extract estrogenic compounds from soil, sediment, sludge, or biosolids. See, for example, Furbacker *et al*., (1999); Korner *et al*., (2000); Matsui *et al*., (2000); Ternes *et al*., (2002); and Holbrook *et al*., (2002). Holbrook *et al*. (2002) extracted raw and digested sludge in pentane, leading to measurement of total estrogenic activities using a reporter-gene assay. Removal of estrogenic activity from raw wastewater via secondary treatment was 55-70 percent. Relatively little of the estrogenic activity lost during secondary treatment, however, was recoverable from waste activated sludge, suggesting that most of the observed loss of activity was due to biodegradation. This result is at apparent odds with theoretical considerations that suggest hydrophobic estrogenic compounds (Table 1) should partition with organic-rich solids. A more likely explanation is that the extraction procedure used did not produce complete desorption of estrogenic compounds from sludge particles, leading to low recoveries. Both anaerobic and aerobic digestion processes increased the mass of extractable estrogenic compounds detected on residual biosolids. The survival of specific estrogenic contaminants during anaerobic treatment of sludge and sediments has been reported by others (Fauser *et al*., 2003).

B. Methodology

Sampling Sites

The fate of estrogenic compounds that are separated with sludge was determined by subjecting solids to the same extraction/bioassay procedures before and after sludge digestion, dewatering and composting. This work was carried out using sludges and biosolids produced at the Ina Road Wastewater Pollution Control Facility (IRWPCF) (Pima County, Arizona), Joint Water Pollution Control Plant (Carson, CA), and Hyperion Wastewater Treatment Plant (Los Angeles, CA). Additional data was taken from the Ina Road and JWPCP plants (raw wastewater, primary effluent, secondary effluent) to support a crude through-plant balance on estrogenic activity. Sampling points (numbered as 1, 2, 3) at the Ina Rd WWTP are shown in Figure 1.

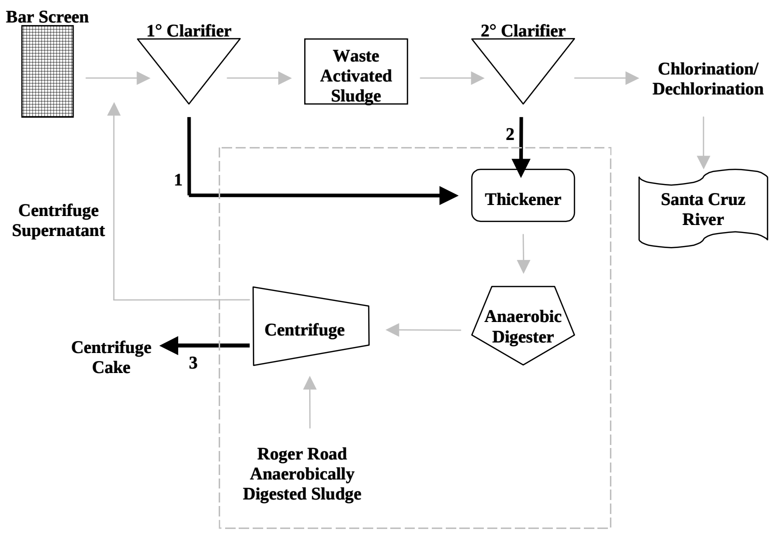


Figure 1. Simplified schematic of the Ina Road Wastewater Treatment Plant (Tucson, AZ) showing sampling points (numbered as 1, 2, 3).

The JWPCP is a 350 million gallon per day (MGD) municipal wastewater treatment plant owned and operated by the County Sanitation Districts of Los Angeles County (CSDLAC), which serves a heavily industrialized section of Los Angeles County, including a population of about 3.5 million. The Hyperion Treatment Plant receives sewage from a 515 square mile area covering most of the greater Los Angeles area, with a capacity of approximately 450 MGD and a current inflow of about 360 MGD. The sludge digestion processes used at the JWPCP and Hyperion Plants are classified as mesophilic and thermophilic respectively. Wastewater, solids, etc., samples were taken at the JWPCP points indicated (Figure 2) by CSDLAC personnel and shipped overnight to University of Arizona for analysis.

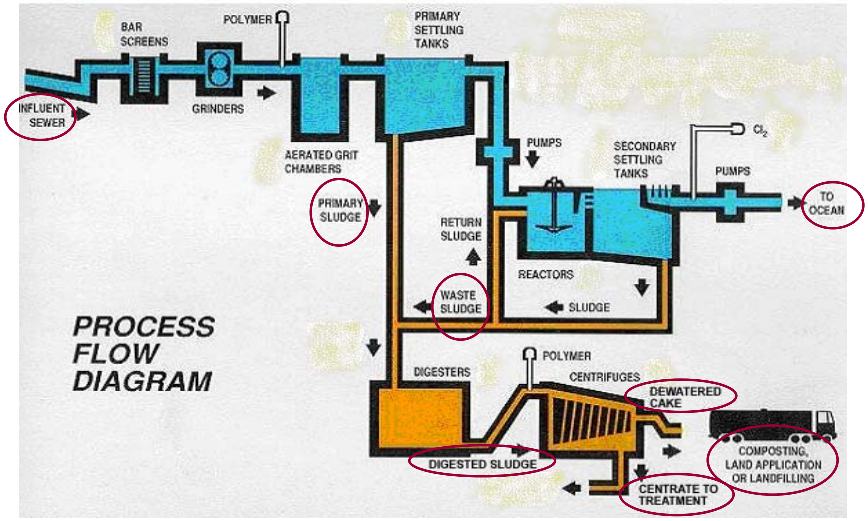


Figure 2. Sampling locations at JWPCP. The circle at lower right represents composted biosolids.

At the Hyperion WWTP, samples of primary and waste activated sludges, a digested blend of primary and waste-activated sludges, and dewatered sludge (four samples total) were collected by plant personnel and shipped overnight to the University of Arizona for analysis. At each plant, one set of grab samples were obtained for analysis within this study. Thus, this project provides only a "snapshot" of sludge digestion performance at each plant.

Laboratory Procedures

In general, samples were stored at 40° - 60°C for 48 hours to produce a dry residual for extraction and analysis. Water content was determined using subsamples that were dried for 12 hours at 103°C. Influent and effluent liquid samples were passed through a 0.80 μm membrane filter, and filtrate was stored for subsequent analyses of total nonylphenol and total estrogenic activity.

<u>*Sample Extraction*</u>. Organic extracts from sludges/biosolids were obtained using a microwave assisted extraction (MAE) procedure in a CEM MDS-2100 Microwave Digestion System. In general, 0.1 g of the dry solid was extracted in 20 mL of reagent grade methanol using the following program. Pressure was ramped from 0 to 20 psig over five minutes by heating the closed extraction vessel and held constant for 30 minutes. Reactor contents were then allowed to cool for 45 minutes. Liquid-phase subsamples were subsequently taken for further processing leading to analyses of nonylphenol and total estrogenic activity.

Post-extraction sample clean-up steps were designed to separate estrogenic compounds from other organic material that might compromise measurements of total estrogenic activity. Methanol-based extracts were diluted to ~1% methanol in Nanopure water.

Hydrophobic organics in the dilute mixture were then adsorbed on C-18 SPE cartridges. Adsorbed organics were separated via differential elution in a methanol/water gradient that initially varied in volume fraction methanol from 0.2 to 1.0 by increments of 0.2. Only the 0.60 and 0.80 v/v methanol fractions proved to be estrogen. Consequently, a standard protocol was adopted in which 5 mL of 0.2 v/v methanol/water was passed through the C-18 cartridge and discarded. Estrogenic compounds were then eluted in 10 mL of 0.8 v/v methanol and saved. The extracts so obtained were directly analyzed for total extractable nonylphenol via HPLC with fluorescence detector.

The process blank was derived using a blank microwave extraction step (methanol only), dilution of the methanol "extract" in Nanopure water, adsorption on a C-18 cartridge and elution, per above.

The organic separation process for samples that were predominantly liquid (raw wastewater, secondary effluent, centrate from sludge dewatering, etc.) was different. At times, the entire sample in its original form was dried and resuspended in methanol for MAE, etc. Occasionally, samples were filtered, and then applied directly to the C-18 disks without a solids extraction step.

Nonylphenol Measurement. Total nonylphenol was determined via high performance liquid chromatography (HPLC) following sample preparation/concentration. Samples in which dry solids comprised a significant fraction of the total mass were dried for 12 hours at 103°C and resuspended in methanol. The ratio of dry sample mass to methanol volume was a function of the expected contaminant mass in the original sample (0.1 g dry weight to 20 ml reagent for most sludge samples). The Hewlett-Packard HPLC-FLD system used for nonylphenol measurement consisted of an autosampler, solvent delivery system, reverse-phase C18 column and a fluorescence detector (1046A). The mobile phase was an acetonitrile (ACN) gradient in ultrapure water and a flow rate of 1mL/min. The ACN gradient program was 0.30 ACN/0.70 water from 0 - 5 min; 0.40 ACN/0.60 water from 5 - 10 min; 0.60 ACN/0.40 water from 10 - 20 min; 0.80 ACN/0.20 water from 20 -25 min; and an isocratic purge from 25 - 30 min after which the eluent composition was returned to 0.30 ACN/0.7 water. The injection volume was 25μL and the excitation and emission wavelengths were 230nm and 305nm.

Estrogenic Activity Measurement. Total estrogenic activity in extracts was measured by using a trans-activation reporter gene assay. A portion of each extracted sample was dried and re-dissolved in Nanopure water for measurement of total extractable estrogenic activity using the yeast estrogen screening (YES) protocol of Routledge and Sumpter (1996). The yeast estrogen screen is an *in vitro* transactivation bioassay based on estrogen-dependent synthesis of β-galactosidase by a recombinant strain of *Saccharomyces cerevisiae*. When applied to chemically complex samples, results can be converted to equivalent concentrations of 17α-ethinyl estradiol (EE_2) via reference to a suitable EE_2 standard response curve. Positive (EE_2) and negative controls were run with each sample set. The process blank was derived using a blank microwave extraction step (methanol only), dilution of the methanol 'extract' in Nanopure water, adsorption on a C-18 disk and elution, per above.

For the YES protocol, sample organics were ultimately redissolved in the yeast growth medium. For liquid samples, the overall procedure consisting of adsorption, elution, drying and redissolution resulted in nominal concentration factors of 200-500 based on the ratio of initial to final sample volumes. When applied to chemically complex samples such as those encountered here, results can be converted to equivalent concentrations EE_2 via reference to a suitable EE_2 standard response curve. Positive (EE_2) and negative controls were run with each sample set. The process blank was derived using a blank microwave extraction step (methanol only), dilution of the methanol "extract" in Nanopure water, adsorption on a C-18 cartridge and elution, per above.

C. Principal Findings and Significance

Ina Road WWTP (Tucson, AZ). At the Ina Road WWTP, analyses of samples collected from raw influent, final effluent, digested sludge, and centrate indicated overall reductions of estrogenic activity and nonylphenol of 49% and 32%, respectively (Figure 3).

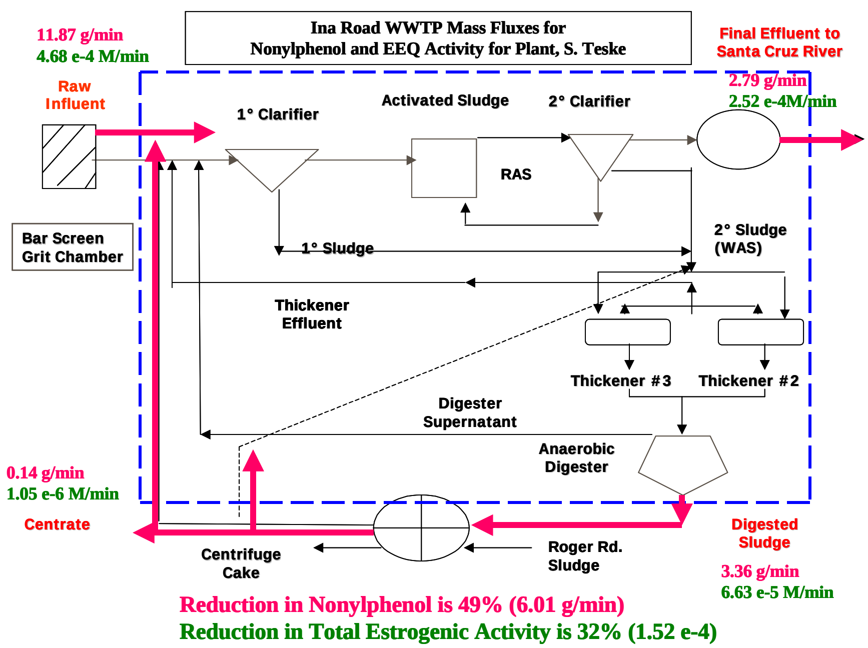


Figure 3. Mass fluxes for nonylphenol and total estrogenic activity at the Ina Rd WWTP, Tucson, AZ.

JWPCP (Los Angeles County, CA). Nonylphenol measurements were combined with mass (solids) or volume (liquids) fluxes corresponding to various points at JWPCP as shown (Table 2) to yield daily mass fluxes of nonylphenol at those positions. From the results of the analysis (Figure 4), it is evident that secondary treatment is capable of lowering the flux of nonylphenol from influent to effluent by more than 90 percent (here, 93%). Of the 93 percent through-plant loss, however, more than two-thirds (72%) was accounted for as extractable nonylphenol in the dewatered sludge. Considering both the dewatered cake and effluent as sinks for nonylphenol at JWPCP, all but a fourth of the influent nonylphenol is accounted for, and (net) biotransformation removed at most 26%

of the influent nonylphenol. There were more circumscribed balances around the anaerobic digester and JWPCP sludge dewatering operations. These show that mesophilic digestion and physical dewatering have a very limited effect on nonylphenol mass. That is, total extractable nonylphenol was essentially unchanged by digestion and centrifugation. A comparison of nonylphenol in primary and waste activated sludges shows that primary sludge accounts for almost 90 percent of the nonylphenol that enters the anaerobic digester. There was little or no loss of extractable nonylphenol during mesophilic anaerobic digestion. As expected, dewatering had little effect on nonylphenol levels or fluxes. A balance around the composting operation, which precedes sale of composted sludge and fertilizer/soil conditioner, suggests that perhaps 75% of the nonylphenol that enters the composting process associated with the dewatered cake is lost, perhaps due to aerobic biochemical activity. This encouraging result should be further examined and verified in future research studies to establish the efficacy of using aerobic decomposition processes, such as composting, for nonylphenol destruction.

Table 2. Measurements and calculations leading to mass balance analyses of nonylphenol fate at JWPCP.

Sample Description	Flow Rate or Mass Flux	Water Content (Mass Fraction)	Nonylphenol Concentration	Nonylphenol Flux (kg/day)
Influent	350 MGD	1.0	0.59 mg/L	775
Effluent	350 MGD	1.0	0.04 mg/L	53
Primary sludge	3.5 MGD	0.968	1150 μg/g	486.2
Thickened waste (activated sludge)	1.1 MGD	0.944	286.6 μg/g (dry wt.)	66.8
Digested sludge (pre centrifugation)	4.6 MGD	0.975	1190 μg/g	520.9
Dewatered cake (post centrifugation)	1650 wet tons/day	0.737	1320 μg/g (dry wt.)	520.5
Centrate	4.16 MGD	1.0	2.74 mg/L	43.2
Composted biosolids	550 wet tons/day	0.169	314.0 μg/g (dry wt.)	130.2

Overall, the balance on nonylphenol fluxes at the JWPCP indicates that two-thirds of the nonylphenol that enters the treatment plant leaves with the dewatered cake. Aerobic biodegradation during secondary treatment may remove as much as 25 percent of the influent nonylphenol.

By comparing nonylphenol levels in filtered versus unfiltered influent and effluent samples, about 80 percent of the nonylphenol in the JWPCP influent was associated with particles larger than 0.8 μm. Thus, relatively low nonylphenol levels in the plant effluent (40 μg/L, equivalent to the highest level recorded in the USGS nationwide survey, Kolpin *et al*., 2002) are more a product of suspended solids removal than of biochemical treatment of nonylphenol.

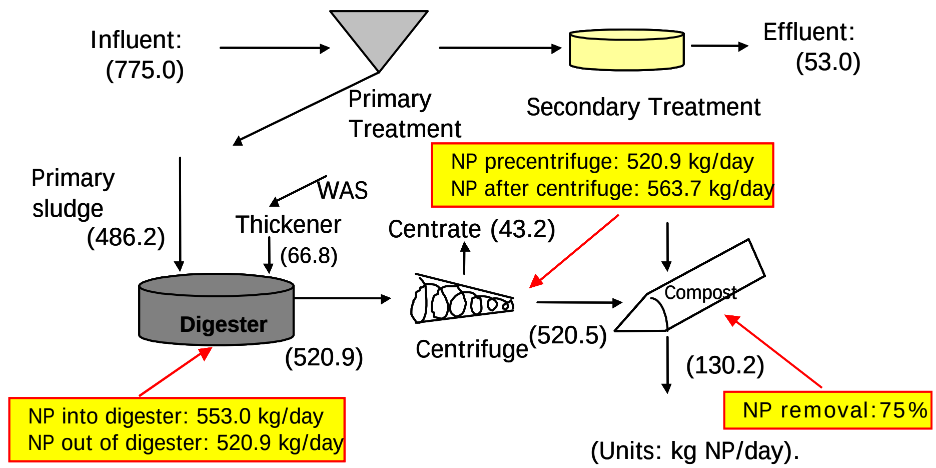


Figure 4. Nonylphenol fluxes throughout JWPCP. Fluxes were calculated based on mass flux or volume rate of flow, water content and NP concentration at the points shown.

A similar overall picture emerges from total estrogenic activity data at JWPCP (Table 3, Figure 5). That is, the EE_2 equivalent mass fluxes indicated that 93 percent of the total estrogenic activity in the plant influent was missing from JWPCP effluent. The EE_2 equivalent concentration of estrogenic activity in JWPCP effluent was 8.9×10^{-11} M (26.3 ng/L), still much higher than minimum levels known to disrupt estrogen physiology in exposed animals. Again, more than 90 percent of the influent estrogenic activity was associated with particles removed on a 0.8 μm filter. In this case, >50 percent of the estrogenic activity lost (based on comparison of JWPCP influent and effluent concentrations) was accounted for in an extract derived from the dewatered sludge. There is some evidence of experimental error in the total extractable estrogenic activity in sludge samples. Estrogenic activity in the dewatered cake was low compared to that of digested sludge before centrifugation. Nevertheless, it is probable that the fraction of total estrogenic activity lost to biochemical processes (≤45 percent based on Figure 5 data) was larger than the fraction of nonylphenol biodegraded (≤26 percent). The balances on total estrogenic activity around anaerobic digestion, dewatering and composting were suspect, probably due to error that is essentially unavoidable in the YES assay. The flux of estrogenic activity out of the digester seems particularly high. As a consequence, it was not possible in this limited study to estimate the efficiencies of individual unit operations (anaerobic digestion, dewatering and composting) for removal of total estrogenic activity.

It may be significant that the total estrogenic activity in sludges entering the digester was just half the activity measured in the digested solids and three-fourths of the activity in the dewatered cake. While this at first seems incongruous and perhaps a consequence of error in sampling, extraction or application of the YES bioassay, the increase in estrogenic activity through the digester may also result from destruction of anti-estrogenic compounds during anaerobic digestion. That is, a primary source of anti-estrogenic activity in the YES bioassay consists of compounds that bind to hERα (human estrogen receptor) without stimulating synthesis of β-galactosidase. A number of natural and synthetic organics have this property. If anti-estrogens are removed or transformed to some extent during anaerobic digestion, those reactions would produce an apparent increase in estrogenic activity, as competition for hERα by anti-estrogen decreased. Other explanations are possible, however.

Table 3. Measurements and calculations for mass balance analysis of total estrogenic activity at JWPCP.

Sample Description	Total estrogenic activity (equivalent EE_2 concentration)	Flux of estrogenic activity (mol EE_2/day)
Influent	1.25 nM	1.66
Effluent	0.089 nM	0.12
Primary sludge	0.8 nMol/g dry wt.	0.34
Thickened WAS	1.0 nMol/g dry wt.	0.23
Digested sludge (precentrifugation)	2.5 nMol EE_2/g dry wt.	1.1
Dewatered cake (post centrifugation)	2.0 nMol EE_2/g dry wt.	0.79
Centrate	no data	—
Composted biosolids	0.15 nMol EE_2/g dry wt.	0.062

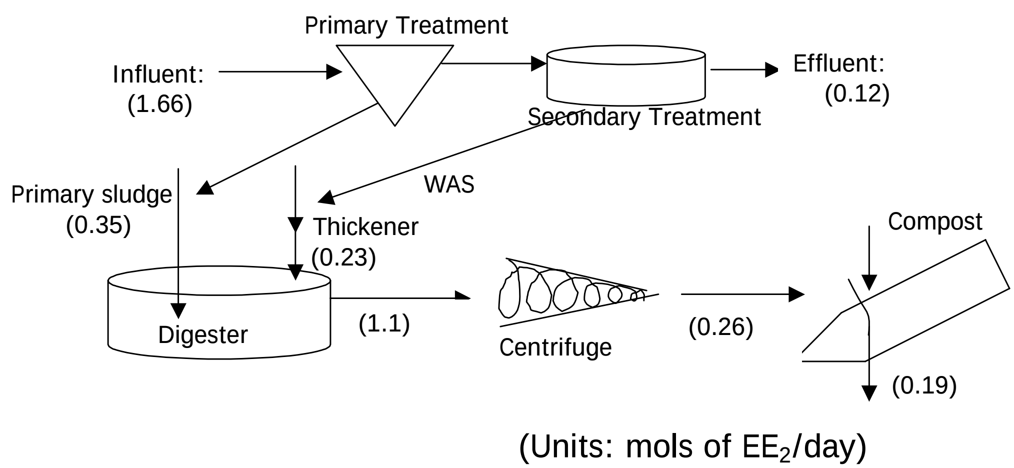


Figure 5. Total estrogenic activity flux at different stages of treatment at JWPCP. Total estrogenic activity fluxes were calculated based on flow rate, water content and estrogenic activity as mol EE_2 equivalents/day at each position shown.

The contribution of nonylphenol to total estrogenic activity is also of some interest. YES bioassays with pure 17α-ethinyl estradiol and a mixture of nonylphenol isomers indicated that EE_2 is 5000 – 10,000 times more estrogenic than nonylphenol (data not shown). The difference in potency is compensated, at least in part, by the relatively large expected concentration of nonylphenol in wastewater and wastewater effluent. Thus, by expressing total estrogenic activity in terms of an equivalent EE_2 concentration, it is possible to speculate on the contribution of nonylphenol to the YES bioassay response. Here we applied a factor of 1/7500 to convert nonylphenol measurements to EE_2-equivalent concentrations. In the JWPCP influent, for example, the nonylphenol concentration was 5.90×10^5 ng/L, for an EE_2-equivalent concentration of 79 ng/L. Total estrogenic activity in the same sample, expressed as an EE_2-equivalent concentration, was 370 ng/L, so that the measured nonylphenol concentration accounted for just over 20 percent of the total estrogenic activity. In the plant effluent, nonylphenol accounted for just 2 percent of the total estrogenic activity. Results suggest that nonylphenol is removed with greater efficiency than other components of estrogenic activity during conventional wastewater treatment, perhaps because the affinity of nonylphenol for organic-rich solids is greater than those of most other estrogens and estrogen mimics. The analysis ignores the possibility of synergy or antagonism among compounds contributing to total estrogen activity and, consequently, should be considered cautiously.

The nonylphenol concentration was estimated at 1300 μg/g in the dried dewatered cake, and the total extractable estrogenic activity was 600 ng EE_2/g in the same sample. Consequently, nonylphenol accounted for perhaps 30 percent of the extractable estrogenic activity in the dewatered cake. Evidently, nonylphenol is an important component of estrogenic activity in the JWPCP wastewater and solid products derived from its treatment.

Hyperion WWTP (Los Angeles, CA). At the Hyperion Treatment Plant in Los Angeles, the one-time sampling effort indicated that there is little loss of estrogenic activity during thermophilic sludge digestion and subsequent dewatering operations (Figure 6; Table 4). The daily mass flux values obtained for total nonylphenol and estrogenic activity suggest that thermophilic sludge digestion probably offers little advantage in terms of estrogen and particularly nonylphenol destruction. Nonylphenol concentrations were probably not affected by the digestion process. The through-digestion increase in total nonylphenol could have arisen from the nature of the experimental design (one-time grab samples), or from conversion of ethoxylated nonylphenol forms to nonylphenol during digestion. Error introduced by sample preparation steps is also a possibility, although no such error was evident in the JWPCP samples reported above. A modest reduction in total estrogenic activity is apparent in the data, and this could be real. Additional data collection is warranted to confirm this result before it is accepted on the basis of a one-time monitoring effort.

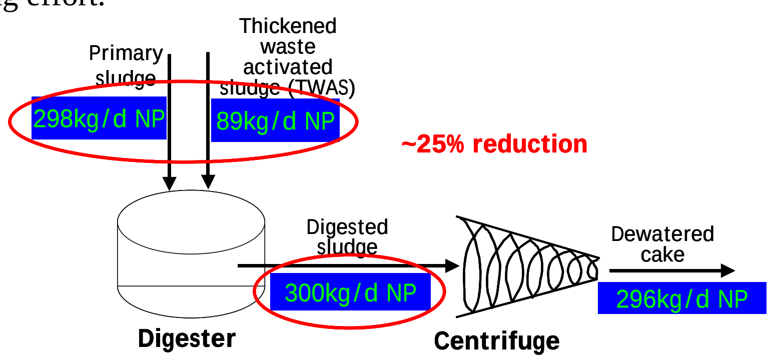


Figure 6. Total daily flux of nonylphenol (NP) during sludge digestion processes at the Hyperion wastewater treatment plant, City of Los Angeles, CA.

Table 4. Measurements and calculations leading to mass balance analyses of nonylphenol and total estrogenic activity at Hyperion WWTP.

Sample Description	Flow Rate or Mass Flux	Water Content (Mass Fraction)	Nonylphenol Concentration (μg/g dry sludge)	Nonylphenol Flux (kg/day)
Hyperion Treatment Plant				
Primary sludge	2.17MGD	0.961	915	298.4
Thickened waste activated sludge	0.93MGD	0.965	715	88.8
Digested sludge	3.1MGD	0.980	1289	299.5
Dewatered cake	800 tons/day	0.683	1286	296.1
	Total estrogenic activity (ng EE2 Equivalent per g dry sludge)		Flux of estrogenic activity (g/day)	
Primary sludge	223		72.6	
Thickened waste activated sludge	465		57.8	
Digested sludge	620		144	
Dewatered cake	544		125	

Publication Information

Dissertations

Teske, S. 2004. Mass flux of total estrogenic activity and nonylphenol for a wastewater treatment plant. *Unpublished M.S. Thesis*. Chemical and Environmental Engineering. The University of Arizona.

Conference Proceedings.

Arnold, R. Quanrud, D., Ela, W., Conroy, O., Zhang, J., and Leung, C. 2004. Total estrogenic activity and nonylphenol concentration at the Roger Road Wastewater Treatment Plant—fates during effluent polishing and implications for water reuse. In: *Proceedings, Seventeenth Annual Symposium of the Arizona Hydrological Society*. Tucson, AZ. September 15-18, 2004.

Quanrud, D., Zhang, J., Teske, S., Dong, H., Orosz-Coghlan, P., Littlehat, P., Conroy, O., Arnold, R., Ela, W., and Lansey, K. 2004. The fate of nonylphenol and total estrogenic activity during wastewater treatment and sludge digestion: a mass balance analysis. In: *Proceedings, 4th International Conference on Pharmaceuticals and Endocrine Disrupting Chemicals in Water*. Minneapolis, MN. October 13-15, 2004.

S. Teske, J. Zhang, H. Dong, P. Orosz-Coghlan, D. Quanrud, R. Arnold, W. Ela. 2004. Estrogenic Compounds in Wastewater Treatment. Arizona Water Pollution Control Association 77th Annual Conference, Mesa, Arizona, May 5-7, 2004.

Zhang, J., Dong, H., Arnold, R. Ela, W., Quanrud, D., and Lansey, K., 2004. The fate of nonylphenol and total estrogenic activity during sludge treatment processes. In: *Proceedings, Seventeenth Annual Symposium of the Arizona Hydrological Society*. Tucson, AZ. September 15-18, 2004.

Controlling Salt Accumulation to Enhance Sustainability of Subsurface Drip Irrigation

Basic Information

Title:	Controlling Salt Accumulation to Enhance Sustainability of Subsurface Drip Irrigation
Project Number:	2004AZ52B
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End Date:	2/28/2005
Funding Source:	104B
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Research Category:	None
Focus Category:	Water Use, Agriculture, Solute Transport
Descriptors:	None
Principal Investigators:	Thomas L Thompson, Arthur W. Warrick

Publication

1. Roberts, Trent, "Salt accumulation with subsurface drip irrigation: modeling and field results," School of Natural Resources, University of Arizona, thesis in process.

A. Problem and Research Objectives

Irrigation water often contains significant concentrations of dissolved salts. Effective management practices for controlling salt accumulation are needed so that the efficiency of water use with subsurface drip irrigation (SDI) can be maximized. It is well-known that adequate leaching is the only permanent solution to prevent salt accumulation. However, the very nature of SDI effectively prevents leaching of salts from the zone of soil above the drip tubing. This problem is exacerbated in arid climates where there is insufficient rainfall to leach salts. Hence, periodic leaching with sprinklers is needed with SDI to leach salts from shallow soil depths. However, the use of supplemental sprinkler irrigation is expensive and labor-intensive. Furthermore, the need to periodically use sprinklers threatens the long-term economic sustainability of SDI, because of the increased costs. More research is urgently needed to enable prediction of the optimum amount and timing of supplemental sprinkler irrigation with SDI systems so that growers can effectively implement salinity management practices. Solving this problem will encourage growers to adopt this proven, efficient, and sustainable cropping system, with benefits for all citizens of Arizona.

The objectives of this project were to 1) identify those factors potentially influencing salt accumulation with SDI, 2) pursue modeling to predict salt accumulation and forecast needed management practices, and 3) validate predictions with data collected in field experiments.

B. Methodology

<u>Field Experiment</u>

The purpose of the field experiment was to validate output from the model, and provide the physical basis for adjustment of model parameters. The field experiment will be conducted at the University of Arizona Maricopa Agricultural Center. The surface soil texture was sandy loam. The experiment included all possible factorial combinations of two irrigation water salinities (ECw 1.6 and 2.5 dS m^{-1}), two depths of drip tubing installation (18 cm, 25 cm), and crop germination with and without sprinkler irrigation. Each treatment was replicated three times in a split-split plot design, for a total of 24 plots. Each plot was 4 m x 5 m (4 beds wide). Drip irrigation tubing was injected at the appropriate depths in raised beds located 1.0 m apart. Cantaloupe (*Cucumis melo* L. Reticulatis group) was planted on 26 March, 2006 on the raised beds and was germinated by irrigating with the SDI system, or with sprinklers, depending on the treatment. Sprinkler irrigation was terminated on 14 April, 2004 and all plots were irrigated with the SDI system after that date. Amounts of water applied are shown in Table 1. Harvests were conducted during June 2005. Yield and quality (%brix) were measured within each plot.

Table 1 Water use in the cantaloupe experiment.

Depth of SDI tubing (cm)	**Sprinklers**	**Water Salinity (dS/m)**	**Water Applied (cm)**
18	-	1.6	53.2
	-	2.5	62.0
	+	1.6	37.1
	+	2.5	41.0
25	-	1.6	56.0
	-	2.5	51.1
	+	1.6	38.3
	+	2.5	32.7

Broccoli (*Brassica olearacea* L. Italica group) was planted in the same plots on 29 September, 2004, after appropriate soil tillage operations. Again, the crop was germinated by using either the SDI system, or with sprinklers, depending on the treatment. Broccoli was harvested on 2 February 2005, and yield and quality were evaluated in each plot.

The low salinity irrigation water (ECw = 1.6 dS m^{-1}) is the normal water supplied at MAC. The high salinity treatment was achieved by constant injection of a concentrated NaCl solution using an injection pump. The resulting salinity was 2.5 " 0.4 dS m^{-1}. Bromide was used as a tracer for monitoring solute movement in soil. The Br^- ion is a biologically conserved tracer that is present in only trace concentrations in most soils. Potassium bromide was injected into all irrigation water delivered through the drip tubing, at a constant concentration of 5 mg Br L^{-1} by using a proportioning pump.

Soil samples were collected within each plot in 3-cm increments to a depth of 30 cm. Samples were collected near the bed center and at each bed shoulder. Thus, a total of 30 soil samples were collected per plot at each sampling event. Samples were collected after cantaloupe and broccoli harvests. The samples were extracted with distilled water (2:1, water:soil), and electrical conductivity was measured using a conductivity cell, and Br^- concentrations were measured by using an ion chromatograph.

Modeling

Irrigation system, soil, and environmental factors that could affect solute distribution will first be identified. Initially, we expect the most important factors to be depth of drip tubing installation, spacing of laterals, soil texture, initial soil salinity, irrigation water salinity, amount of water applied, amount of rainfall, and potential evapotranspiration. In addition, crop salt tolerance will influence the optimum timing of leaching irrigation with sprinklers.

The HYDRUS-2D model (Simunek et al., 1999) will be used to predict solute distributions as a function of SDI system, soil, and environmental conditions. Water flow will be described using the Richards' equation:

$$\frac{\partial \theta}{\partial t} = \nabla \cdot (K \nabla h) - \frac{\partial K}{\partial z} \qquad (1)$$

where *2* is the volumetric water content, *h* is pressure head (negative for unsaturated conditions), *K* is the unsaturated hydraulic conductivity, *t* is time, and *z* is depth. There are limited cases for which Richards' equation can be solved using analytical techniques (Warrick, 2003). However, because Richards' equation is highly nonlinear, most cases of interest must be solved by numerical methods. The initial plan is to fully utilize HYDRUS-2D to carry out the numerical computations for water flow.

Solute flow will be described using the advective (convective) dispersion equation (ADE) (Warrick, 2003):

$$\frac{\partial c}{\partial t} = \nabla \cdot (D \nabla c) - v \cdot \nabla c - S \qquad (2)$$

∇ with *c* a solute concentration, a vector gradient operator, $\cdot$ a vector dot product, *D* an apparent diffusion coefficient (or hydrodynamic dispersion, accounting for dispersion and diffusion), *v* an apparent (vector) velocity and *S* a sink/source term. For the simpler cases dealing with conservative solutes and well-defined sink/source terms (such as linear adsorption and first order kinetics), HYDRUS-2D (Simunek et al., 1999) will be used, and water and solute transport are coupled automatically.

C. Principal Findings and Significance

The lowest salt concentrations (measured by soil EC) were found near and below the SDI tubing (Figs. 1 and 2), because irrigation with SDI effectively dilutes salts near the tubing and leaches salt below the tubing. Salt concentrations near the soil surface were lower when the drip tubing was installed at 25 cm depth, compared to 18 cm depth. The use of sprinklers for germination resulted in lower water use (Table 1), and resulted in approximately a 20% yield increase (data not shown). Examination of Figs. 1 and 2 reveals that in most cases use of sprinklers at the beginning of the season resulted in lower soil salt concentrations at the end of the season. Despite the 50% higher salt content of water in the high salinity treatment, this did not appear to result in higher salt concentration in the soil.

Salt concentrations were high near the soil surface (<10 cm) in all treatments, which could inhibit germination of subsequent crops unless sprinklers are used. In the subsequent broccoli experiment, percent emergence and yield of broccoli were lower without sprinklers than where sprinklers were used (data not shown).

A limited number of HYDRUS-2D model runs have been completed. Two examples are shown in Fig. 3. Figure 3A should be compared to the actual data shown in Fig 2A. The predicted salt distribution was similar to the actual distribution, although salt concentrations were under-predicted at very shallow soil depths. Figure 3B should be compared to the actual data shown in Fig. 2C. In this case it is evident that the model predicted significantly higher salt concentrations than actually occurred.

Because of the short duration of this study, not all analyses have been completed by this time. The second cropping season was completed in February 2005, and samples have not yet been analyzed. The following still remains to be completed: 1. Br analyses from season 1. 2. EC and Br analyses from season 2. 3. Further comparisons of field data with predictions from HYDRUS 2-D.

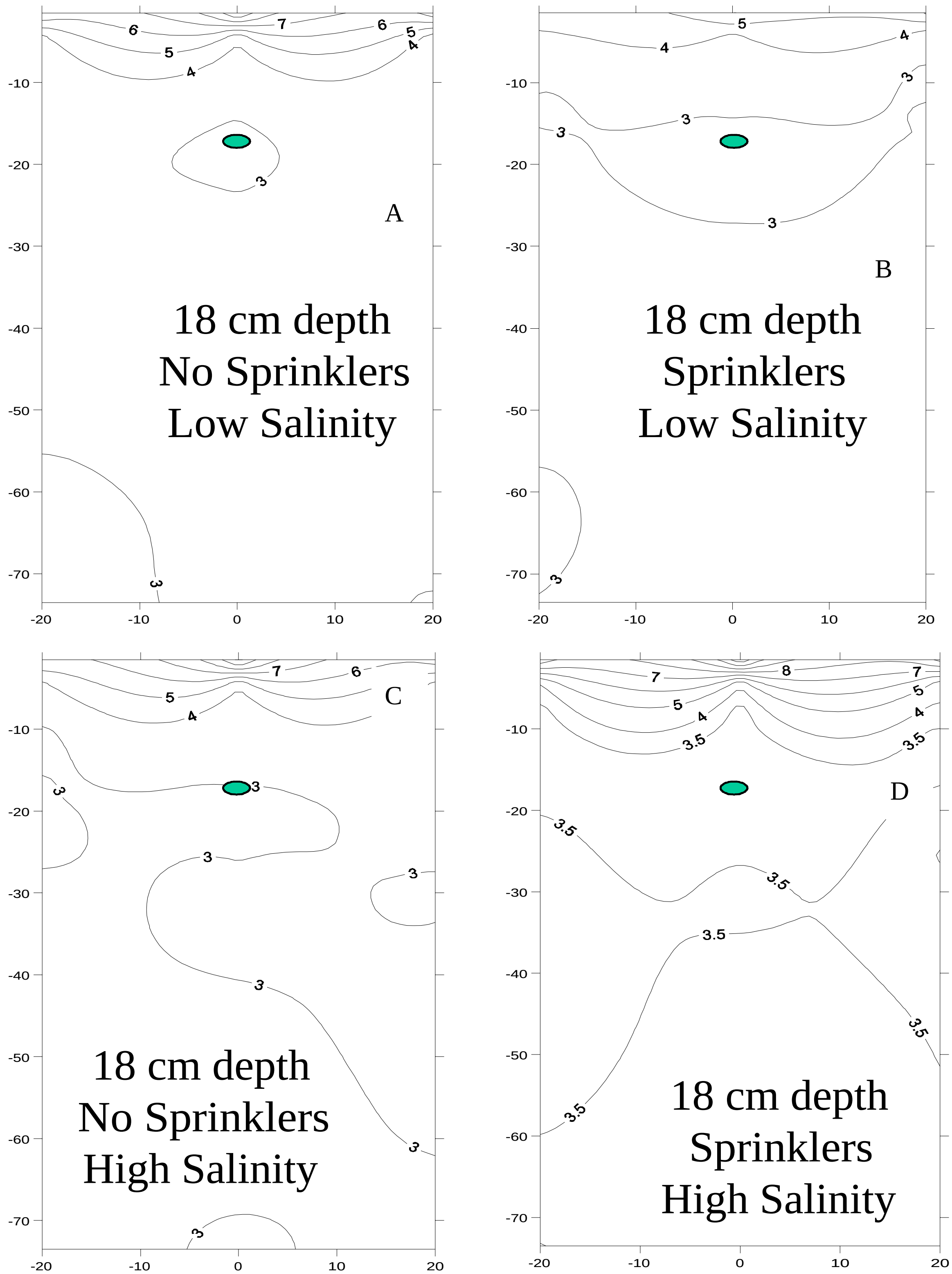

Figure 1 Distribution of electrical conductivity in plots with SDI tubing installed at 18 cm depth, from the first cropping season.

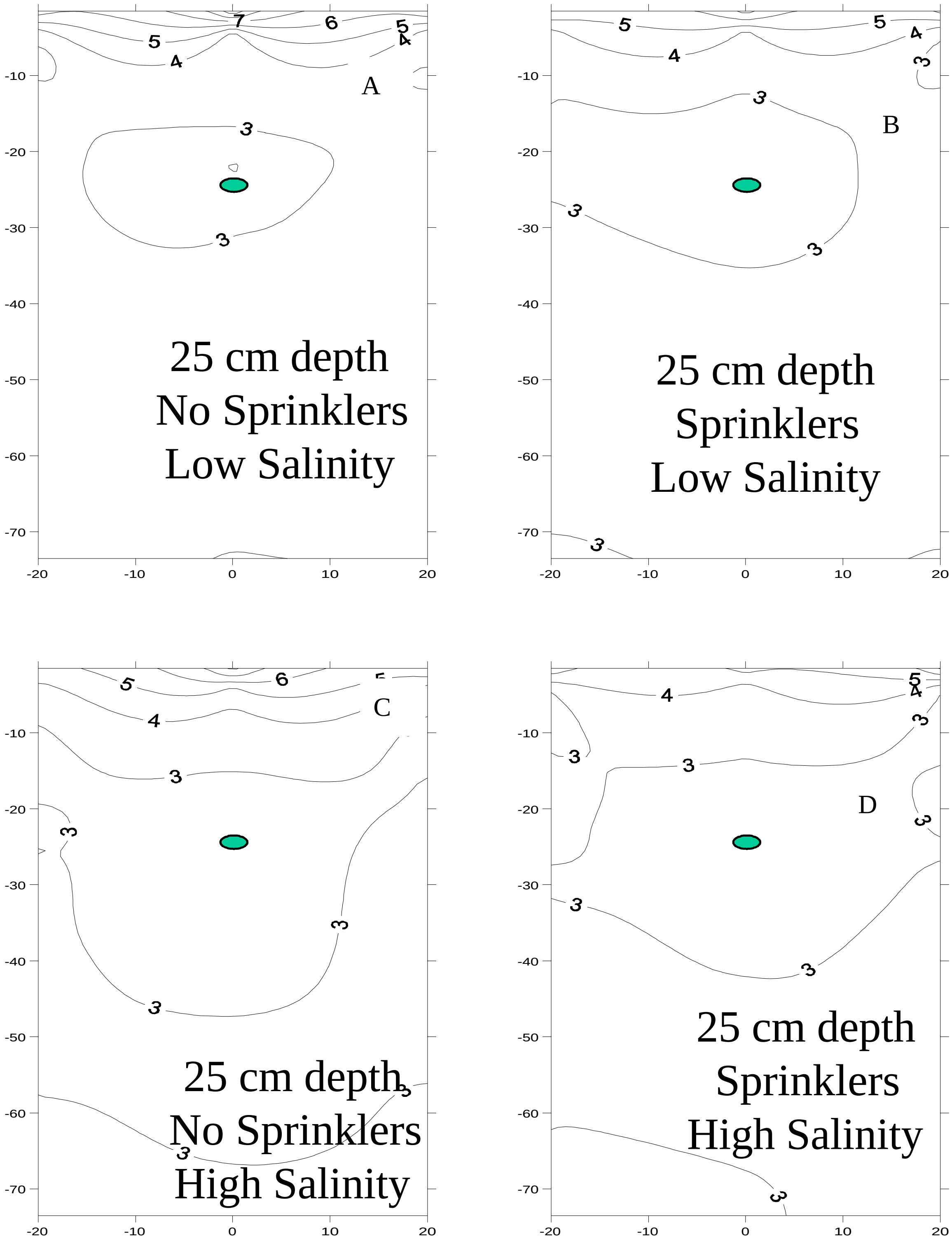

Figure 2 Distribution of electrical conductivity in plots with SDI tubing installed at 25 cm depth.

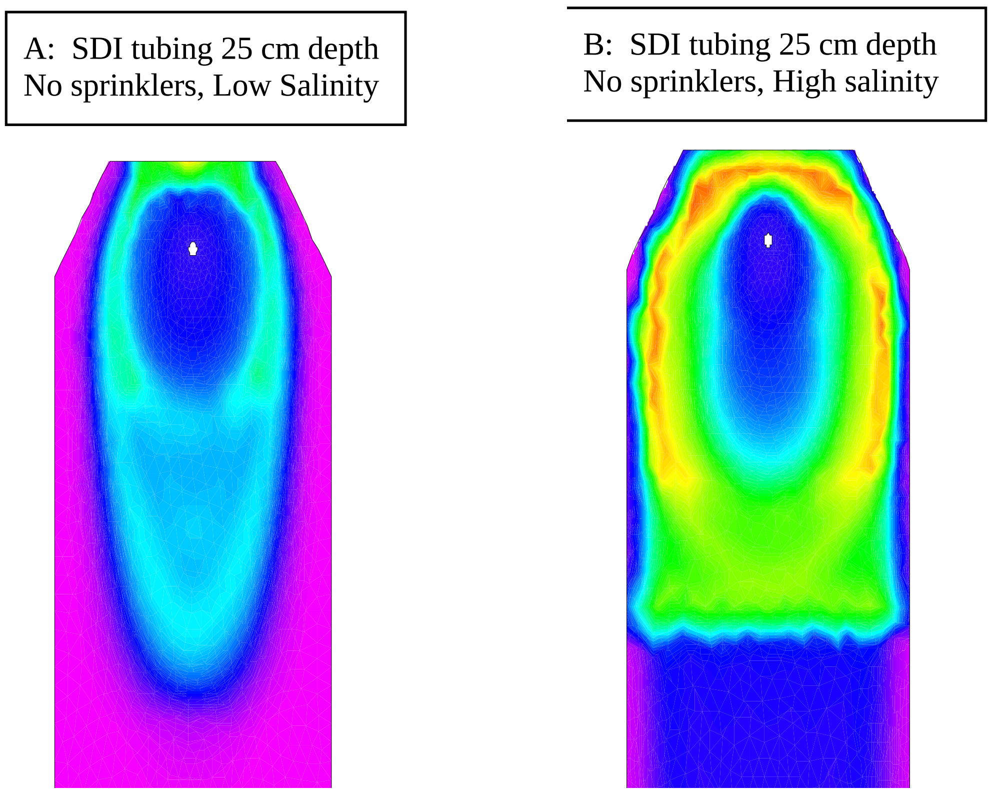

Figure 3. Simulated salt concentration profiles for the end of the first season, generated using HYDRUS 2-D.

Estimation of acute upper lethal water temperature tolerances of native Arizona fishes

Basic Information

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A. Problem and Research Objectives

Streams in the southwestern United States often experience unpredictable fluctuations in their physical conditions (Meffe and Minckley 1987). Deterioration in the quality of streams over the past 100 years has resulted in the decline or complete loss of many unique and ecologically sensitive endemic fish species. Interactions with exotic organisms, introduction of novel diseases, permanent changes in stream velocity and volume, deterioration of water quality, and alteration of habitat have all contributed to declines in abundance and distribution (Lowe et al. 1967; Moyle et al. 1986; Rinne et al. 1986; Douglas et al. 1994). Rising water temperatures have become a common concern, compelling fisheries mangers to evaluate the effects of rising stream temperatures on fish health and survival (Barber et al. 1970; Poole and Berman 2001; Chatterjee et al. 2004). In Arizona, where groundwater and snow melt have been the main drivers of stream temperature, extreme reductions in stream volume combined with loss of riparian vegetation have resulted in the loss of a buffer for temperature fluctuations. Consequently, stream temperatures are more likely to be influenced by the atmospheric temperature trends. Stream temperatures have been rising in Arizona since the early 1900s (Miller 1961), and across North America, incidences of heat death in fishes are becoming more common (Bailey 1955; Zimmerman and Kucera 1977; Matthews et al. 1982; Mundahl 1990). Mortalities related to high water temperatures occur frequently in Arizona, particularly in stagnant shallow waters with high ambient temperatures and direct sun exposure. Stream temperatures ranging from 35-40.3°C are recorded frequently in small Arizona streams during July and August (Deacon and Minckley 1974), and a few native fish species are thought to be living at temperatures close to their upper thermal limits (Lowe and Heath 1969).

Alteration of natural water temperature regimes can create a wide variety of life history, behavioral, and physiological responses in aquatic organisms (Brett 1956; Myrick and Cech 2000; Lass and Spaak 2003), and small changes in water temperature can have considerable consequences for freshwater fish (Morgan et al. 2001). Thermal tolerance in an organism is determined by a wide variety of biotic and abiotic factors, with acclimation temperature and thermal history being among the most important (Chung 2001). A temperature increase beyond the optimal range for any species can influence the capacity to function properly. Elevated temperature can diminish swimming ability in fishes (MacNutt et al 2004) and result in poor body condition by reducing cardiac performance and limiting the amount of available oxygen (Brett 1956; Fry 1967). Temperature can influence metabolic activities and have lasting effects on behavior such as predator avoidance, migration, and spawning.

Although native fishes in Arizona are considered tolerant to high temperature because their evolutionary history is rooted in a desert environment, little is known about the effects of temperature on native fishes in the Southwest. Available information is limited to field observations and a small number of laboratory studies (John 1964; Lowe and Heath 1969; Minckley and Barber 1971; Deacon and Minckley 1974). Few attempts have been made to quantify the upper lethal tolerance of multiple fish species in a single study (Deacon 1987; Smale and Rabeni 1995). Differences in acclimation temperatures, heating rates, undefined behavioral endpoints, and other factors make comparisons among thermal tolerance values derived by a variety of methods ambiguous and difficult to interpret (Lutterschmidt and Hutchison 1997b). Lethal limits typically are comparable only when repeated when tests are run under similar conditions and similar acclimation temperatures are used (Brett 1956).

The objective of this research was to compare the upper thermal tolerance of 11 native and 7 nonnative freshwater fishes found throughout Arizona. Tests were conducted using the critical thermal method.

B. Methodology

Fish Collection

We collected eleven native and eight nonnative fish species (Table 1). Thermal tolerance data existed for the majority of nonnative fishes in our study. However, we retested these species, as it was important to use the same method to compare all species and to ensure that thermal tolerance of a species does not vary geographically. For this reason, we repeated tests for a variety of nonnative species to guarantee we had thermal tolerance data for the populations in this region.

We used small seine nets (1.6 mm) to collect wild fish in spring and summer 2003 and 2004 from Aravaipa Creek, Bonita Creek, the San Pedro River, the Verde River and Buenos Aires National Wildlife Refuge stock tanks. We collected fish of similar total length (30-70 mm) however; fathead minnow *Pimephales promelas*, yellow bullhead *Ameiurus natalis*, bluegill sunfish *Lepomis macrochirus* and green sunfish *Lepomis cyanellus* measured 80-116 mm TL. Gila topminnow *Poeciliopsis occidentalis* and Gila chub *Gila intermedia* were artificially propagated at the University of Arizona. Razorback suckers *Xyrauchen texanus* and bonytail chub *Gila elegans* were obtained from the Willow Beach National Fish Hatchery, Arizona. Spikedace *Meda fulgida* were obtained the Gila River (fish were propagated at the University of New Mexico) and Aravaipa Creek, to test for differences among stocks. We used guidelines provided by Arizona Game and Fish (In review) to transport fish to the laboratory. All species were treated for *Ichthyophthirius* with formaldehyde for at least 10 d upon arrival to the laboratory. Yellow grub *Clinosotnum complanatum* was present in approximately 20% of the Aravaipa Creek spikedace and in the majority of the loach minnow collected at the west end of Aravaipa Creek. We tested the temperature tolerance of these fishes separately to evaluate the effects of yellow grub on thermal tolerance.

When possible, we limited tests to summer so that photoperiod and other variables would be similar among tests. Long photoperiods are typically associated with increased heat tolerance (Fry 1967; Lutterschmidt and Hutchison 1997b). We tested spikedace, loach minnow and desert sucker several times throughout the year to evaluate differences in heat tolerance throughout the year.

Acclimation and Critical Thermal Method

The critical thermal method (CTM), as described by Becker and Genoway (1979) is the most common method used to quantify fish tolerance to extreme high and low temperature. CTM provides a standard for evaluating the thermal requirements of an organism and is often used to make comparison among species (Lutterschmidt and Hutchison 1997b). CTM is a preferred method in the field of thermal ecology due to the small number of animals needed and short period required to complete a test. The use of similar acclimation temperatures and heating rates also facilitates comparisons of data from several studies.

We used 200W Ebo-jager aquarium heaters to maintain ten to twelve fish of each species in well-aerated 75 L glass aquaria at 25°C and 30°C ($\pm$ 0.1°C) for a minimum of 14 d. Due to limited availability, fathead minnow, largemouth bass and yellow bullhead, were tested only at 25°C. Large windows in the laboratory provided natural light cycles. Fish were fed daily to satiation a combination of brine shrimp, daphnia, bloodworms, spirulina and tropical fish food flakes. Diet composition varied by species as it does in nature. Unconsumed food was removed every other day by siphon. Approximately 20% of the tank water was removed 3-4 times each week and replaced with dechlorinated (Stresscoat®) tap water to prevent the accumulation of ammonia, nitrates and nitrites. Small water changes ensured that water temperature fluctuations were less than 1.0°C. Fish were not fed 24 h prior to testing.

We tested four fish of each species per trial. Each fish was randomly selected from the holding tank and placed in a 1-L beaker filled with water from the acclimation tank. Four beakers were placed in a 42 cm x 28 cm x 11 cm metal basin filled with water. Beakers were elevated on a metal grate, which allowed water flow to reach all sides of the beakers. A powerhead (Rio 1100) was placed in the basin to mix the water. Temperature within the basin was maintained at the acclimation temperature for 30 min prior to testing to avoid confusing handling stress with thermal stress. We used portable aerators and air stones to keep test water continually mixed and aerated. Once a testing period commenced, the basin was placed on a Fisher Scientific, 120-V, 5.4-A hotplate and temperature was increased at a constant rate of 0.3°C min^{-1}, a rate of change recommended for small-bodied fish (Beitinger et al. 2000). We adjusted settings on the hotplate at predetermined intervals to ensure a linear rate of change. Rate of change can vary within a given test (0.3 $\pm$ 0.2°C min^{-1}). We tested each species using a minimum of three trials to increase the amount of variation for upper lethal tolerance values of each species, to avoid any variation due to slight differences in rates of change. The rate of change is critical. An ideal rate will allow body temperature to follow water temperature without a significant time lag and avoid overshooting the upper lethal (Becker and Genoway 1979). During each experiment, one person observed the fish while a second person recorded data and maintained correct hotplate settings.

We recorded sub-lethal and lethal endpoints. Sub-lethal endpoints are referred to as CTMax endpoints and defined as the point where an animal loses the ability to escape from conditions that will ultimately lead to its death (Cowles and Bogert 1944). We recorded several commonly cited endpoints including initial loss of equilibrium, no response to prodding, and flaring opercules. Loss of equilibrium, defined as the temperature where a failure of righting occurs, has been the most commonly reported endpoint in CTM trials (Becker and Genoway 1979). We reported initial loss of equilibrium as the CTMax value. However, because of the subjective nature of non-lethal endpoints when comparing multiple species, we also reported upper thermal limit at death. Death was defined as the cessation of opercular movements and was reported as the upper lethal limit. All fish were weighed, measured, examined for parasites, and preserved.

Data Analysis

CTMax and upper lethal temperature values were reported with 95% confidence limits. Multiple regression was used to evaluate the relationship among acclimation temperature, time of year (trial), size (TL), CTMax, and upper lethal temperature. Trial was included in the model, because for some species, individuals were caught and tested at different times throughout the year. Therefore, we tested for effects of time of year on thermal tolerance. Within species, two-sample t-tests were used to assess differences between CTMax and upper lethal temperature at different acclimation temperatures. Analysis was conducted using JMP Version 4.0.4.

Acclimation response ratio (ARR) was calculated for both CTMax and upper lethal tolerance values (Claussen 1977). The acclimated response ratio estimates the ability of fish to alter CTMax values (or other endpoints) with changing acclimation temperature. We estimated ARR values by calculating the difference between endpoint temperatures at each acclimation temperature (i.e. ΔCTM) and then dividing by the differences in the acclimation temperatures ($\Delta T = 5°C$).

C. Principal Findings and Significance

Precision of CTMax Endpoints and Death

The critical thermal method provided a precise method for assessing thermal tolerance of the 18 species tested. The most precise endpoint was flaring opercules (SE = 0.36°C) followed by the upper lethal temperature (SE = 0.41°C) and loss of equilibrium (SE = 0.54°C). Loss of equilibrium, a widely cited endpoint, was not easily observed in some species, and signs of disorientation varied greatly by species.

Effects of Time of Year and Total Length

Total length of fish and time of year did not significantly impact CTMax and upper thermal tolerance values for any species (F- tests $P < 0.05$), except spikedace. Time of year was significant for upper thermal tolerance of spikedace ($P = 0.007$), when we included acclimation temperature and length in our model. Testing was conducted from February through October. No individuals were removed from analyses due to large size or time of year tested.

CTMax and Upper Lethal Tolerance

Upper thermal tolerance values ranged from 41.8 (± 0.2°C) for desert pupfish to 36.0 (± 0.4°C) for speckled dace when acclimated at 25°C (Table 3). Comparison of CTMax and upper lethal tolerance values indicated a strong difference among species (ANOVA *F*-ratio = 133.3, $P < 0.0001$) for both acclimation temperatures. Upper lethal tolerance did not seem to be grouped by taxa. Cyprinids comprised the largest family tested and had the widest tolerance, ranging from the lowest (19^{th}) to the 4^{th} highest tolerance of the eighteen species tested. The most abundant nonnative species found throughout Arizona comprised 5 of the top 8 positions with respect to thermal tolerance, all surviving temperatures close to or above 40°C.

Within species, increasing acclimation temperature increased both the CTMax (two-sided $P < 0.0001$) and upper lethal tolerance (two-sided, $P < 0.0001$). Average mean CTMax and upper lethal tolerance were higher when acclimation temperature was increased from 25°C and 30°C (1.9 ± 0.4°C and 1.5 ± 0.5°C, respectively).

Acclimation Response Ratio

The ability of fish to thermally acclimate varied greatly among species. Increases in thermal tolerance were most extreme within the cyprinid family, varying from 0.3°C (loach minnow) to 2.2°C (longfin dace) (Table 4).

Significance of Results

The CTM provided a precise measurement of upper thermal tolerance for all species tested. The CTM is not a test meant to mimic natural conditions, but to demonstrate relative differences in the ability to withstand high temperature among species. Under natural conditions, stream temperatures rise at a slower rate than the rate used in this study, sometimes taking 12 h to increase 5-10°C. Fish typically are exposed to heterogeneous thermal environments and latency in body temperature change, when exposed to high temperatures, affords the organism time to escape potentially lethal conditions (Beitinger et al. 1977). The CTM utilizes a rapid increase in temperature (i.e. 0.3°C/min), eliminating the opportunity for acclimation to changing temperature. For this reason CTM tests typically overshoot the actual upper lethal temperature by 3-4°C (Selong et al. 2001; Carveth 2004; Widmer 2004). Consequently, our results would not accurately reflect temperatures that these species can withstand in the wild unless temperatures in the wild increase at a similar rate to the one used in this study.

According to Beitinger et al. (2000), loss of equilibrium is an ecologically significant endpoint because this is where a fish losses the ability to escape conditions that will ultimately lead to death. Loss of equilibrium is the most widely cited CTM endpoint (Mundahl 1990; Smale and Rabeni 1995; Benfrey et al. 1997; Currie et al. 1998; Diaz and Buckle 1999; Selong et al. 2001), and, as most fish recover once placed into cooler water, is the most logical endpoint when testing endangered and threatened species.

However, loss of equilibrium was the least statistically precise endpoint of the four endpoints we used. All fish experienced loss of equilibrium, but signs were more subtle in some species, specifically loach minnow and yellow bullhead, which remained on the bottom of the beaker for the majority of the test period. Several species demonstrated obvious disorientation only when prodded with a glass rod. In a similar study conducted by Lutterschmidt and Hutchison (1997a), loss of equilibrium had significantly more variance than another endpoint, the onset of spasms. The species we used did not consistently experience the onset of spasms and this endpoint was not recorded. Reaction to prodding and death were more precise endpoints than loss of equilibrium. However, flaring opercules was the most precise endpoint and most consistent among all species. We recommend that flaring opercules be used as an endpoint where precision is important especially when making comparisons among multiple species.

Body size can influence the upper thermal tolerance of fishes due to either ontogenetic differences in physiology or due to the area: volume ratio. Body temperature in poikilotherms is influences by external changes in temperature and therefore larger organisms may experience a slower rate of heat penetration, affecting upper CTM values (Becker and Genoway 1979). In a study conducted by Smale and Rabeni (1995), several fish species, including a variety of cyprinids, centrarchids, catastomids, ictalurids and poeciliids showed no indication of size-dependent variation in upper lethal tolerance. Barrionuevo and Fernandes (1995) did not find a significant effect of body size when testing the CTMax of curimbata *Prochiludos scofra,* but body size did affect the CTMin. Cox (1974) found a difference in the upper thermal tolerance of bluegill sunfish when temperatures were increased at a rapid rate, but not when temperatures were increased at a slower rate. It is unlikely that temperatures increase at such a fast pace in nature, making it unlikely that size impacts the thermal tolerance of a species in the wild. Although, we attempted to limit the size of the individuals tested, for certain species including fathead minnow, largemouth bass, yellow bullhead, green sunfish and bluegill sunfish larger fish (80-116 mm) were used. Because there was little variation in the size of these individuals, related to the other species tested, it is unknown whether the size of individuals of these species influenced results.

Desert pupfish were the most tolerant species to high temperature, with a CTMax of 41.3 $\pm$ 0.32°C when acclimated to 30°C. Tolerance to extremely high temperature is common among cyprinodons, and these fishes are typically found inhabiting water with high temperatures. Desert pupfish can live at 38.9°C and exhibit discomfort and death at 40.6°C (Deacon and Minckley 1974). Using the same acclimation temperature as in our study, Lowe and Heath (1969) determine the CTMax for desert pupfish to be 42°C (SD = 0.3°C). Speckled dace had the lowest tolerance, with a CTMax of 35.8 $\pm$ 0.57°C. John (1964) reported maximum thermal tolerance of 33°C for speckled dace in the wild, when fluctuations of 10-15°C were present.

In our study, the thermal tolerance of some cyprinid species approached the tolerance of the desert pupfish. Although Brett (1956) concluded that ictalurids have the highest tolerance and cyprinids have intermediate tolerance, we found that thermal tolerance was not grouped by taxa. Thermal tolerance of the cyprinids tested ranged from 35.8°C for speckled dace to 40.5°C for longfin dace when acclimated at 30ºC. Although previously studied, we re-tested several nonnative species to ensure that CTMax values were estimated for fish from this geographic area. Our results were consistent with other studies. Red shiner ranked within the top five of the eighteen species tested in this study and is known to successfully cope with extreme pH, salinity and temperature (Matthews and Hill 1977). King et al. (1985) report that red shiners experience loss of equilibrium at 36.5-38.0°C (SD = 0.41-94) when acclimated at 25°C. Similarly, loss of equilibrium was reported at 39.6°C (SD = 0.23) when red shiners from a Texas population were acclimated at 30°C (Rutledge and Beitinger 1989) (Table 2). The red shiner has become a notorious invader throughout streams in Arizona, and has been cited in the decline of several native species (U.S. Fish and Wildlife Service 1991a; 1991b). Red shiner is able to persist in environments that have been rendered inhospitable for many native fishes (Douglas et al. 1994), and can tolerate thermal shock at high and low temperatures (Matthews and Hill 1977). Data for other nonnative species was consistent with our findings. Currie et al. (1998) reported CTMax values as 36.7 ± 0.59°C (SD) and 38.5 ± 0.34°C, for largemouth bass from Oklahoma acclimated at 25 and 30°C, respectively, at a rate of change of 0.3°C/min. Similar consistencies exist for mosquito fish data presented by Otto (1973), from a population taken from Arizona State University (Table 2). Among the nonnative species tested, geographical variability does not exist when compared to the literature. Similarities in CTMax values indicate that thermal tolerance is consistent for species from different regions. This provides us with some confidence that results from different studies can be compared when similar rates of change are used.

Temperature tolerance acclimation is the process of re-establishing internal homeostasis allowing for survival in heterogeneous thermal environments. Stauffer et al. (1984) demonstrated that cyprinids possess a greater ability to acclimate to changing temperature than do salmonids. Among the eighteen species we studied, there was a large amount of variability in the ability to extend the CTMax and upper lethal limits by acclimation to higher temperatures as shown by the variation in ARR values. A limited ability for temperature tolerance acclimation indicates that the realized tolerance of a species is similar to the fundamental tolerance regardless of the acclimation temperature (Beitinger and Bennett 2000). Red shiner and woundfin *Plagopterus argentissimus* can greatly shift their CTMax in response to acclimation temperature (Deacon et al. 1987). This provides these species with an advantage in warmer, shallower and thermally variable environments. Loach minnow, Gila chub, desert pupfish and speckled dace have a limited ability to extend upper thermal tolerance, compared to Gila topminnow, bluegill sunfish and green sunfish. These data suggest that species with low ARR would be most sensitive to increasing thermal regimes, as these species have little ability to extend their thermal range. If temperatures were increased at a much slower rate than 0.3°C/min, species with high ARR values would likely have the ability to extend their upper thermal tolerance and remain active during periods of rapid temperature increase, permitting escape from stressful situations. Under natural conditions, species with low ARR values may not be able to withstand large daily and annual fluctuations in temperature. For example, loach minnow have been described as widely adaptable to varying physical conditions, including flow and substrate (Rinne 1989). As loach minnow have a limited ability to adapt to high temperature, relative to other species, temperature may be a factor limiting their range. This may also be true for desert pupfish, which has evolved in a stable thermal environment. Desert pupfish from Quitobaquito Springs, Arizona commonly and preferentially occupy waters with temperatures from 40-41°C when water with temperatures of 30°C is immediately available to the fish (Lowe and Heath 1969). These high temperatures are only 1-2 °C below their thermal limit indicating that behavioral thermoregulation is occurring (Hutchison and Manness 1979).

Overall our results indicate that native cyprinids were less tolerant to high temperature than nonnative cyprinids and centrarchids. Evolutionary history was cited as the reason for the success of the Arroyo chub *Gila orcutti* in displacing the native Mojave tui chub *Gila bicolor mohavensis* (Castleberry and Cech 1986). Due to their evolution in fluctuating environmental conditions, including temperature, Arroyo chub are better adapted for dealing with fluctuating environmental conditions in the Mojave River. Changing thermal regimes throughout Arizona may be negatively impacting native species by exposing native fish to temperature fluctuations outside of their tolerance range and favoring some of the more heat tolerant nonnative species, which evolved with larger temperature extremes. Historically, desert streams and rivers experienced small daily and annual temperature fluctuations. Anthropogenic alteration of stream channels and riparian areas have increased the amount of exposed surface area and increased the amount of solar radiation reaching the stream, resulting in substantially elevated water temperatures (Dickerson and Vinyard 1999). Today stream temperatures throughout Arizona regularly reach 34-35ºC in summer and have been as high as 40.3ºC during July and August (Deacon and Minckley 1974). Although surface temperatures may have achieved such high temperatures in the past, water depth provided a buffer with water temperatures at greater depths being much cooler (John 1964; Deacon and Minckley 1974).

Temperature is only one of many abiotic factors that impacts the distribution and survival of native fishes. However, changing thermal environments in Arizona streams and rivers, such as elevated daily and annual mean temperatures, may be adversely impacting native desert fishes. Our results provide baseline data concerning the thermal tolerance of these species. More research is needed to determine the thermal requirements of native and nonnative fish species, specifically to determine interactions occurring among these species at high temperatures.

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Table 1. Fish species, sorted by family, used in critical thermal maximum tests, with total length (mm) and status in Arizona.

Family and Scientific Name	Common Name	Total Length (mm)	Status in Arizona
Cyprinodontidae			
Cyprinodon macularius	Desert Pupfish	27 - 42	Native
Cyprinidae			
Rhinichthys osculus	Speckled Dace	37- 68	Native
Tiaroga cobitis	Loach minnow	34 – 49	Native
Agosia chrysogaster	Longfin Dace	29 – 44	Native
Meda fulgida	Spikedace	34 – 51	Native
Cyprinella lutrensis	Red Shiner	38 – 60	Nonnative
Pimephales promelas	Fathead Minnow	72 - 80	Nonnative
Gila intermedia	Gila Chub	49 - 64	Native
Gila robusta	Roundtail Chub	34 - 51	Native
Gila elegans	Bonytail Chub	24 - 43	Native
Catostomidae			
Catostomus clarki	Desert Sucker	36 – 60	Native
Xyrauchen texanus	Razorback Sucker	44 - 64	Native
Centrachidae			
Lepomis macrochirus	Bluegill Sunfish	92 – 113	Nonnative
Lepomis cyanellus	Green Sunfish	48 - 109	Nonnative
Micropterus salmoides	Largemouth Bass	51 - 79	Nonnative
Peociliidae			
Poeciliopsis occidentalis	Gila Topminnow	25 – 50	Native
Gambusia affinis	Mosquito Fish	26 – 46	Nonnative
Ictaluridae			
Ameiurus natalis	Yellow Bullhead	49 - 116	Nonnative

Table 2. Upper lethal tolerance of selected species found throughout Arizona. Where available, CTM results are presented with loss of equilibrium as the endpoint. Thermal tolerance is presented with a 95% confidence interval unless otherwise noted.

Species	**Method**	**Rate of Change (^{0}C)**	**Acclimation temperature (^{0}C)**	**Upper lethal (^{0}C)**	**Reference**
Pimephales promelas	CTM	0.3	32	40.4 (SD=0.25)	Richards and Beitinger, 1995
Ictalurus punctatus	CTM	0.3	25	38.7 (SD=0.36)	Currie et al. 1998
			30	40.3 (SD=0.29)	
Catostomus latipinnis	CTM	0.24	25	37.0 (SD=0.29)	Deacon et al. 1987
Plagopterus argentissimus	CTM	0.24	25	39.5 (SD=0.21)	
Lepidomeda mollispinis mollispinis	CTM	0.24	25	37.0 (SD=0.44)	
Lepomis cyanellus	CTM	0.017	26	32.7 (SD = 0.75)	Smale and Rabeni, 1995
Lepomis macrochirus	CTM	1.0	25	36.3- 37.0	Holland et al. 1974
			30	37.4-39.6	
Micropterus salmoides	CTM	0.3	25	36.7 (SD = 0.59)	Currie et al. 1998
			30	38.5 (SD = 0.34)	
Cyprinella lutrensis	CTM	0.3	25	36.5 – 38.0 $\pm$ 0.41-0.94	King et al. 1985
			30	39.6 (SD = 0.23)	Rutledge and Beitinger, 1989
Rhynicthys osculus	CTM	0.3	20	32.4 $\pm$ 1.90	Castleberry and Cech, 1992
	CTM	0.24	25	36.8 (SD=0.63)	Deacon et al. 1987
Gambuisa affinis	CTM	0.3	25	39.5	Otto 1973
			30	42.3	
Cyprinodon macularius	CTM	0.5	30	42 (SD 0.30)	Lowe and Heath, 1969

Table 3. Loss of equilibrium temperatures for native and nonnative species found throughout Arizona. Loss of equilibrium was recorded for two acclimation temperatures including 25°C and 30°C. Confidence intervals (95%) are indicated for each value. Species are ranked by CTMax value for an acclimation temperature of 25°C.

Species	**CTMax at 25^{0}C**	**CTMax at 30^{0}C**	**Upper Lethal Tolerance at 25°C**	**Upper Lethal Tolerance at 30°C**
Desert Pupfish	40.0 ± 0.26 °C	41.3 ± 0.32 °C	41.8 ± 0.23 °C	42.7 ± 0.28 °C
Mosquitofish	39.5 ± 0.32 °C	41.4 ± 0.76 °C	40.7 ± 0.22 °C	42.1 ± 0.44 °C
Gila Topminnow	38.4 ± 0.35 °C	41.1 ± 0.35 °C	39.4 ± 0.33 °C	42.1 ± 0.28 °C
Longfin Dace	38.2 ± 0.08 °C	40.5 ± 0.16 °C	38.9 ± 0.14 °C	41.1 ± 0.13 °C
Yellow Bullhead	38.0 ± 0.44 °C	-------	39.8 ± 0.92 °C	------
Largemouth Bass	37.8 ± 0.28 °C	-------	39.1 ± 0.40 °C	------
Red Shiner	37.6 ± 0.62 °C	39.7 ± 0.27 °C	39.5 ± 0.32 °C	40.9 ± 0.23 °C
Green Sunfish	37.4 ± 0.53 °C	40.2 ± 0.50 °C	39.3 ± 0.20°C	41.5 ± 0.21 °C
Bonytail Chub	37.2 ± 0.42 °C	39.0 ± 0.26 °C	38.7 ± 0.36 °C	40.2 ± 0.21 °C
Gila Chub	37.0 ± 0.20 °C	38.1 ± 0.30 °C	38.3 ± 0.24 °C	39.0 ± 0.29 °C
Razorback Sucker	36.7 ± 0.23 °C	39.1 ± 0.33 °C	39.1 ± 0.17 °C	40.3 ± 0.12 °C
Roundtail Chub	36.6 ± 0.11 °C	------	38.0 ± 0.30 °C	------
Fathead Minnow	36.1 ± 1.17 °C	------	36.9 ± 1.07 °C	------
Bluegill Sunfish	35.8 ± 0.38 °C	38.7 ± 0.27 °C	37.3 ± 0.32 °C	39.6 ± 0.19 °C
Loach Minnow	35.3 ± 0.20 °C	36.1 ± 0.32 °C	36.5 ± 0.14 °C	36.8 ± 0.47 °C
Desert Sucker	35.1 ± 0.30 °C	36.7 ± 0.28 °C	36.9 ± 0.16°C	37.6 ± 0.17 °C
Spikedace	34.7 ± 0.48 °C	36.9 ± 0.68 °C	37.0 ± 0.35 °C	39.1 ± 0.35 °C
Speckled Dace	34.4 ± 0.42 °C	35.8 ± 0.57 °C	36.0 ± 0.44 °C	36.9 ± 0.28 °C

Table 4. Acclimation response ratio for all species acclimated at 25 and 30°C ($\Delta T = 5°C$). Acclimation response ratio determined for both CTMax ($\Delta CTM/\Delta T$) and upper lethal tolerance ($\Delta ULT/\Delta T$) values.

Species	ΔCTM	ΔCTM/ ΔT	ΔULT	ΔULT/ΔT
Bluegill Sunfish	2.9°C	0.58	2.3°C	0.46
Green Sunfish	2.8°C	0.56	2.2°C	0.44
Gila topminnow	2.7°C	0.54	2.7°C	0.54
Razorback Sucker	2.4°C	0.48	1.2°C	0.24
Longfin dace	2.3°C	0.46	2.2°C	0.44
Spikedace	2.2°C	0.44	2.1°C	0.42
Red Shiner	2.1°C	0.42	1.4°C	0.28
Mosquito fish	1.9°C	0.38	1.4°C	0.28
Bonytail Chub	1.8°C	0.36	1.5°C	0.30
Desert Sucker	1.6°C	0.32	0.7°C	0.14
Speckled Dace	1.4°C	0.28	0.9°C	0.18
Desert Pupfish	1.3°C	0.26	0.9°C	0.18
Gila Chub	1.1°C	0.22	0.7°C	0.14
Loach minnow	0.8°C	0.16	0.3°C	0.06

Forward and Inverse Transient Analytic Element Models of Groundwater Flow

Basic Information

Title:	Forward and Inverse Transient Analytic Element Models of Groundwater Flow
Project Number:	2004AZ68G
Start Date:	9/1/2004
End Date:	8/31/2006
Funding Source:	104G
Congressional District:	7th
Research Category:	Ground-water Flow and Transport
Focus Category:	Groundwater, Hydrology, Models
Descriptors:	Groundwater flow models, analytic element modeling, steady state and transient flow
Principal Investigators:	Shlomo P. Neuman

Publication

Mr. Kris Kuhlman, a doctoral student in the department of Hydrology and Water Resources, has been appointed Research Associate to work 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. Dr. Hsieh visited us to discuss the project on January 7, 2005.

Work began by conducting a thorough literature survey to establish the state-of-the-art analytic element modeling (AEM) of steady-state and transient groundwater flow problems. We have also conducted a review of numerical inverse Laplace transform methods. The latter review has benefited greatly from a face-to-face discussion of the topic with Dr. Knight, an expert in this field who has been visiting the University of Arizona. It has become clear to us that no numerical inverse Laplace transform algorithm is ideal for all function types we wish to handle, yet the algorithms we have been considering (developed in part by Dr. Knight) should work well for most if not all of these functions.

To date we have implemented steady-state and transient versions of an AEM program with circular inclusions of material having properties other than the background material (circular inhomogeneities). Our purpose in implementing a steady state version was to benchmark our approach against other existing AEM codes such as Tim^{ML}. We explored and developed theoretical approaches for the use of circular and spherical inclusions under steady-state to prescribe hydraulic head and flux at material boundaries. Our current implementation of this idea makes it possible to nest point and circular elements inside circular elements, allowing us to consider finite domains, a capability that standard AEM methods do not possess. We started extending the idea to transient flow and developing a corresponding theory for elliptical and ellipsoidal inclusions.

Our numerical implementation has thus far been done using Matlab, but we plan to convert all our algorithms into the Fortran-90 language. In preparation for this conversion, we have investigated the Fortran implementation of several special functions (Bessel and Mathieu) and of the inverse Laplace transform, all needed for the final working implementation of our Laplace transform based AEM approach.

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

Basic Information

Title:	Pharmaceutically Active Compounds: Fate in Sludges and Biosolids Derived from Wastewater Treatment
Project Number:	2004AZ70G
Start Date:	9/1/2004
End Date:	8/31/2006
Funding Source:	104G
Congressional District:	7th
Research Category:	Water Quality
Focus Category:	Groundwater, Non Point Pollution, Toxic Substances
Descriptors:	Pharmaceutically active compounds, endocrine disrupting compounds, sludge, biosolids
Principal Investigators:	David Matson Quanrud, Robert Arnold, Jon D Chorover, Gail Cordy, Wendell Ela

Publication

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 first 8 months of this study, the following tasks were performed to satisfy project objectives:

1. A preliminary comparison of sample extraction techniques for methods used at University of Arizona (microwave assisted extraction, MAE) and at USGS (accelerated solvent extraction, ASE) has been performed using samples obtained locally from the Ina Road Wastewater Pollution Control Facility, Tucson, AZ. 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, Denver, Colorado.

2. As part of the knowledge transfer objective of the project, two PhD students from the environmental engineering program at the University of Arizona (UA) traveled to Edward Furlong's USGS laboratory in December 2004 for a two-week visit to learn the LC-MS and GC-MS analytical techniques used to measure PhACs and EDCs and then apply these methods on the MAE and ASE sample extracts derived from the Ina Rd sludge/biosolid samples.

3. Three 1-m long stainless steel soil columns were set up at UA containing mixtures of locally obtained agricultural soils and biosolids from the Ina Road plant. Two additional columns are being prepared for additional studies. These columns will be irrigated and leachates collected for analysis of EDCs and PhACs. A master's student in the environmental engineering program at UA is performing this work. Soil samples from experimental plots at the University of Arizona's Marana farm have been collected and archived. These plots have received annual applications of biosolids for the past 20 years. Extraction and analysis of these soils will provide information on the long-term fate of EDCs and PhACs originating from biosolid application.

4. Conference calls among project participants were conducted to

Activities Planned for next six months:

1. A second visit to Edward Furlong's USGS laboratory by the two UA PhD students will take place in summer 2005. Activities will include additional comparison of MAE and ASE sample extraction methodologies and extraction/analyses of sludge/biosolid samples obtained from each of the three wastewater treatment plants included in the study. Sampling points will include raw and digested sludges, dewatered sludges, finished biosolids, as well as liquid phase samples of plant influent and effluent to support mass

balance calculations. Collection and analysis of these samples will be repeated at least four times (quarterly) at each plant during the course of the project.

2. Soil column studies will commence. Column leachates will be analyzed for presence of EDCs and PhACs. Samples of column soils will be extracted and analyzed for the same sets of compounds to determine whether compounds degrade or become unavailable over time.

Information Transfer Program

Information Transfer

Basic Information

Title:	Information Transfer
Project Number:	2004AZ58B
Start Date:	3/1/2004
End Date:	2/28/2005
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, joe gelt, Kathy Jacobs, Jackie Moxley, Kerry Schwartz, Terry Wayne Sprouse

Publication

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Information Transfer

Introduction

The University of Arizona's Water Resources Research Center continues its involvement in water policy research and analysis and in information transfer activities, such as publications, conferences, lectures, seminars, and other formats to inform and educate water professionals, elected and appointed officials, students and the public.

In October 2004, the Center welcomed the addition of Justin Ferris to its ranks as the Coordinator for Applied Research. Dr. Ferris served previously as a Natural Research Council Postdoctoral Fellow with the U.S. Geological Survey in Denver, Colorado.

Outreach and Education

WRRC places great importance on utilizing its experience and expertise to be actively involved in statewide water issues. Water Center staff reaches out to the community through presentations and lectures, service on boards, committees and panels, written articles and research activities. Applied research serves as a foundation for outreach and education.

In particular, Director Sharon Megdal and Katherine Jacobs have both made numerous presentations on topics related to water management, drought planning, environmental restoration, climate and rural water resources issues to audiences ranging from undergraduate classes to keynote addresses at conferences. Dr. Medgal and Ms. Jacobs jointly developed and taught a spring semester 2005 graduate seminar in Arizona Water Policy.

Dr. Megdal made presentations to both state and national groups and organizations. Groups within Arizona that Dr. Megdal addressed included the Arizona Hydrological Society, Pima Association of Governments, League of Arizona Cities and Towns, agribusiness groups and rural watershed organizations. On the national level, she gave presentations at the National Institutes for Water Resources meeting in Washington D.C, the Western Regional Science Association meeting in San Diego and the Universities Council on Water Resources/National Institutes for Water Resources meeting in Oregon. Dr. Megdal presented a paper in the Strategies for Success: Building Public, Private, and Institutional Organizations for Change Panel, at the Second International Symposium on Urban Design in Arid Regions. She also was available to local media appearing in two interviews on KUAT television in Tucson; her column, Public Policy Review, appears regularly in the Arizona Water Resources newsletter. New funding agreements she negotiated include one with the U.S. Army Corps of Engineers, two with the U.S. Bureau of Reclamation, and one with the U.S. Geological Survey. Dr. Megdal became WRRC director on July 1, 2004.

Ms. Jacobs 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. She is project manager on a grant for a major project with the U.S. Bureau of Reclamation on incorporation of climate information into modeling activities associated with managing the Colorado River. Ms. Jacobs serves as chair of the Education and Outreach Committee of the University's Water Sustainability Program, linking four water centers and enhancing connections between university research activities and stakeholders. Ms. Jacobs has been involved in three National Academy panels over the past two years.

Ms. Jacobs gave presentations to the Tucson Water Citizen's Advisory Committee, the Arizona Hydrological Society, the Legislative Education Workshop, Southwest Strategy (a consortium of federal natural resources agencies in Arizona and Nevada), a Bureau of Reclamation-sponsored conference on the Verde Watershed, a drought planning meeting comprised of 31 different organizations, and spoke to the Government Finance Officers of Arizona Summer Training on Colorado River water supply issues of interest to municipalities. She was a panelist on a media briefing which examined impacts of warming on urban water supply and demand, an event covered by television and newspaper reporters. Ms. Jacobs also made a presentation on Decision Maker's Needs for Uncertainty Information to the National Research Council in Washington, DC.

WRRC was instrumental in preparing the background report for the 85th Arizona Town Hall. WRRC co-issued with the University of Arizona Office of Economic Development the report, entitled, "Arizona's Water Future: Challenges and Opportunities." Included among the state and regional issues discussed in the report were: climate variability and water planning; Indian water rights; Colorado River issues, including operation of the Yuma Desalting Plant; water sustainability for rural communities; and environmental issues. The findings and recommendations of the three-day, invitation only, Town Hall forum are already serving as the foundation for the water policy development, legislative proposals, and water management deliberations. Ms. Jacobs was co-author of eight of the 11 chapters and Dr. Megdal co-authored three chapters. This reference document was very well received by Town Hall participants and subsequent recipients.

WRRC researcher, Terry Sprouse continued work on his Fulbright Grant to study bi-national effluent management in Nogales, Sonora and Nogales, Arizona. He presented some of the results of his research at the 2nd International Symposium on Transboundary Waters Management. Sprouse also presented results from a National Park Service water quality study at the conference on Biodiversity and Management of the Madrean Archipelago II.

In addition, Sprouse made two presentations at the Tumacacori National Park Information Exchange Day hosted by USGS and spoke with representatives of the Arizona State Parks on issues of how Mexican effluent in southern Arizona could affect plans to expand state park holdings. Sprouse also represented the WRRC on the International Boundary and Water Commission's Southeast Arizona Citizen's Forum, Board of Directors.

Jackie Moxley, Program Coordinator for the Water Sustainability Program, co-authored the very useful new publication, "Arizona Know Your Water: A Consumer's Guide to Water Sources, Quality Regulations, and Home Water Treatment Options." This handy guide has proven to be very popular with the public. Over 5000 copies have been distributed to County Extension offices, public libraries, state agencies and through individual requests. A downloadable web-based version is also available on the WSP web site www.uawater.arizona.edu.

Brown Bag Seminars

The Center's Brown Bag Luncheon Seminar Series provides a forum for university personnel and other experts from around the state. Included among the presentations in 2004-2005, was the presentation by Bureau of Reclamation Regional Director, Robert Johnson on "Colorado River Issues." This seminar addressed several topics that have been prominent in the news. These issues included drought impacts on the river, proposed operation of the Yuma Desalting Plant, and Mexican water concerns.

Arizona Water Resource Newsletter

The "Arizona Water Resource" newsletter is published six times per year. With a mail circulation of over 2,500 people, the 12-page newsletter focusing on Arizona state and regional water issues is distributed free of charge. Most of its readers are from Arizona, but the newsletter also is sent to 42 states and 14 foreign nations. A feature story, guest view, public policy column and other shorter features are included. Feature articles for the past year addressed issues of special concern to the state including Indian water right settlements, resolution of Central Arizona Project affairs, Arizona-Nevada water banking agreement and efforts to take advantage of the marketing potential of university water research. The newsletter has been providing special coverage of the drought, with news about the development of the state's drought plan along with coverage of other drought related topics. Three news articles and two "Guest Views" have been republished in the Arizona Capital Times.

The newsletter regularly includes supplements from outside agencies. These are four-page inserts that are included within the newsletter. The supplements serve various purposes: 1) they are a cooperative venture between WRRC and outside agencies and as such involve building working relationships between the WRRC and other programs; 2) they provide a service to outside agencies enabling them to publicize their work and research; and 3) the money agencies pay to have the supplement published generally pays for the newsletter. This past year the U.S. Bureau of Reclamation and the UA Water Sustainability Program each published a newsletter supplement; the U.S. Geological Survey provided two supplements.

The WRRC web page provides access to the newsletter and other WRRC papers. It is updated regularly to include presentations made by WRRC faculty, who are in great demand as speakers within Arizona, nationally and internationally. Annual reports from 104B funded research projects and from Water Sustainability Program grants are posted on the web page. The web site includes links to many state and national water related web sites, including the NIWR homepage.

Annual WRRC Water Conference

"The Future of Agricultural Water Use in Arizona" was the title and theme of the UA's WRRC April 28, 2004 conference in Casa Grande. The WRRC and the Department of Agricultural and Resource Economics co-sponsored this well-attended conference (over 250 registrants). Financial sponsors included numerous agricultural and government organizations.

A theme running through the conference was the importance of agriculture in the U.S. and Arizona economies, and its likelihood of maintaining its importance in the future. USDA Assistant Secretary Jim Butler, the opening keynote speaker, announced that U.S. agriculture adds $4 trillion to the nation's economy. Arizona Farm Bureau President Kevin Rogers stated that Arizona agriculture generates more than $6 billion annually to the state's economy, not counting the $1 billion nursery industry that is part of agriculture. Although agricultural activity in some parts of the state is on the decline, it is on the upswing in other areas. Agriculture's bottom line has benefited from productivity improvements. Rogers touted the agricultural gains that have resulted with the application of biotechnology, specifically noting its effectiveness at reducing the input costs for cotton.

Agricultural water supplies are threatened by urban water users who often seek additional water resources at the expense of farmers. Conference speakers decried preferences given to urban and industrial water users over agricultural users. Young farmers' panel member Brian Hogue expressed fears that Tucson will some day seek to tap into water supplies close to his family ranch in Wilcox.

A special feature of this conference was the preparation and dissemination of written materials at the conference, most of which were prepared specifically for the conference by water experts from the UA, USGS, Salt River Project and the Arizona Department of Water Resources. These materials as well as many of the presentations are posted on the WRRC web site. Two speakers from Indian Nations were on the program. The audience for the conference included a broad mix of water users, water professionals, university faculty and staff, elected and appointed public officials, representatives of Indian Nations, and the interested public.

The WRRC newsletter provided both pre-conference information and post-conference results and commentary. A newsletter article written after the 2004 conference presented an exposition and summary of lessons learned from the conference. The article provided a broad sampling of the information presented at the conference, and was specifically directed to the lay reader.

Planning for the 2005 WRRC Conference was done in late 2004 and early 2005. The 2005 conference, held April 6, 2005 in Tucson, covered the prominent topic, “Water and the Environment: The Role of Ecosystem Restoration.” Student participation at WRRC conferences is strongly encouraged. Of the 312 people registered for the hugely successful 2005 conference, 30 were students.

Virtual Water University

Governor Napolitano has proposed the establishment of a virtual water university to combine the resources of the three state universities: UA, Arizona State University and Northern Arizona University. The proposed virtual water university will be without a campus and will unite academic researchers to help focus their work on state water problems. WRRC is involved in preliminary discussions regarding establishment of the virtual water university.

Water Briefing for State Legislators and Public Officials

As part of the University of Arizona’s Water Sustainability Program, the WRRC organized a briefing on water for Arizona Legislators and other invited agency guests. The successful program was held on March 23, 2004 in Phoenix. Educational briefings were provided by Sharon Megdal and Kathy Jacobs. WRRC Project WET Director, Kerry Schwartz, was a member of a panel of experts who provided an overview of their water programs. Dr. Megdal provided an overview of state water issues at a special session of the Navajo County Board of Supervisors. She has been invited to organize a session on water for the annual meeting of the Arizona League of Cities and Towns.

Water EXPO at the Arizona State Capitol

Water Expo - 2005 was an opportunity for Arizona lawmakers to obtain information about projects in the state concerned with water sustainability. Conducted January 25, 2005 on the Senate lawn of the Arizona State Capitol, the event was sponsored by the UA’s Water Sustainability Program, with support from the Central Arizona Project and the Salt River Project. (The Water Resources Research Center along with three other UA water centers make up the Water Sustainability Program.)

A lunchtime program, hosted by Dr. Megdal, featured Arizona Governor Janet Napolitano and prominent speakers from the water community. Forty-six legislators as well as a number of legislative staff members attended the event. The 40 exhibitors participating in the event included the Agri-Business Council of Arizona, U.S. Bureau of Reclamation, County Graham Cooperative Extension, Northern Arizona University Fossil Creek Initiative and various cities throughout the state, from Flagstaff to Tucson.

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 other National Science Foundation funded water centers. WSP's origin was 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 six other UA initiatives. The new funding was designated as the Technology and Research Initiative Fund (TRIF).

WSP, now in the fourth 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 a grants and fellowship program, and will continue to do so. In this role, WRRC personnel interact with faculty and staff throughout the University of Arizona, including Cooperative Extension offices. Expectations are that the program will continue to be funded by the Arizona Board of Regents after the first five-year funding cycle ends on June 30, 2006. This will allow the four centers and the large UA water community to continue to expand its water resources research, education and outreach.

A key component of the WSP is the competitive grants program. Thirty-one projects have been selected in two cycles of the competitive grants program through an expert review process and have received a total of $2.2 million. About 70 faculty and staff from 22 departments/schools/units, across five colleges are working on these projects in cross-disciplinary collaborations. Over 80 student opportunities through paid positions or research assistantships have been created. Over 75 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 close to $600,000 in direct dollar support from off-campus sponsors.

In 2004, the TRIF WSP competitive grants program funded 11 new proposals and 16 continuing multi-year projects, for total funding of $1.2 million. In 2005, eight new projects were selected, for a total of $1.1 million in funding for both new and on-going multi-year projects. Each project funded through the TRIF WSP competitive grant process is associated with one of the four University of Arizona water centers. More information on the grants can be found at www.uawater.arizona.edu. The following projects were hosted by the WRRC, with the date of award in parenthesis.

1. Arizona: Know Your Water, A Consumer's Guide to Water Sources, Quality, Regulations, and Home Water Treatment Options (2003). Dr. Janick Artiola, Department of Soil, Water, and Environmental Science; Dr. Kathryn Farrell-Poe, Department of Agricultural and Biosystems Engineering; Ms. Jackie Moxley, Water Resources Research Center.
2. Arizona Water and Pesticide Safety CD (2003). Mr. Louis Carlo, Department of Entomology and Cooperative Extension; Dr. Paul Baker, Department of Entomology and Cooperative Extension.
3. Evaluation of M&I Water Conservation Measures Through Actual Water Savings And Cost Benefit Analysis (2003). Ms. Val Little, Drachman Institute;
4. Tailored Drought Research and Educational Outreach for Arizona (2003). Dr. Greg Garfin, Institute for the Study of Planet Earth; Dr. Barbara Morehouse, Institute for the Study of Planet Earth/Department of Geography and Regional Development; Dr. Andrew Comrie, Department of Geography and Regional Development; Dr. Sharon Megdal, Water Resources Research Center; Ms. Katharine Jacobs, Water Resources Research Center; and Dr. Donald Wilhite, National Drought Mitigation Center.
5. Improved Turf and Landscape Irrigation Management for Northern Arizona (2003). Dr. Paul Brown, Department of Soil, Water and Environmental Science; Dr. Peter Waller, Department of Agricultural and Biosystems Engineering; Ms. Abigail Roanhorse, Department of Agricultural and Biosystems Engineering.
6. The Water Wagon: A Mobile Laboratory and Education Center (2003). Dr. Randall Norton, Graham County Cooperative Extension; Dr. Lee Clark, Safford Agricultural Center; Ms. Sue Martin, Graham County Cooperative Extension; Ms. Jonie Burge, Safford Agricultural Center.
7. Spanish Language Landscape Water Conservation Program for the Green Industry (2004). Ms. Vicki Richards, Pima County Cooperative Extension.
8. Early Termination of Cotton as a Drought Mitigation Strategy (2004). Dr. Russell Tronstad, Department of Agricultural & Resource Economics; Dr. Jeffrey C. Silvertooth, Department of Soil, Water and Environmental Science.
9. Enhancing Water Supply Reliability through Improved Predictive Capacity and Response (2004). Ms. Kathy Jacobs, Water Resources Research Center; Dr. Bonnie Colby, Agricultural and Resource Economics; Dr. David Meko, Laboratory of Tree Ring Research; Dr. Bart Nijssen, Department of Hydrology and Water Resources/Civil Engineering.

10. The Value of Binational Effluent and Sustainable Watershed Management in the Upper Santa Cruz Basin (2005). Dr. Terry Sprouse, Water Resources Research Center; Dr. George Frisvold, Department of Agricultural and Resource Economics.
11. Arizona Project Wet Evaluation: Examining Impact And Developing Water Education Assessment Tools For Students (2005). Dr. Jerome D'Agostino, Department of Educational Psychology; Kerry Schwartz, Water Resources Research Center.
12. Promoting The Adoption Of Subsurface Drip Irrigation By Arizona's Farmers (2005). Dr. Thomas L. Thompson, Department of Soil, Water, and Environmental Science; Dr. Edward Martin, Department of Agricultural and Biosystems Engineering; Patrick Clay, Maricopa County Cooperative Extension; Dr. Mary Olsen, Department of Plant Pathology; Dr. Russell Tronstad, Department of Agricultural and Resource Economic; Dr. James Walworth, Department of Soil, Water, and Environmental 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 good solid pedagogy has been developed in writing workshops involving 350 teachers and resource specialists working together. The lessons are interactive, multidisciplinary, research-based, constructivist and promote critical thinking.

Presently WRRC has two full time Water Education Coordinators, one housed at the WRRC in Tucson, and a second housed at Maricopa County Cooperative Extension in Phoenix and funded with TRIF funds. The two water coordinators from WRRC train facilitators who, in turn, train educators in one- to two-day workshops all over the state. 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. With a combined effort from coordinators and facilitators 753 teacher/educators were trained in 42 workshops affecting a reported 36,650 students (with 234 educators not having reported the number of students reached) during the reporting period.

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 4th grade students and their teachers and are held on a school day. Content is in accordance with the 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 five locations reaching 2,700 students, 102 teachers, 30 schools and 704 volunteers in 2004. The addition of a Program Coordinator allowed a Water Festival to be held in more places including Ganado on the Navajo Nation, where 500 Navajo students from five area schools were reached.

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, Salt River Project and Central Arizona Project as well as other local sponsors resulted in a budget of $27,900 for 2004 Water Festivals. In-kind donations for 2004 from over 40 organizations totaled $82,884.

Professor Aaron Wolf to join WRRC as Associate Director

Recruitment began during the reporting period for a faculty member to be jointly funded for a period of time by the WRRC (using TRIF funds) and the Department of Geography. Professor Aaron Wolf will join the University of Arizona in August 2006 as Professor of Geography, with tenure, and Associate Director, WRRC. Professor Wolf comes to the WRRC from Oregon State University, Department of Geosciences, where his research focused on Transboundary Water Conflicts and Conflict Resolution, Water Basin Technical and Policy Analysis, and Environmental Policy Analysis. He was also instrumental in developing an interdisciplinary masters-level curriculum in water resources management at Oregon State. The WRRC looks forward to having Professor Wolf's enthusiasm and experience at the Water Center.

WRRC Becomes Research/Extension Unit

In recognition of its research and extension programs, the WRRC officially became a Research/Extension unit of the College of Agriculture and Life Sciences. It previously had been classified as an administrative unit. Prior to that, it had been located within the Department of Soil, Water and Environmental Sciences. The WRRC Director, who reports directly to the Dean of the College of Agriculture and Life Sciences, is a Specialist with Cooperative Extension and a Professor with the Department of Agricultural and Resource Economics.

Student Support

Student Support					
Category	**Section 104 Base Grant**	**Section 104 RCGP Award**	**NIWR-USGS Internship**	**Supplemental Awards**	**Total**
Undergraduate	12	0	0	0	12
Masters	10	0	0	0	10
Ph.D.	0	0	0	0	0
Post-Doc.	0	0	0	0	0
Total	22	0	0	0	22

Notable Awards and Achievements

Sharon Megdal presided over a successful and well attended University of Arizona-sponsored Water Expo for state legislators and public officials. This was an opportunity for Arizona lawmakers to obtain information about projects in the state concerned with water sustainability. Conducted January 25, 2005 on the Senate lawn of the Arizona State Capitol, the event was sponsored by the University of Arizonas Water Sustainability Program, with support from the Central Arizona Project and the Salt River Project. (The Water Resources Research Center along with three other UA water centers make up the Water Sustainability Program.). A lunchtime program, hosted by WRRC Director Megdal, included Governor Janet Napolitanoand other prominent speakers from the water community.

The annual statewide WRRC water conference was held on April 28, 2004 on the important topic of the future of agricultural water use in Arizona. Over 250 people attended the conference.

Kathy Jacobs chaired the National Research Council Panel Reviewing the GAPP Water Cycle Research Program. Jacobs has been involved in three National Academy panels over the past two years.

Kathy Jacobs was a lead staff person in developing Arizonas first drought plan in cooperation with the Arizona Department of Water Resources and the Climate Assessment for the Southwest.

Sharon Medal and Kathy Jacobs are team-teaching a graduate level course at the University of Arizona on Arizona Water Policy.

WRRC was instrumental in preparing the background report for the 85th Arizona Town Hall. WRRC co-issued with the University of Arizona Office of Economic Development the report, entitled, Arizonas Water Future: Challenges and Opportunities. The findings and recommendations of the three-day, invitation only, Town Hall forum are already serving as the foundation for the water policy development, legislative proposals, and water management deliberations. Ms. Jacobs was co-author of eight of the 11 chapters and Dr. Megdal co-authored three chapters.

Kerry Schwartz serves on the Project WET USA Council, and served on the leadership team for the newly published (April 2004) educators guide, Discover a Watershed The Colorado.

Kerry Schwartz, after five years of success conducting water education festivals, was recognized for exceptional work in the field of water conservation by the U.S. Bureau of Reclamation in 2004.

Kerry Schwartz was elected a Board Member 2005-2006 for the Arizona Association for Environmental Education.

Joe Gelt had three of his Arizona Water Resource newsletter articles republished in the Arizona Capital Times.

Jackie Moxley co-authored the highly-successful publication, Arizona Know Your Water, distributed to 5000 persons.

Terry Sprouse received a certificate of Official Recognition and Appreciation from the International Boundary and Water Commission for his on-going work with the Southeast Citizens Forum Board.

Terry Sprouse continued his study of effluent utilization on the U.S. Mexico border with support from a Fulbright Grant.

As part of the University of Arizonas Water Sustainability Program, the WRRC organized a briefing on water for Arizona Legislators and other invited agency guests. The successful program was held on March 23, 2004 in Phoenix. Educational briefings were provided by Sharon Megdal and Kathy Jacobs. WRRC Project WET Director, Kerry Schwartz, was a member of a panel of experts who provided an overview of their water programs. Dr. Megdal provided an overview of state water issues at a special session of the Navajo County Board of Supervisors. She has been invited to organize a session on water for the annual meeting of the Arizona League of Cities and Towns.

WRRC officially became a Research/Extension unit of the College of Agriculture and Life Sciences. It previously had been classified as an administrative unit.

Publications from Prior Projects

1. 1999AZ5B ("Physical Effects of Flood Flows on Seedling Growth and Survivorship: Comparative Responses of Native Riparian Trees and Shrubs to Saltcedar") - Articles in Refereed Scientific Journals - Levine, C. M. & J. C. Stromberg, (2001), Effects of flooding on native and exotic plant seedlings: implications for restoring southwestern riparian forests by manipulating water and sediment flows, Journal of Arid Environments 49:111-131.
2. 1999AZ5B ("Physical Effects of Flood Flows on Seedling Growth and Survivorship: Comparative Responses of Native Riparian Trees and Shrubs to Saltcedar") - Dissertations - Levine, C., (2001), Physical effects of flooding on native and exotic plant seedlings: implications for restoring riparian forests by manipulating flow regimes, M. S. Thesis, Arizona State University, Tempe.
3. 2000AZ03B ("Multiobjective Optimization of a Public Wellfield using a Neural Network and Non-Linear Programming") - Dissertations - Coppola, Emery A., Jr., (2000). Optimal Pumping Policy for a Public Supply Wellfield using Computational Neural Network with Decision-Making Methodology, Ph,D. Dissertation Hydrology, University of Arizona, Tucson.

4. 2000AZ05B ("Field Studies of Virus Transport Through Unsaturated Alluvium and Fractured Rock") - Dissertations - Blanford, William James, (2001) Characterization of Remediation of Pathogen, Solvent, and Petroleum Contaminated Aquifers, Ph,D. Dissertation, Hydrology, University of Arizona, Tucson.
5. 2000AZ5B ("Field Studies of Virus Transport Through Unsaturated Alluvium and Fractured Rock") - Articles in Refereed Scientific Journals - Blanford, W. J., Brusseau, M. L., Yeh, T. C. J., Gerba, C. P., and Harvey, R. Influence of Water Chemistry and Travel Distance on Bacteriophage PRD-1 Transport in a Sandy Aquifer. Water Research, in press.
6. 2001AZ901B ("Measurement of Estrogenic Activity And Volume Contribution of Treated Wastewater In Water from Wells along the Santa Cruz River") - Dissertations - Quast, Konrad William, (2003) Boron Isotopes as Intrinsic and Artificial Hydrologic Tracers, Ph.D. Dissertation, Hydrology, University of Arizona, Tucson
7. 2001AZ982B ("New Approaches to Addressing Tribal Water Rights") - Other Publications - Colby, B., J. E. Thorson and S. Britton, (2005), Negotiating Tribal Water Rights, Fulfilling Promises in the Arid West, (Tucson: University of Arizona Press).
8. 2002AZ3B ("Microbial Mediated Mobilization of Arsenic from Drinking Water Treatment Residuals in Landfills") - Articles in Refereed Scientific Journals - Field, J.A., R. Sierra-Alvarez, I. Cortinas, G. Feijoo, M. T. Moreira, M. Kopplin and A. J. Gandolfi, (2004), Facile reduction of arsenate in methanogenic sludge, Biodegradation 15:185-196.
9. 2002AZ3B ("Microbial Mediated Mobilization of Arsenic from Drinking Water Treatment Residuals in Landfills") - Articles in Refereed Scientific Journals - Sierra-Alvarez R., Field, J. A., Cortinas, I., Feijoo, G., Moreira, M. T., Kopplin, M., Gandolfi, and A. J., (2005), Anaerobic microbial mobilization and biotransformation of arsenate adsorbed onto activated alumina. Water Res. 39(1):199-209.
10. 2002AZ3B ("Microbial Mediated Mobilization of Arsenic from Drinking Water Treatment Residuals in Landfills") - Articles in Refereed Scientific Journals - Sierra-Alvarez, R., Cortinas, I., Yenal, U. and Field, J. A, (2004), Methanogenic inhibition by arsenic compounds, Appl. Environ. Microbiol. 70:5688-5691
11. 2002AZ4B ("The effect of mycorrhizae on competitive ability and drought tolerance of cottonwood (Populus fremontii) and saltcedar (Tamarix ramosissima)") - Articles in Refereed Scientific Journals - Beauchamp, V.B., J.C. Stromberg and J.C. Stutz, Interactions between Tamarix ramosissima (saltcedar), Populus fremontii (cottonwood), and mycorrhizal fungi: effects on seedling growth and plant species coexistence, Plant and Soil, in press.
12. 2002AZ4B ("The effect of mycorrhizae on competitive ability and drought tolerance of cottonwood (Populus fremontii) and saltcedar (Tamarix ramosissima)") - Dissertations - Beauchamp, Vanessa B, (2004), Effects of flow regulation on a Sonoran riparian ecosystem, Verde River, Arizona, Ph.D. Dissertation, Arizona State University, Tempe.
13. 2002AZ4B ("The effect of mycorrhizae on competitive ability and drought tolerance of cottonwood (Populus fremontii) and saltcedar (Tamarix ramosissima)") - Other Publications - Beauchamp, V.B., Stromberg, J.C. and J.C. Stutz, (2004), Interactions between Tamarix ramosissima (saltcedar), Populus fremontii (cottonwood), and arbuscular mycorrhizal fungi: effects on seedling growth and plant species coexistence. Poster presentation. Ecological Society of America conference, Portland, OR, August 1-6, 2004.
14. 2002AZ4B ("The effect of mycorrhizae on competitive ability and drought tolerance of cottonwood (Populus fremontii) and saltcedar (Tamarix ramosissima)") - Other Publications - Beauchamp, V.B. and J.C. Stutz, (2003), (Arbuscular Mycorrhizal Fungal and Dark Septate Endophyte Interactions with Saltcedar (Tamarix ramosissima)). Poster presentation. Fourth International Conference on Mycorrhizae, Montreal, Canada, August 10-15, 2003.

15. 2003AZ15B ("Selection of High Performance Microalgae fro Bioremediation of Nitrate-Contaminated Groundwater") - Other Publications - Case, N. M. Sommerfeld, and Q. Hu, (2005), Utilizing microalgae to remediate nitrate-contaminated groundwater, The 7th Annual Poster Symposium of Central Arizona-Phoenix Long-Term Ecological Research (CAP LTER), January 19, Arizona State University.
16. 2003AZ12B ("Attenuation of Estrogenic Activity in Reclaimed Water and Stormwater During Impoindment in Natural Systems") - Conference Proceedings - Quanrud, D., Bjolseth, I.M., Karpiscak, M., Ela, W., Lansey, K., and Arnold, R., (2004), Fate of estrogenic activity in constructed wetlands receiving wastewater effluent, Proceedings, Seventeenth Annual Symposium of the Arizona Hydrological Society, Tucson, AZ, September 15-18.
17. 2003AZ19B ("Integrating Research and Education to Assist Watershed Initiatives: A Survey of Three Arizona Watershed Organizations") - Other Publications - Browning-Aiken, A. J.E. de Steiguer, and D. Young. 2004. Integrating Research and Education to Assist Watershed Initiatives: A Survey of Three Arizona Watershed Organizations. Tucson, AZ: Udall Center for Studies in Public Policy, University of Arizona. 31 pp.