Penn State Institutes of the Environment
Annual Technical Report
FY 2003

Introduction

This report summarizes the water research and information transfer activity supported by or administered through the Pennsylvania Water Resources Research Center during FY 2003. The PA WRRC is located at Penn State University and receives funding from an annual base grant program and occasional grants awarded through a national competitive grants program. Priority in funding in the annual base grant program is given to projects that involve new and innovative research ideas, participation by undergraduate and graduate students, and participation by faculty representing a wide range of educational institutions across the state. The FY 2003 base grant funding was used to support three exploratory research projects and one information transfer program (Sharpe and Swistock). In addition, one competitive grant research project (Stolz) was also administered through PA WRRC during FY 2003. Projects supported were:

Using Crumb Rubber Filtration for Ballast Water Treatment by Dr. Yuefeng Xie at Penn State University, Capital Campus

Development of Passive Treatment System Monitoring and Research Program by Dr. Fred Brenner, Margaret Dunn, and Shaun Busler with Grove City College and Stream Restoration Inc.

Spruce Creek Watershed Keystone Project by Lysle Sherwin and Charles Cole with Penn State University, University Park Campus.

Water Conservation Training and Public Education by Dr. William E. Sharpe and Bryan Swistock with Penn State University, University Park Campus.

Enrichment, Biochemical, and Molecular Techniques for Assessing Microbial Arsenic Reduction by Dr. John Stolz at Duquesne University.

Research Program

Research projects funded through the Pennsylvania Water Resources Research Center during FY 2003 largely dealt with high-priority water quality issues in our state and region. Research conducted by Dr. Yuefeng Xie was directed at improving water quality of ship ballast water through the use of an innovative crumb-rubber filtration system that uses ground waste rubber tires. Dr. Fred Brenner and colleagues studied the performance of various types of passive treatment systems used to reduce pollution from abandoned coal mines in western Pennsylvania. Dr. John Stolz investigated new laboratory methods for studying the microbial reduction of arsenate compounds that renders arsenic soluble in water. Finally, a continuing project on Spruce Creek in central Pennsylvania conducted by Lysle Sherwin and Andrew Cole is directed at monitoring, modeling, and managing the impact of agricultural and suburban land uses on water quality. All of these projects deal with water quality issues that are of significance to Pennsylvania and regions beyond.

Using Crumb Rubber Filtraton for Ballast Water Treatment

Basic Information

Title:	Using Crumb Rubber Filtraton for Ballast Water Treatment
Project Number:	2003PA11B
Start Date:	3/1/2003
End Date:	2/28/2004
Funding Source:	104B
Congressional District:	17th of PA
Research Category:	Engineering
Focus Category:	Treatment, Ecology, Water Quality
Descriptors:	ballast water treatment, crumb rubber medium, invasive species, waste tires, water filtration
Principal Investigators:	Yuefeng Xie, Hsin-hsin Tung

Publication

1. Chen, Peng, 2004, Ballast Water Treatment Using Crumb Rubber Filtration, Environmental Pollution Control Program, Penn State Harrisburg, Middletown, PA, 65.
2. Xie, Yuefeng; Peng Chen, 2004, Crumb Rubber Filtration for Ballast Water Treatment: A Preliminary Study, in Proceedings of the 2nd International Ballast Water Management Conference and Exhibition, Singapore.

Abstract:

Ballast water is an important way of transferring aquatic nuisance species (ANS) all over the world. Due to the lack of natural predators in the new environment, they are often able to thrive and outgrow the native species. Their domination can cause massive ecological, economic, and public health problems. Currently the only ballast water treatment option is mid-ocean ballast water exchange. Due to the safety concern and low efficiency, alternative options such as filtration, ultraviolet irradiation, biocides/chemicals, and heat treatment are under study. Crumb rubber, a scrap tire-derived material, is an innovative filter medium that can be used to treat ballast water. Millions of scrap tires are generated in the United States each year. These discarded tires present both health and environmental problems.

A side-by-side comparison between a crumb rubber filter and a sand/anthracite filter was conducted with water from Swatara Creek at a local water treatment plant. Experiments were conducted with different filtration rates and with/without polyaluminum chloride. The filter performance was evaluated by head loss, turbidity removal, particle removal, zooplankton and phytoplankton removal. The backwash performance was also studied.

The results show that the performance of the crumb rubber filter for turbidity, suspended particle, phytoplankton, and zooplankton removal was similar to that of the sand/anthracite filter. However, the crumb rubber filter allowed a greater filtration rate and longer filter run. Both filters were effective in removing larger particles (>10 μm). Adding 10 mg/L polyaluminum chloride did not improve the removal of large particles (>10 μm) but did significantly increase the filter head loss and reduced the filter run time.

The crumb rubber filtration is a potential shipboard technology for ballast water treatment because of its low head loss, high filtration rate, light-medium weight, and low expensive. Further study should be conducted at much higher filtration rates to investigate the removal of large particles (>10 μm and >50 μm) in ballast water. Our group has been awarded a grant by National Oceanic and Atmospheric Administration to investigate the crumb rubber filtration for ballast water treatment in a pilot scale study.

Statement of Critical Need:

Ballast water is an important way of transferring aquatic nuisance species (ANS) all over the world. ANS include algae, shellfish, developing larvae, eggs and other microorganisms. Due to the lack of natural predators in the new environment, they are often able to thrive and outgrow the native species. This domination can cause massive ecological, economic, and public health problems such as degradation of habitat, alteration of water quality, blockage of flow in drainage and irrigation canals, or even transition of diseases to humans. One of the most notorious ANS is the Zebra Mussel, which caused great damage in the Great Lakes. The International Maritime Organization (IMO) regards the introduction of harmful aquatic organisms and pathogens to new environments via ballast water, as one of the four greatest threats to the world's oceans.

Currently, ballast water exchange is the only method which is practiced to reduce the risk of ballast-mediated invasion. Ballast water exchange involves replacing coastal water with open-ocean water during a voyage. This process reduces the density of coastal organisms in ballast tanks that may be able to invade a recipient port, replacing them with oceanic organisms with a lower probability of survival in near shore waters. However, conducting ballast water exchange at open sea puts many ships at risk especially in bad weather conditions. Therefore, less than 50% of ships are practicing ballast water exchange. In addition, ballast water exchange achieves only 65 to 95% effectiveness in the exchange of the original ballast water. Conventional sand/anthracite water filtration is an effective process for removing organisms. However, it is not economically and technologically feasible for ballast water treatment because of its heavy weight, low filtration rate, and large space requirement. A cost effective treatment technology is needed for ballast water treatment.

Crumb rubber, a scrap tire-derived material, is an innovative filter medium. Millions of scrap tires are generated in the United States each year. These discarded tires present both health and environmental problems. Scrap-tire reuse or recycling is an effective way to reduce these problems. Crumb rubber is currently used in highway pavement, athletic track surfaces, landfill lines, compost bulking agents, energy recovery and artificial reefs for aquatic life. As a filter medium, crumb rubber allows the porosity of the filter bed to decrease through the filter, resulting in the smallest pore size at the bottom and largest pore size on the top, which is ideal for down-flow filtration. The crumb rubber filter allows greater depth filtration and it then allows a greater filtration rate. In addition, the relatively low density of crumb rubber allows a lower filter back wash rate and simple back-wash procedure. The significantly light weight of crumb rubber filters make this an ideal on-board treatment technology for ballast water filtration.

Statement of Results of Benefits:

The proposed project will demonstrate a treatment technology for ballast water treatment that can be used to protect the precious coastal water resources in Pennsylvania and the nation. Because of its high filtration rate and light weight, the crumb-rubber filter could be used as an in-vessel treatment facility in cargo ships or cruise ships. The crumb-rubber filters could also be mounted on a barge as a mobile treatment unit. For land based treatment facilities, using crumb rubber filtration could significantly reduce the land requirements and the capital and operational cost for ballast water treatment. This technology could also be developed for storm water and combined effluent treatment. The use of the crumb rubber will also minimize the waste tire piles and promote green technology concepts.

Nature, Scope and Objectives:

This study was a preliminary study on the use of the crumb rubber filtration for ballast water treatment. A side-by-side comparison of the crumb rubber filter and sand/anthracite filter was conducted using a fresh water source. The objective of this

preliminary study was to explore the use of crumb rubber filtration in ballast water treatment. The filtration efficiency was evaluated based on head losses, removal of turbidity, particles, zooplankton, phytoplankton and heterotrophic bacteria.

Methods, Procedures and Facilities:

Pilot filters

Two 9 feet × 2 inches (i.d.) transparent polyvinyl chloride (PVC) columns were built and installed in a local water treatment plant. The sand/anthracite filter contained 1 ft of gravel as support media, 1 ft of sand, and 2 ft of anthracite. The crumb rubber filter contained 1 ft of gravel as support media and 3 ft of crumb rubber medium. The filtration rate was controlled by a rotameter installed in the filter outlet pipe. The water from Swatara Creek was taken as ballast water.

Analytical Methods

1. Head loss was measured using a static pressure indicator. The head loss was expressed in inches.
2. Turbidity was measured according to Standard Method 2130 with a Hach 2100p turbidimeter. The level of turbidity was expressed as nephelometric turbidity units (NTU).
3. Particles (size and number) were measured according to Standard Method 2560 using a Hach 2200 PCX particle counter. The particle counting was expressed as number per 100mL.
4. Phytoplankton was measured according to Standard Method 10200 F. A SPENCER microscope with a magnification of 100 and a Sedgwich-Rafter (S-R) counting chamber were used for phytoplankton counting. Two categories of phytoplankton based on sizes (less than 10 μm and larger than 10μm) were identified and counted. The phytoplankton was expressed as number per mL sample.
5. Zooplankton was measured according to Standard Method 10200 G. Each sample was concentrated from 100mL to 10mL using the method suggested by the standard method. The zooplankton was expressed as number per mL sample.

Principal Findings and Significance:

1. Both the sand/anthracite and crumb rubber filters were effective in removing large particles (>10 μm and >15 μm). For the sand/anthracite filter, the removal efficiencies for particles larger than 10 μm and 15 μm were 89.4% and 94.5%, respectively. For the crumb rubber filter, the removal efficiencies for particles larger than 10 μm and 15 μm were 86.8% and 93.6%, respectively.
2. Both the sand/anthracite and crumb rubber filters resulted in a moderate reduction in small particles (>2 μm) and turbidity. For the sand/anthracite filter, the removal efficiencies for small particles (>2 μm) and turbidity were 43.5% and 51.7%, respectively. For the crumb rubber filter, the removal efficiencies for small particles (>2 μm) and turbidity were 23.6% and 47.6%, respectively.
3. Adding 10 mg/L of polymerized aluminum chloride (PACl) significantly enhanced the removal of small particles (50.0%) and turbidity (70.8%) in crumb

rubber filter and sand/anthracite filter (64.3% for small particles and 74.8% for turbidity). However, adding the coagulant did not significantly enhance the removal of large particles (>10 μm and >15 μm).

4. Adding the coagulant dramatically increased the filter head losses and shortened filter runs for both filters. For the crumb rubber filter, adding the coagulant reduced the filter run time at 16 gpm/ft^2 from 24 hours to 6 hours.
5. For particle and turbidity removal, there was no significant difference between the sand/anthracite and crumb rubber filters. In comparison to the sand/anthracite filter, however, the crumb rubber filter had much lower head losses and longer filter run times.
6. For phytoplankton and zooplankton removal, the analytical results were determined to be unreliable because of the small sample size used. Future studies should be conducted by concentrating the samples using 10 and 50 μm plankton sampling nets.
7. This study indicates that the crumb rubber filtration is a potential treatment technology for ballast water. As an on-board treatment technology, however, the water production capacity of the crumb rubber filter needs to be significantly increased. The future study should be conducted at much higher filtration rates (e.g., 80-120 gpm/ft^2) for the removal of large particles (>10 μm and >50 μm) in ballast water.

Students Supported: (name, major, degree)

Chen, Peng, Environmental Pollution Control, Master in Environmental Pollution Control
Tung, Hsin-hsin, Environmental Engineering, PhD in Environmental Engineering
Jain, Anuj, Environmental Engineering, MS in Environmental Engineering

Presentations and Other Information Transfer Activities:

- Chen, Peng and Yuefeng Xie, Ballast Water Treatment Using Crumb Rubber Filtration (poster presentation)**,** the 19th Penn State Graduate Exhibition, University Park, PA, 28 March 2004

- Xie, Yuefeng and Peng Chen, Crumb Rubber Filtration for Ballast Water Treatment: A Preliminary Study (poster presentation)**,** the 2nd International Ballast Water Management Conference and Exhibition, Singapore, 19-21 May 2004

Awards:

Research Grant, National Oceanic and Atmospheric Administration, A Pilot-Scale Study on Crumb Rubber Filtration for Ballast Water Treatment, $156,473, 9/1/2004-8/31/2006.

Spruce Creek Watershed Keystone Project

Basic Information

Title:	Spruce Creek Watershed Keystone Project
Project Number:	2003PA14B
Start Date:	3/1/2003
End Date:	4/30/2005
Funding Source:	104B
Congressional District:	5th of PA
Research Category:	Water Quality
Focus Category:	Management and Planning, Water Quality, Education
Descriptors:	education, management, nonpoint source pollution, planning, trout fishery, water quality, watershed stewardship
Principal Investigators:	Lysle S. Sherwin, Charles Andrew Cole, Lysle S. Sherwin

Publication

Abstract:

This proposal seeks support for a graduate assistant working with two interdisciplinary teams of graduate students and faculty engaged in a two year watershed assessment and planning practicum (Keystone Project) in the Spruce Creek watershed of the Little Juniata River, Pennsylvania. One team of eight students will be assigned to the first phase (2003/2004) and a second team of nine students will be engaged in the second phase (2004/2005). Through participation in a watershed case problem, the students will develop competence in scientific data collection techniques and problem analysis tools directed at quantifying, analyzing, and ultimately mitigating widespread types of polluted runoff. The Keystone Project will also afford the graduate assistant an enhanced education in community-based, team-oriented watershed management and will provide outreach to the host watershed community. The project itself will address water quality issues in Spruce Creek, a high quality trout stream threatened by land development, agricultural enterprises, and in-stream flow reductions from groundwater withdrawals. The entire Spruce Creek watershed is designated as a High Quality-Cold Water Fishery by the PADEP Chapter 93 Water Quality Standards. However, two major tributaries and part of the mainstream were recently listed as impaired for suspended solids by the PADEP 303(d) list, attributable to agriculture and land development activities. Watershed stakeholders are concerned that other stream segments may be impaired, and there are emerging problems with inadequate treatment of sewage effluent from on-lot systems. Data on stream flow and water chemistry are insufficient to calculate pollutant loadings as the baseline to determine appropriate reductions needed to achieve water quality standards and protect designated uses. Modeling polluted runoff in the impaired reaches will serve to target critical contributions and serve as a basis for development of a watershed restoration plan.

Project Objectives:

a) Conduct an assessment of biophysical and cultural factors in the watershed related to protection and restoration of water quality to support designated uses and progressive land use.
b) Analyze assessment data to identify problems and opportunities and engage a broad base of stakeholders in developing shared watershed restoration and protection goals.
c) Produce a watershed stewardship plan responsive to those goals and that incorporates implementable recommendations and strategies for cooperative action. A model Total Maximum Daily Load (TMDL) would be prepared for the impaired reach of Halfmoon Creek.
d) Produce a Watershed Restoration Plan for Halfmoon Creek
e) Replicate the TMDL analysis for the second impaired tributary (Warriors Mark Creek) as a precursor for future restoration planning in that subwatershed.

Methods:

Water samples will be collected beginning August 2003 at five stations on Halfmoon Creek - three within the impaired reach, one upstream of the impaired reach, and one reference station in the upper watershed. A sampling location will also be established on an unnamed tributary, locally known as Loveville Creek. Analysis will be done for total suspended solids, total nitrogen, and total phosphorus. Runoff event samples will also be collected randomly through

the project period. Measurement of stream flow will be done concurrently using portable current meters. Pennsylvania Spatial Data land cover and other data will be retrieved and clipped using Arc-View GIS. The Keystone team and the research assistant will be engaged in organizing a series of community stakeholder input meetings. A day-long watershed planning workshop of experts will be convened in Spring 2004 to review and critique the proposed pollution abatement recommendations developed by the student team and to develop strategies for implementation of management practices. These recommendations would be presented at a public forum at the completion of the first phase of the Keystone Project in April 2004. The graduate research assistant funded by this proposed grant would have lead responsibility of communicating the data findings and the analysis to layperson and technical audiences at the various forums and workshop and in direct meetings with private landowners and representatives of collaborating agencies involved in implementation of watershed restoration practices.

Over the summer, 2004, the graduate assistant will be involved in digitizing site maps and coordination with landowners and partner organizations relevant to a site specific restoration plan for Halfmoon Creek. Beginning in the fall 2004 semester, the full student team will commence work on a more extensive assessment and field data collection at a number of sites throughout the Spruce Creek. Methods will include an IBI, Rapid Bioassessments, electrofishing, Arc-View GWLF modeling and a variety of other data collection and analytical tools. The team will also work in collaboration with watershed stakeholders to define task areas that would be supportive of an emerging community-based watershed protection and restoration program in the Spruce Creek watershed. The 2004/2005 team will utilize a similar schedule of planning workshops and meeting and produce a report document and community presentation in April 2005.

Statement of Critical Need:

Spruce Creek is a nationally recognized trout fishery potentially threatened by residential and commercial land development pressure, a variety of agricultural enterprises, and in-stream flow reductions from groundwater withdrawals. The entire Spruce Creek watershed is designated as a High Quality-Cold Water Fishery by the PADEP Chapter 93 Water Quality Standards, which mandates special protection under the “non-degradation” criteria of the Federal Clean Water Act. However, a 1.4-mile reach of Halfmoon Creek, a major tributary, a 16 mile segment of Warriors Mark Run, and two miles of the main stem of Spruce Creek were recently listed as water quality impaired for suspended solids by the PADEP 303(d) list, attributable to poor livestock pasture management practices at several beef cattle, dairy, and horse stable operations and to residential land development activities. There are concerns among watershed stakeholders that other stream segments may be similarly impaired, or are becoming so, as well as emerging problems with excessive nutrient loadings and pathogens associated with inadequate treatment of domestic sewage effluent from on-lot systems. Quantitative data on stream flow and water chemistry are insufficient to calculate current pollutant loadings as the baseline to determine appropriate reductions required to achieve water quality standards and protect designated uses. Despite deep-seated concerns about future water resources trends and strong motivation among stakeholders to protect the water resource and fishery, there is a lack of a cohesive watershed initiative grounded in the community or collaborative approaches and restoration strategies and programs in place to address the problem.

Statement of Results or Benefits:

We will produce a watershed stewardship plan and a watershed restoration plan in two phases which are responsive to watershed residents' goals and incorporates implementable recommendations and strategies for cooperative action. Deliverables at the conclusion of the Keystone Project in May 2005 will include: printed color copies of the final plan document; CD-ROM files of all related work products such as public meeting information and educational display posters and digital presentations, GIS data layers, field data, and relevant analyses used on specific assessment topics; documentation of community focus group sessions, planning meetings and workshops to develop management recommendations and; a model TMDL prescription for suspended solids impairment of Halfmoon Creek in the form of a restoration plan

Nature, Scope, and Objectives of the Project, including a Timeline of Activities:

The nature and scope of the project essentially entails experiential training of graduate students in water resources disciplines through a scientific watershed assessment utilizing existing and original data and a team-based, case problem planning approach conducted in close collaboration with community stakeholders, governmental agencies, and academics. The project objectives are to: a) Collect original and existing data on water chemistry and flow, land cover and land use, aquatic biota, and other information relevant to the restoration of impaired water quality and the protection of water resources to meet designated uses in the study watershed; b) Analyze data to assess problems and engage a broad base of stakeholders in developing shared watershed restoration goals; c) Produce a watershed stewardship plan that is responsive to those goals and that incorporates recommendations and strategies for cooperative action. A model Total Maximum Daily Load (TMDL) prescription would be prepared for the impaired reach of Halfmoon Creek; d) Produce a Watershed Restoration Plan for Halfmoon Creek; and, e) Replicate the TMDL analysis for the second impaired tributary (Warriors Mark Creek) as a precursor for future restoration planning in that subwatershed.

Timeline of Major Project Activities:

March 2003	Initiate on-going water sampling and flow measurements; retrieve existing data from DEP, EPA Mid-Atlantic Assessment, and other sources.
April 2003	Establish working group of community stakeholders and involved agency staff.
June 2003	Commence organization and scheduling watershed stakeholder meetings.
Aug. 2003	Commence Keystone Project practicum, (Fall semester, "Assessment"). Semester objective is to produce a quantitative and qualitative assessment of watershed conditions with particular emphasis on the range of pollutant loadings causing water quality impairment and probable sources.
Dec. 2003	Draft Watershed Assessment Report complete
Jan. 2004	Keystone Project practicum (Spring semester, "Analysis and Planning"). Semester objective is to engage students, faculty, agency and university

	technical advisors in analysis of assessment findings and development of management recommendations.
March 2004	Planning Workshop Format involves expert critique and refinement of proposed management strategies and recommendations to be included in Watershed Stewardship plan. A TMDL proposal for impaired stream segment will be a primary plan element.
April 2004	Delivery of completed plan to watershed stakeholders and working group. Deliverables include printed and digital versions with supporting data, a public presentation of recommendations, and educational outreach materials generated to assist in implementation by local sponsors.
May-Aug. 2004	Base mapping and field evaluation of non-point contributors. Identification and access agreements with landowners. Restoration Plan coordination with partner agencies and organizations.
Fall 2004	Assessment phase-qualitative and quantitative characterization of surface water, groundwater, and other resources. Interaction and input with community stakeholders on implementing restoration/protection practices and programs.
Spring 2005	Analysis and plan development
April/May 2005	Results and findings presented to watershed stakeholders. Recommendations for actions.

Methods, Procedures, and Facilities:

Water samples will be collected beginning August 2003 at five stations on Halfmoon Creek, including three stations within the water quality impaired reach, at one station upstream of the impaired reach, and at one reference station in the upper watershed. A sampling location will also be established on an unnamed tributary, locally known as Loveville Creek. Analysis by standard methods will be done for total suspended solids, total nitrogen, and total phosphorus by the Water Quality Laboratory at the Penn State Institutes for the Environment. Samples will be routinely collected approximately monthly during the project period. Runoff event samples will also be collected randomly through the same time period. Measurement of stream flow will be done concurrently with water quality sampling using portable Price-type current meters. These data are essential in determining the magnitude of pollutant loading to the impaired tributary and receiving stream and enabling the development and implementation of abatement measures. Once the baseline monitoring network is in place, ongoing data collection will be useful in evaluating the accuracy of the pollutant reduction formula and the effectiveness of management practices. Existing published reports and field data from state, federal, county and local agencies will be acquired for background information. Pennsylvania Spatial Data (PASDA) land cover and other data will be retrieved and clipped to the Spruce Creek watershed in the CWS Arc-View GIS lab. The Keystone Team and the research assistant will be engaged in organizing a series of community stakeholder input meetings. A day-long watershed planning workshop of invited experts will be convened in Spring 2004 to review and critique the proposed pollution abatement recommendations developed by the student team and to develop strategies for implementation of management practices. These recommendations would be presented at a public forum at the completion of the first phase of the Keystone Project in April 2004. The graduate research assistant funded by this proposed grant would have lead responsibility of communicating the

data findings and the analysis to laypersons and technical audiences at the various forums and workshop and in direct meetings with private landowners and representatives of collaborating agencies involved in implementation of watershed restoration practices.

Over summer 2004, the graduate assistant will be involved in digitizing the site maps and coordination with landowners and partner organizations relevant to a site specific restoration plan for Halfmoon Creek. Beginning in the fall 2004 semester, the full student team will commence work on a more extensive assessment and field data collection at a number of sites throughout the Spruce Creek. Methods will include an IBI, Rapid Bioassessments, electrofishing, Arc-View GWLF modeling and a variety of other data collection and analytical tools. The team will also work in collaboration with watershed stakeholders to define task areas that would be supportive of an emerging community-based watershed protection and restoration program in the Spruce Creek watershed. The 2004/2005 team will utilize a similar schedule of planning workshops and meetings and will produce a report document and community presentation in April 2005.

This project will occur under the sponsorship of the Center for Watershed Stewardship at Penn State University. Penn State is the land-grant institution for Pennsylvania and has a full set of facilities available for our use (library, computing, water analysis laboratories, dry and wet lab space, office space).

Principal Findings and Significance:

Water quality data and modeling (ARC-View GWLF) on the impaired segment of Halfmoon Creek reconfirmed the 2002 designation by PA DEP on the 303(d) list of impaired waters due to sediment. Further, our water quality/stream flow data indicates that Nitrogen (N) exceeds EPA-recommended levels at all stations, at all flows (base, median, and flood event) and that Phosphorus (P) and Total Suspended Solids (TSS) exceed recommended levels during flood events, and with few exceptions, during median flow periods. Similarly, a fish species Index of Biotic Indicators, macroinvertebrate collection and electrofishing indicated a dominance of generalist feeders tolerant of pollution. Although wild brown trout were collected at all stations on Halfmoon Creek, young of the year were found at only one station (HM-5) near the junction with Spruce Creek.

The significance of these findings is that a more extensive biological degradation of Halfmoon Creek exists than is reflected in the 1.4 mile segment presently identified on the 303(d) list as not meeting the HQ Cold Water Fishery designation criteria of being capable of maintaining a self-reproducing trout fishery. A restoration plan for Halfmoon Creek to be undertaken in the second phase of the Keystone Project partially funded by the USGS grant is expected to begin to mitigate the environmental conditions causing the stream's impairment.

The project has also raised stakeholder awareness of watershed stewardship issues and has served as a catalyst for the formation of a steering committee of watershed residents interested in organizing a Spruce Creek watershed group. The various public meetings, planning workshop, community presentation and open house, had a direct positive influence in stimulating a broader

engagement of community stakeholders and the participation of governmental entities in addressing restoration and protection of the Spruce Creek watershed.

Students Supported:

Cristina Maria Torres, a MS candidate in Environmental Pollution Control, was directly supported in her thesis research with assistance in field data collection by her Keystone Project peers. Her analysis contributed significantly to the findings described above. Seven other master's students in Landscape Architecture, Ecology, and Forest Resources degree programs received training through their participation in the Keystone Project.

Presentations and Other Information Transfer:

Students made public presentations at three public meetings during the assessment phase, a day-long planning workshop held in March 2004, and a concluding presentation for the first year held in April 2004. The format of the latter included a "poster session" at topical stations, e.g Groundwater Resources, involving seven large format color posters describing assessment findings, watershed management issues, and recommended strategies and programs for stewardship of the watershed's environmental and cultural assets. Representatives of various public and private entities participated in the open house with displays and handout educational materials to inform watershed residents of available programs and resources to assist local efforts. Participants include Penn State Cooperative Extension, PA Master Well Owners Network, Centre County Conservation District, and Juniata Clean Water Partnership.

A Spruce Creek Watershed website was created by a Keystone Project team member to facilitate communication and information exchange among people and organizations involved in protection and management of the watershed.

Awards:

None

Development of Passive Treatment System Monitoring and Research Program

Basic Information

Title:	Development of Passive Treatment System Monitoring and Research Program
Project Number:	2003PA16B
Start Date:	3/1/2003
End Date:	2/29/2004
Funding Source:	104B
Congressional District:	21st of PA, 4th of PA
Research Category:	Water Quality
Focus Category:	Non Point Pollution, Water Quality, Ecology
Descriptors:	passive treatment, wetlands, cid mine drainage, restoration
Principal Investigators:	Fred Brenner, Margaret Dunn

Publication

Abstract:

Stream Restoration Inc. and Grove City College propose to develop a training program to monitor passive treatment systems and receiving streams in the headwaters of Slippery Rock Creek. Since 1995, a public-private partnership effort consisting of non-profit corporations, colleges and universities, private industries, and governmental agencies have cooperated in the restoration of the 27-sq. mi. headwater area of the Slippery Rock Creek Watershed. The quality of the headwaters was documented in 1970 by Operation Scarlift to be heavily impacted by abandoned mine drainage. To date, 15 passive treatment systems have been constructed within the project area including every major type of component, such as vertical flow ponds, aerobic wetlands, anoxic limestone drains, open limestone channels, and horizontal flow limestone beds. Even though the passive systems are low maintenance, periodic inspection and monitoring will help insure the timely resolution of minor maintenance issues before larger problems develop. In addition, decreasing effectiveness in water treatment will also be documented in order to improve the design of future passive systems. This program will continue the extremely important outreach and educational opportunities to students and interns, vital not only to the professional growth of individual students but also to sustaining the watershed restoration concept.

Statement of Critical Water Problem:

Problems associated with abandoned minelands are so extensive that alone neither federal, state, or local governments nor the mining industry nor watershed groups can adequately address the impacts. In Pennsylvania, $2{,}500^{+}$ miles of streams are impacted (equal to the distance from Pittsburgh, PA to Los Angeles, CA) and $250{,}000^{+}$ acres are unreclaimed. In northern Butler County, western Pennsylvania, coal mining was conducted in a 27-sq. mi. area of the Slippery Rock Creek headwaters for over 100 years. Mining communities which once flourished are now abandoned and what remains are polluted streams, coal refuse, and spoil. The residents that stayed called Slippery Rock Creek, “Sulfur Creek”, due to the affects of mine drainage. In 1970 during the Commonwealth’s Operation Scarlift, the quality of the headwaters was documented to be “the most severe condition of coal mine drainage... Indeed, very little drainage from this region is produced exclusive of contact with, or issuance from mine workings.” (About 4000 acres are underlain by mine workings and 8000 acres were included in surface mine permits.) Within the 410 square miles of the Slippery Rock Watershed, streambed sediments in the headwaters have the highest heavy metal concentration.

Since 1995, a public-private partnership consisting of non-profit corporations, colleges and universities, private industries, and governmental agencies have cooperated in the restoration of the 27-sq. mi. headwater area of the Slippery Rock Creek Watershed. To date, 15 passive treatment systems have been constructed within the project area including every major type of component, such as vertical flow ponds, aerobic wetlands, anoxic limestone drains, open limestone channels, and horizontal flow limestone beds. These systems remove 30% of the acid loading and contribute 193 kg/day of alkalinity to Slippery Rock Creek and its tributaries along with removing over 27% of the iron and aluminum loading. Without proper monitoring and maintenance, the effectiveness of these systems is not certain.

Objectives:

Since these passive systems include all technologies currently available for the treatment of acid mine drainage, they provide a unique opportunity for the development of educational training programs for undergraduate students. These students will be given "hands-on" experience and training from professionals in the field of passive treatment.

Specifically, students will:

- Conduct monitoring and maintenance of 15 passive treatment systems and their receiving streams in the headwaters of Slippery Rock Creek
- Conduct field and lab analysis of the passive systems and receiving streams
- Upload data to a website for investigators and others to view
- Evaluate the performance of the passive systems

Timeline of Activities

Milestones Project Tasks	**Responsible Parties**	**Start Date**	**End Date**
Grant Administration	SRI	03/2003	04/2004
Project Development Compile or complete O&M Plan for each of 15 passive treatment systems Develop website in order to download blank inspection forms and enter site data collected Develop inspection sheet w/site schematic for each site Purchase field test labs(2) and digital cameras(1)	BMI, GCC, SRI, 241	03/2003	04/2003
Site Monitoring Monthly-passive systems Annually-land reclamation sites (growing season to Sept.) Annual wetland monitoring	BMI, GCC, SRI	04/2003	04/2004
Aquatic Survey (Fish and macroinvertebrates) Annual "fish-shocking" of six stream stations	GCC, SRI	07/2003	07/2003

Abbreviations: BioMost, Inc. (BMI); Grove City College students (GCC); Stream Restoration Inc. (SRI); 241 Computer Services (241)

Methods:

Students will begin by creating site-specific monitoring and maintenance plans based on an existing template of a passive treatment system. The plan would consist of a site inspection schedule and field and laboratory testing of selected intermediate points between passive components in order to document the effectiveness of each and to determine if maintenance or retrofitting is necessary. Field and laboratory testing (including flow measurements) and inspection are to be conducted monthly. Both total and dissolved metals will be analyzed

according to Standard Method procedures. During inspections digital photos would be taken of selective features in order to assist in determining the need for maintenance. Simple maintenance tasks, such as removal of excessive vegetation from inlet or outlet structures, will be performed in the field at the time of the evaluation. Inspection of passive treatment systems may take up to an hour or more while inspection of land reclamation sites may require only a few minutes to photograph the site. Passive treatment experts will assist the students with the creation of the monitoring and maintenance plan and during the first monitoring event.

Wetlands will be monitored annually for functionality and diversity. Success of vegetative establishment will be documented by estimating percent coverage of plant species. Survival rates will be estimated using species counts that are correlated to the original species planted. Vegetative densities and survival rates will be used to document successful establishment of the wetland or identify problems requiring corrective measures. Invasive species will be identified, monitored closely, and removed as determined to be necessary. Extremely aggressive invasive species, such as Phragmites or purple loosestrife, will be removed immediately upon identification.

An aquatic survey will be conducted annually. Last year, Dr. Fred Brenner, Grove City College biology professor, documented the return of fish in the upper portion of Seaton Creek, which just the year before had a 4.5 pH and now has a 6.5 pH. Documenting the return of fish in other portions of Seaton Creek and the main branch of Slippery Rock Creek would be invaluable in the determination of the success/failure of the entire headwaters restoration effort.

All plans created and information obtained through this program will be placed in an online database to document the performance of the passive systems. The online database will provide three basic functions (1) to download site-specific monitoring and maintenance plans, (2) to upload information gathered in the field or from laboratory reports, and (3) to provide meaningful, accessible reports on demand. The database will be MySQL format, stored on a server (600MB of space) and accessed via the world wide web. Data will be uploaded in comma delimited format. The data will automatically be inserted into the database and will be immediately accessible. The data will be able to contain objective measurements as well as subjective comments and photographs. Reports specified by Stream Restoration Inc. and Grove City College will be generated on demand from the latest data available. The reports will be available in a printable format or for download as comma delimited text files. Professionals will assist the students in the creation of this database.

The Slippery Rock Watershed Coalition has a unique opportunity to research a variety of passive treatment systems and land reclamation techniques in the headwaters of Slippery Rock Creek. Coalition participants, having been involved in the restoration effort, are extremely familiar with the design of these systems. The following innovative passive systems have been successfully implemented (See also attached map):

Site	Year	Restoration Effort	Special Challenge	Innovative Solution	Comments
SR114	1995	1450 T ALD	125-gpm upwelling	vertical collection	largest ALD in PA (1995)
Bertha	1996	900 T ALD	lake proximity	ALD encapsulation	Bald Eagle use of lake
Tipple	1996	alkaline addition	100,000 T gob	175,000 T coalash	21-ac floodplain
Ferris	1997	four VFP	5 flows(170 gpm)	compost/LS & LS-only	1st two-step VFP in PA
Able	1998	land reclamation	acidic spoil	90,000 T coalash	56-ac "no-cost" project
SR109	1998	VFP	limited space	decreasing depth pond	diverse wetland T
SR101A	1998	900 T ALD	sloughing soils	piezometers-head data	old railroad grade used
Brookville	2001	alkaline addition	gob piles in stream	gob neutralization	22 ac reclaimed
DeSale I	2000	3000 T VFP(2)	high dissolved Al	VFP in parallel	1st two-tier piping in PA
DeSale II	2001	4400 T VFP (2)	treat entire stream	controlled intake	1 acre wetland
Goff	2001	8200 T VFP (4)	5 flows (400 gpm)	2 VFP in parallel	bat hibernaculum
SR96	2002	700 OLD	oxygen in AMD	Flushing option	1st known in W. PA
SR81	2002	1300 T ALD	relocated discharge	in-situ collection	add. land reclamation
DeSaleIII	2002	1700 VFP (2)	high acidity	3 alk gen. in series	use existing facilities
Erico	2002	12,000 T ALD (3)	Mine pool	Built over coal seam	largest known ALD (2002)

As an example, the Goff Station Restoration Area is an extremely innovative, 15 component system that includes the creation of wildlife habitat. This system is successfully treating the mine discharges and has further developed passive treatment technology. The first full-scale, side-by-side comparison of limestone-only and limestone/compost Vertical Flow Ponds were constructed at this site. The monitoring of these Vertical Flow Ponds will provide valuable information into the design of future passive systems.

As-built drawings exist for most of the restoration sites. An as-built is a detailed post-construction plan drawn to scale that includes survey elevations. As a result of this information, the performance of these systems can be accurately evaluated and the most productive system identified.

Related Research:

During the last decade, vertical flow pond (VFPs) have been used to treat AMD, but the mechanisms operating within these systems are not completely understood. Based on the initial design or these systems proposed by Kepler and McCleary (1994), VFPs provide net addition through the dissolution of limestone and sulfate reduction, resulting in the precipitation of iron and aluminum in the substrate. Brenner (2001a,b) reported that VFPs are effective in removing over 90% of the iron and aluminum, but they are not as effective in removing manganese. Demchak (1998) indicated that the efficiency of some of these systems began to decline after 18-24 months due to a reduction in limestone and iron accumulation in the substrate and similar results were reported by Brenner (2001a,b) and Brenner *et al.* (2002) for a scale model of these

systems. When a scale model was dissected, iron precipitates had accumulated in the upper third of the substrate and efficiency of the system in both alkaline addition and metal removal had declined in the second year of operation (Brenner 2001a,b). Demchak (1998) also reported the overall effectiveness of the VFPs varied seasonally especially as the systems aged.

The objective of the current study is to evaluate the efficiency of passive systems in the headwaters of Slippery Rock Creek while providing an educational experience for undergraduate students. Only sporadic water quality sampling has occurred to date of these systems. This study will monitor monthly each component and document their performance and effects on the receiving streams. This information can then be compared to similar studies by Demchak and Brenner.

References:

Brenner, F.J. 2001a. Use of constructed wetlands for acid mine drainage abatement and stream restoration. Water Science Technology 44:449-454.

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Brenner, F.J., K.D. Kosick, S. Busler, C.A. Gardner, and C. Tippie. 2002. Efficiency of a Scale model vertical flow and aerobic wetland system in treating acid mine drainage. American Surface Mining Association. Lexington, KY pp 818-829.

Demchak. J.L. 1998. Analysis of vertical flow wetlands in the treatment of acid mine Drainage. Unpub. M.S. Thesis. Clarion University of Pennsylvania.

Kepler, D.A. and E.C. McCleary. 1994. Successive alkalinity-producing systems (SAPS) for the treatment of acidic mine drainage. Proceedings International Land Reclamation and Mine Drainage Conference and Third International Conference on the Abatement of Acid Drainage. 1:195-204.

Results:

Fifteen passive treatment systems in the Slippery Rock Watershed were monitored for 12 months beginning in May of 2003 and terminating in May of 2004. Currently, these systems are treating over 700,000,000 gallons of acid mine drainage annually. These combined systems are currently removing 640 tons/yr of acidity and 160 and 30 tons/yr of iron and aluminum, respectively from acidic discharges while providing 70,445 kg/year of alkalinity to Slippery Rock Creek and its tributaries. These systems include every major type of component currently used to treat acid mine drainage such as vertical flow ponds, aerobic wetlands anoxic limestone drains, open limestone drains and horizontal flow limestone beds. Although these systems varied in their efficiency, all systems except SR 109 were effective in treating acid mine drainage. In the remaining 14 systems, the pH increased from <3 to between 6.0 and 7.2 and alkalinity exceeded acidity in the final discharge to receiving streams. The systems comprised of a combination of vertical flow ponds and aerobic wetlands were effective in removing iron and aluminum and the concentrations of these metals were < 4 mg/L in the final discharge even in those systems where iron and aluminum exceeded 50 mg/L in the inflows. But only those systems with horizontal flow limestone beds as the final treatment system were effective in removing manganese from

acidic mine discharges. For the two vertical flow pond/aerobic wetland and horizontal flow limestone bed systems, 27 mg/L of alkalinity (as $CaCO_3$) was added to receiving streams and iron, manganese and aluminum concentrations were reduced by 40 mg/L, 16 mg/L and 27 mg/L respectively. In addition, pH units increased from an average of between 2.87 and 3.80 in the inflows to an average of between 6.8 and 7.2 in the discharges to receiving streams. In order to insure adequate alkaline addition and manganese removal to receiving streams, it is recommended that the inclusion of horizontal flow limestone beds be considered as the final treatment in all passive treatment systems. As a result of the improvement in water quality in receiving streams, macroinvertebrates and fish are beginning to re-colonize the headwaters of Slippery Rock Creek and its two major tributaries, Murrin Run and Seaton Creek. During the last year, caddisflies, mayflies, dragonflies, damselflies, crayfish and three fish species have been collected in both Seaton Creek and Murrin Run below the discharges of these passive treatment systems.

Students Supported:

Jeremy Benjamison, Biology Major, Junior, Grove City College
Emily Coughlin, Biology Major, 2004, Graduate, Grove City College
Ty Coulter, Biology Major, Junior, Grove City College
James F. Dunne, Chemistry Major, Junior, Grove City College
Candace McClure, Biology Major, 2003, Graduate, Grove City College, Graduate School of Public Health, University of Pittsburgh
Natalie Johnson, Biology Major, 2004, Graduate, Grove City College.
Peggy Lo, Biology Major, Junior, Grove City College
Justin Treeter, Molecular Biology Major, Sophomore, Grove City College

Papers Presented:

The efficiency of passive treatment systems and their impact on Seaton Creek. Emily Coughlin, Jeremy Benjamison, Justin Treeter, James Dunne, Candace McClure, Fred J. Brenner and Shaun Busler. Poster. American Society of Mining and Reclamation, Morgantown WV and Sigma XI Grove City College

Evaluation of the efficiency of passive treatment systems on water quality in the headwaters of Slippery Rock Creek. Emily Coughlin, Jeremy Benjamison, Justin Treeter, Terry Coulter, James Dunne, Candace McClure, Fred J. Brenner and Shaun Busler. Poster. American Society of Mining and Reclamation. Morgantown WV.

Watershed approach to acid mine drainage abatement: A historical prospective. Fred J. Brenner. Pennsylvania Academy of Science. Monroeville, PA.

Evaluation of passive treatment system efficiency in the headwaters of Slippery Rock Creek. James Dunne, Emily Coughlin, Candace McClure, Fred J. Brenner and Shaun Busler. Pennsylvania Academy of Science, Monroeville, PA

The efficiency of passive treatment systems and their impact on Seaton Creek. Emily Coughlin, James Dunne, Candace McClure, Nicholas Morgan, Fred J. Brenner and Shaun Busler. Pennsylvania Academy of Science, Monroeville, PA and Slippery Rock Watershed Student Symposium, Slippery Rock University.

Enrichment, biochemical, and molecular techniques for assessing microbial arsenic reduction

Basic Information

Title:	Enrichment, biochemical, and molecular techniques for assessing microbial arsenic reduction
Project Number:	2001PA721G
Start Date:	9/1/2001
End Date:	8/31/2006
Funding Source:	104B
Congressional District:	14
Research Category:	Biological Sciences
Focus Category:	Non Point Pollution, Water Quality, Groundwater
Descriptors:	arsenic, bacteria, arsenate reductase, biochemical probes, molecular probes, arsenate reductase
Principal Investigators:	John F. Stolz

Publication

1. Stolz, J.F., P. Basu, and R.S. Oremland, 2002. Microbial transformation of elements: the case for arsenic and selenium. Int. Microbiol. 5:201-207.
2. Afkar, E., J. Liska, C. Saltikov, P. Basu, R.S. Oremland, and J.F. Stolz. 2003. The respiratory arsenate reductase from Bacillus selenitireducens Strain MLS10. FEMS Microbiol. Letts. 226:107-112.
3. Oremland, R.S. and J.F. Stolz. 2003. The ecology of asenic. Science. 300:939-944.
4. Oremland, R.S., J.F. Stolz, and J.T. Holligaugh. 2004. The microbial arsenic cycle in Mono Lake, CA. FEMS Microbiol. Ecol. 48:15-27.
5. Hoeft, S.E., T.R. Kulp, J.F. Stolz, J.T. Hollibaugh, and R.S. Oremland. 2004. Dissimilatory arsenate reduction with sulfide as the electron donor: Experiments with Mono Lake water and isolation of strain MLMS-1, a chemoautotrophic, arsenate-respirer. Appl. Environ. Microbiol. 70:2741-2747.
6. Santini, J.M. and J.F. Stolz. 2004. Prokaryote arsenate and selenate reduction. In M.S. Nakano and P. Zuber (eds.) Strict and facultative anaerobes: Medical and environmental importance. Horizon Scientific Press (in press).
7. Poster Presentation: E. Fisher, A. Dawson, J. Kisak, G. Polshyna, P. Basu, and J.F. Stolz. 2003. Enrichment, isolation, and characterization of a Clostridium species that respires arsenate and selenite

ASM General Meeting, Washington, DC, May 18-22, 2003.

8. Poster Presentation: E. Afkar, C. Saltikov, J. Lisak, P. Basu, R.S. Oremland, and J.F. Stolz. 2003. Purification and characterization of the respiratory arsenate reductase from Bacillus selenitireducens. ASM General Meeting, Washington, DC, May 18-22.
9. Poster Presentation: J.F. Stolz, E. Afkar, J. Lisak, C.Saltikov, R.S. Oremland, and P. Basu. 2003. The respiratory arsenate reductase from the Haloalkaliphile Bacillus selenitireducens: A Molybdoenzyme that functions at high pH and salinity. Gordon Research Conference, Kimball Union Academy, June 29-July 4, 2003.
10. Invited Presentation: Gordon Conference on Molybdenum and Tungsten Enzymes, Kimball Union Academy, Meriden, NH, July 3, 2003.
11. Symposium: Arsenic dynamics within soils and sediments, American Society of Agronomy, Crop Science Society of American, and Soil Science Society of American annual meeting, Denver, CO, November 3, 2003.
12. Departmental Seminar: Geosciences Department, Virginia Polytechnic Institute and State University, (Microbial transformation of arsenic), April 26, 2004.

Problem and Research Objectives:

Arsenic is a naturally occurring element that can cause skin lesions, cancer (e.g., bladder, kidney, lung and skin), diabetes, and cardiovascular disease in elevated concentrations. It has also been shown to act as an endocrine disruptor, affecting the glucocorticoid receptor. Arsenicosis is a global problem that is primarily caused by the consumption of arsenic-contaminated groundwater. Bacteria that grow by using arsenate as a terminal electron acceptor may play a crucial role in the transformation and mobilization of arsenic in groundwater. It has become imperative, therefore, that effective methods be developed for the identification of these organisms in the environment. The purpose of this proposal is to improve enrichment culture methods and develop biochemical and molecular techniques for the identification of arsenate-respiring bacteria in order to assess microbial arsenic mobilization. Specific studies proposed include (1) formulation of enrichment media for culturing freshwater arsenate-respiring bacteria and (2) design and development of biochemical and molecular probes for the detection of Gram positive arsenate-respiring bacteria.

Methodology:

The medium contains per liter K2HPO4 (0.225g), KH2PO4 (0.225g), NaCl (0.46g), (NH4)2SO4 (0.225g), MgSO4.7H2O (0.117g), yeast extract (1g), NaHCO3 (4.2g) Na2S.9H2O (0.1g), 10ml of SL10 trace element solution, 10ml of vitamin solution. Different concentrations of arsenate (1,5, and 10 mM) and different electron donors (e.g., acetate, lactate, pyruvate, formate) are then added. The pH is adjusted to 7.3 prior to autoclaving. The liquid medium is dispensed in crimped septum sealed tubes or bottles (125ml) and degassed with a 80:20% mixture of N2:CO2 for five minutes. The headspace is then degassed for one minute and the vessels sealed, crimped, and autoclaved. The vitamin solution, lactate, reducing agent, and bicarbonate are filter sterilized and added separately. The bottles are inoculated with a slurry made from soil sample (1 gm) in minimal medium lacking electron donor, acceptor, and yeast. After incubation, the bottles are examined for the visible production of arsenic trisulfide (a yellow precipitate). AsIII and AsV are determined with HPLC using a BioRad Aminex HPX-87H ion exclusion column, 16 mN H2SO4 as the eluent, a flow rate of 2ml/min, and UV detection at 195 nm. The morphology of the bacteria are determined using Gram staining and light and electron microscopy.

Enrichment cultures and isolates are screened for arsenate reductase activity using the methyl viologen assay. For the spectrophotometric assay, a reaction mixture containing 2.2 ml of HEPES buffer (50 mM, pH 7), 100 mg of sample (in 100 ml of buffer), 80 ml of methyl viologen (1mg/ml), and 100 ul of 5mM arsenate, is sparged for 5 minutes with oxygen-free dinitrogen in a Thunberg cuvette. A solution containing sodium dithionite (5mM) in bicarbonate buffer (0.01M, 100ml) is simultaneously sparged in the sidearm of the cuvette. The cuvette is then assembled and the contents of the sidearm mixed with the reaction mixture. The control cuvette contains all but the sample and is placed in the reference beam of the spectrophotometer. Activity is measured at 600 nm as the methyl viologen is oxidized by the reductase. For the zymogram, protein samples are solubilized

in SDS sample buffer and loaded onto a 4 - 12% gradient acrylamide gel (BioRad, Hercules, CA). After running the gel for an appropriate time (e.g., 2 hrs at 150 V) the gel is first stained in a solution of dithionite reduced methyl viologen and then developed with a solution of 5 mM sodium arsenate in Tris buffer (pH 8). A duplicate gel is blotted onto nitrocellulose and probed with the antibodies raised against 26 kDa polypeptide from S. barnesii or the 110 kDa subunit of the arsenate reductase from B. selenitireducens.

The procedure for purification of the RasR has been worked out in detail. Starting with 15 g of cells (pooling the harvest of three fermentor batches), the pellet is resuspended in 140 ml of 10 mM Tris -HCl buffer (pH 8.0) containing 1 mM EDTA and 10 µM phenyl methyl sulphonyl fluoride (buffer A). The suspension is then sonicated (150 W, 50/60 Hz) for 10 min on ice. The resulting suspension is centrifuged at 7500 x g for 15 min to remove the unbroken cells. The supernatant is then centrifuged at 200,000 x g for 2hrs. The supernatant is decanted and the reddish pellet resuspended in 50 ml of buffer A containing 0.3 M KCl and centrifuged at 100,000 x g for an additional 1 hr. The resulting pellet is resuspended in 50 ml of buffer A, and Triton X-100 is added to give a final concentration of 2% (wt/ vol). After the suspension is gently stirred for 2hrs at 4°C, the solubilized suspension is centrifuged at 100,000xg for 1 hr. The reddish supernatant is then loaded onto a DEAE-Toyopearl column (3x14 cm) equilibrated with buffer A containing 1 % Triton X-100 (wt / vol) and eluted with a NaCl gradient (0 - 0.5 M) in buffer A. Fractions with the enzymatic activity are pooled, dialyzed against 2 liters of buffer A containing 1% Triton X-100, and then subjected to a second DEAE-Toyopearl column (1.5 x11.5 cm) also eluted with a linear gradient of NaCl (0~0.5M) in buffer A containing 1% Triton X-100. The fractions with arsenate reductase activity are pooled and loaded onto a sephacryl S-300 size exclusion column equilibrated with 10 mM Tris-HCl buffer (pH.0.8) containing 1% Triton X-100 and 0.25 M NaCl. The yellowish-brown fractions containing pure RasR are pooled.

Principal Findings and Significance:

We tested an enrichment technique for assessing the potential for arsenate respiration in environmental samples. Using sediments from the Ohio River in Pittsburgh we have assessed the impact of arsenic concentration (1, 5, 10, and 20 mM) and electron donor (hydrogen, acetate, formate, pyruvate, lactate) of the enrichment of arsenate-respiring bacteria. Our results suggest that while electron donor may not matter significantly, the concentration of arsenic does. We found that 5 mM sodium arsenate produced the greatest amount of arsenic trisulfide (within 48 to 72 hours), an indication of arsenate and sulfate reduction. While the bottles containing 10 and 20 mM arsenate did not show copious production of arsenic trisulfide (Figure 8), HPLC analysis indicated that indeed the enrichments had transformed a significant quantity of AsV to AsIII. Those enrichments containing 1 mM arsenate did eventually show indication of arsenite production (in the form of arsenic trisulfide) but after much longer incubation periods (weeks to a month). We are currently in the process of tabulating this latest result and are preparing a publication on the methodology. This matrix, in conjunction with our

biochemical and molecular probes should be quite useful for assessing the potential for arsenate respiration in other environments.

We have also completed the characterization a new species of *Clostridium* that we isolated from sediments of the Ohio River. Designated strain OhILAs, it is able to couple the oxidation of acetate to the reduction of AsV to AsIII. While it can grow on medium containing up to 40 mM arsenate, it can only reduce 10 mM as the concomittant production of 10 mM arsenite is inhibitory. It can use either acetate or lactate as electron donors and in addition to arsenate, use selenate, nitrate, sulfate, and thiosulfate as electron acceptors. We are investigating further whether OhILAs can respire selenite, as the HPLC analysis was inconclusive. We have, however, established that it has a respiratory arsenate reductase similar to the arsenate reductase from *B. selenitireducens* (see below). Of greater significance is our finding that OhILAs is not only resistant to the poultry feed additive roxarsone (4 hydroxy,3 nitrophenol arsonic acid) but readily transforms it. It is resistant to high concentrations (at least 5 mM) and depending on growth substrates will transform it within 24 h (1g/L yeast) to a week (glycerol). We are currently analyzing the end products.

In the past year we have purified the dissimilatory arsenate reductase from the haloalkaliphile *Bacillus selenitireducens*. This enzyme is comprised of two subunits of 110 and 34 kDa. N-terminal sequence analysis has revealed a high degree of homology (50% identity, 85% similarity) with the ArrA and ArrB of the arsenate reductase of *Crysiogenes arsenatis*. It also exhibits a higher affinity for arsenate with an apparent Km of 38 mM. The enzyme is robust and maintains activity in SDS-PAGE gels as long as the sample is not heated. Using the methyl viologen assay with SDS-PAGE, we can readily detect arsenate reductase activity in bacteria with this type of enzyme. Thus it will be useful for rapidly screening new isolates. We have successfully cloned and sequenced the gene encoding the catalytic subunit (ArrA), and in collaboration with Dianne Newman's group at CalTech, are designing oligonucleotide probes and PCR primers. We are also in the process of raising antibodies against the catalytic subunit of the enzyme for use as an environmental probe.

Student Supported: (name, major, degree)

Anna Polshyna, chemistry and biochemistry, Masters degree (RA supported by NIWR) Edward Fisher, biology, Masters degree (research supported), Miru Thangavelu, biology, masters degree (research supported).

Presentations and Other Information Transfer Activities:

Departmental seminar, University of Oklahoma, Norman OK, Department of Botany and Microbiology, October 18, 2002.

Departmental seminar, St. Vincent College, Department of Biology, January 24, 2003

A manuscript describing the purification and characterization of the arsenate reductase from *B. selenitireducens* has been submitted to FEMS Microbiology Letters. A manuscript describing and the isolation and characterization of *Clostridium* sp. Strain OhILAs is in preparation.

Information Transfer Program

Water resources education programs are viewed as a very high priority activity along with research in the annual program sponsored by the Pennsylvania Water Resources Research Center. One major outreach program supported during FY 2003 was a water conservation program conducted by Dr. William Sharpe and Bryan Swistock. Several of the research projects previously described also involved aspects of information transfer, especially the Spruce Creek project by Sherwin and Cole. Without public understanding of the results of water research, widespread adoption of new practices simply can not be achieved.

Activities of the Director of the Pennsylvania Water Resources Research Center also contributed to water resources information transfer. One notable activity during FY 2003 was the organization of a Watershed Acidification Workshop in cooperation with personnel with the U. S. Forest Service, Northeastern Research Station held on October 20 and 21, 2003 at Deep Creek MD. The workshop brought 20 scientists together for the first time to discuss their watershed acidification research conducted at the Fernow Experimental Forest in West Virginia. The outcome of the workshop was the drafting of an outline for a hardbound book on the Fernow Watershed Acidification Experiments to be written during 2004 and published by Kluwer Academic Publishers Inc. as part of their Environmental Studies Series.

Water Conservation Training and Public Education

Basic Information

Title:	Water Conservation Training and Public Education
Project Number:	2003PA13B
Start Date:	3/1/2003
End Date:	2/29/2004
Funding Source:	104B
Congressional District:	5th of PA
Research Category:	Climate and Hydrologic Processes
Focus Category:	Water Quantity, Conservation, Drought
Descriptors:	water conservation, drought, education
Principal Investigators:	William E. Sharpe, Bryan Reed Swistock

Publication

1. Sharpe, W.E., and B.R. Swistock. 2004. Household Water Conservation. College of Agric. Sci., Agric. Res. and Coop. Extension, Penn State University, 8p.

Abstract:

Serious droughts and increasing competition for water have exacerbated water use disputes in Pennsylvania in recent years. Recently passed water use legislation in the state has focused on water conservation as an important tool for future management of the states water supplies. The Cooperative Extension system provides an attractive vehicle to disseminate water conservation education throughout Pennsylvania. This project provided tools and training to increase public knowledge of water conservation and water use issues through education programs delivered by trained Cooperative Extension educators. A two-day workshop on water conservation was delivered to 25 Extension educators from Pennsylvania in October 2003. The program included speakers from Pennsylvania and California with presentations on drought, water use, home water conservation, youth water conservation education, landscape water conservation, water re-use, agricultural water management, river basin water management, and western water conservation experiences. Each attendee received a binder with written background materials. A new publication was also developed entitled "Household Water Conservation" and 5,000 copies were printed for distribution by Extension educators throughout Pennsylvania. A water conservation web page was also developed to house this publication and other fact sheets related to water conservation. A water conservation PowerPoint was also created and is available on the web page. Finally, ten water conservation table-top displays were created and distributed throughout the state for use by Extension educators. Impacts of this training and the new resources will be measured over the coming years through the Extension web reporting system.

Statement of Critical Water Problem:

Serious droughts have occurred in six out of the last ten years in Pennsylvania. Falling groundwater and stream levels have impacted both community water suppliers and rural homes using private water systems. State-imposed water use restrictions have been imposed in many counties as an emergency measure to conserve water supplies and in extreme situations water conservation measures were mandatory. State government requests for water conservation are usually satisfied through temporary changes in water use habits, such as bans on outdoor water use. These changes in habit, while effective in reducing water use, provide little long-term benefit.

In addition to drought concerns, increasing competition for water in traditionally rural areas has exacerbated water use disputes. These problems along with persistent droughts lead to the passage of legislation (Act 220) in December 2002 to promote water conservation and management of the Commonwealth's water resources. This legislation recognizes that water conservation education is necessary to deal with future water supply and demand in Pennsylvania.

Unfortunately, there is little specific information or education available to the public on comprehensive, long-term water conservation measures. The Cooperative Extension system provides an attractive vehicle to disseminate water conservation education throughout Pennsylvania. Extension agents utilize a combination of professional training and written and electronic resources to disseminate educational programs to citizens throughout Pennsylvania. In many counties, Cooperative Extension agents also serve on mandatory drought task forces that

provide guidance to water suppliers and communities about water supply and conservation. Clearly there is a demand for and interest in water conservation programs in Pennsylvania. During a recent meeting of Cooperative Extension agents from across the state, water conservation was identified as one of the most important needs for agent training and public education in the coming years.

Results and Benefits:

This project provided tools and training for Extension educators to disseminate water conservation education to the citizens of Pennsylvania. Prior to this project, Extension educators were providing water-related educational programs to over 10,000 people in over 40 counties in Pennsylvania. Over 2,000 people benefited from water conservation programs or educational materials from Cooperative Extension such as home water conservation, alternative sources, and farm irrigation conservation in the past year. We anticipate that water conservation education will increase in 2004 in response to this project. The combination of professional training, background resources, publications, displays and web resources gives educators the resources they need to comfortably deliver water conservation programs across the state. Agents are also encouraged to measure the impact of their programs including number of participants and number that initiated water conservation measures. We are utilizing a web-based evaluation tool to determine the number of citizens that participated in educational programs delivered by agents and the number that initiate water conservation practices.

Nature, Scope and Objectives:

This project was intended to increase public knowledge of water conservation and water use issues through education programs delivered by trained Cooperative Extension Agents using displays, publications and presentations created by this project.

Methods, Procedures and Facilities:

A two-day workshop was held at the Ramada Inn in State College, PA on October 14-15, 2003. Twenty-five Extension educators from across the state attended the program. The program agenda and speakers are listed below:

Agenda
Tuesday, October 14, 2003
9:00 – 9:30 **Introduction to Drought and Water Conservation** – Why Are We Here?
Bryan Swistock, Penn State University

9:30 – 10:00 **Water Use and Water Budgeting in Pennsylvania** – past, present and future water use and the concept of water budgets on a watershed scale
Bill Gast, Pennsylvania Department of Environmental Protection

10:00 – 10:30 - **Groundwater Conservation through Green Technology** – encouraging groundwater conservation through enhanced recharge
Al Jarrett, Penn State University, Agricultural and Biological Engineering

10:30 – 10:45 – **Refreshment Break**

10:45 – 11:30 - **Home Water Conservation in Pennsylvania -** measures to reduce home water use, behavior versus fixture replacement, expected benefits, past studies and results.
Dr. William Sharpe, Penn State University

11:30 – 12:00 - **Youth Water Conservation Education** – methods and resources available for youth water conservation programs in Pennsylvania and how to get teachers involved.
Dr. Sanford Smith, Penn State University

12:00 – 1:00 **Buffet Lunch Provided at Ramada Inn**

1:00 – 1:45 – **Western Water Conservation I** – Residential and Landscape Water Conservation Practices in California
Simon Eching – California Department of Water Resources, Office of Water Use Efficiency

1:45 – 2:30 - **Landscape Water Conservation Ideas for PA –** landscaping to reduce water demands (xeroscaping) and irrigation methods to reduce water use.
Gregg Robertson, Pennsylvania Landscape and Nursery Association

2:30 – 2:45 – **Refreshment Break**

2:45 – 3:15 - **Institutional Water Conservation** – leak detection, plumbing retrofitting, and other institutional audits to reduce water use – an example from Elizabethtown College
Joe Metro, Elizabethtown College

3:15 – 4:00 – **Community Water System Leak Detection and Water Auditing –** utilizing leak detection practices and auditing procedures to identify water saving practices in community water systems.
Don Muir, Pennsylvania Rural Water Association

4:00 – 4:45 – **Water Saving Clothes Washers and Dishwashers - Residential Water Auditing** – new developments in dishwashers and clothes washers that save water and energy costs - determining where water is used and wasted in the home.
Bryan Swistock, Penn State University

5:00 PM – **Cook-out at Spring Creek Park**
Wednesday, October 15, 2003

<u>Wednesday, October 15, 2004</u>

8:00 – 8:15 **Introduction to Day Two**
Bryan Swistock, Penn State University

8:15 – 9:00 – **Susquehanna River Basin Commission Water Conservation and Management** – water conservation programs within the Susquehanna River Basin Commission and regulations affecting water use and conservation.
Gil Hershel, Susquehanna River Basin Commission

9:00 – 9:15 – **Alternative Sources of Water in an Emergency** - utilization of home gray water, capturing stormflow, rain barrels and rain gardens, miscellaneous use of swimming pool water, etc.
Bryan Swistock, Penn State University

9:15 – 10:00 - **Current Status of the Water Resources Planning Act** – the status of implementation of this Act, how water use will be registered, forms, water use measuring techniques, future actions under the Act
Stu Gansell, PA Department of Environmental Protection

10:00 – 10:15 – **Registration of Large Water Users under Act 220 –** an update on the pre-registration results and the upcoming registration process for large water users under Act 220
Dave Jostenski – PA Department of Environmental Protection

10:15 – 10:30 – **Refreshment Break**

10:30 – 12:00 – **Panel Discussion – Inter-agency Cooperation for Water Resources Education –** agency perspectives on water resources programs and how we can strengthen future cooperation. 10-minute presentations by each panel member followed by discussion.

Cooperative Extension *– Jack Watson (moderator)*
State Conservation Commission *- Karl Brown*
PA Department of Environmental Protection *– Stu Gansell and Cedric Karper*
Natural Resources Conservation Service *– Barry Frantz*

12:00 – 1:00 – **Buffet Lunch Provided at Ramada Inn**

1:00 – 1:45 - **Western Water Conservation II** – Agricultural water conservation, CIMIS technique to estimate water needs in the home and farm, water reuse and dry year preparation
Simon Eching – California Department of Water Resources, Office of Water Use Efficiency

1:45 – 2:15 **Community Water Re-use** –explanation of the beneficial reuse project in State College as an example of re-use technology.
Cory Miller, University Area Joint Authority

2:15 – 3:15 – **Disseminating Water Conservation Education in PA – Where Do We Go From Here?** – Discussion of existing and planned programs, downlinks, publications, displays and other resources that can be used to provide water conservation and water use education in Pennsylvania.
Jim Weaver and Bryan Swistock, Penn State University

3:15 – **Evaluation and adjourn**

Each attendee received a 2" binder with relevant publications and written background related to each of the presentations. A new water conservation publication was created for use by the trained educators. This eight-page publication is entitled "Household Water Conservation". It was added to several other fact sheets already in existence related to water conservation. 5,000 copies of this publication were printed for use by Extension educators.

Ten vinyl tabletop displays were designed and created for use by Extension educators during water conservation or other programs. These displays are often used during county fairs, Farm Show, Ag. Progress Days, and other county events to publicize Extension programs and resources. One display was delivered to each of the eight Extension regions in Pennsylvania. Two additional displays are housed at University Park for use by educators.

A water conservation web page was developed and added to the existing water resources Extension site to serve as a clearinghouse for available resources related to water conservation. Various fact sheets and information about the publication and display created from this project were added to the web page. A PowerPoint presentation was also created and linked on the web page for use by Extension educators during water conservation programs. This web page is available at:
http://www.sfr.cas.psu.edu/water/water%20conservation.htm

Principal Findings and Significance:

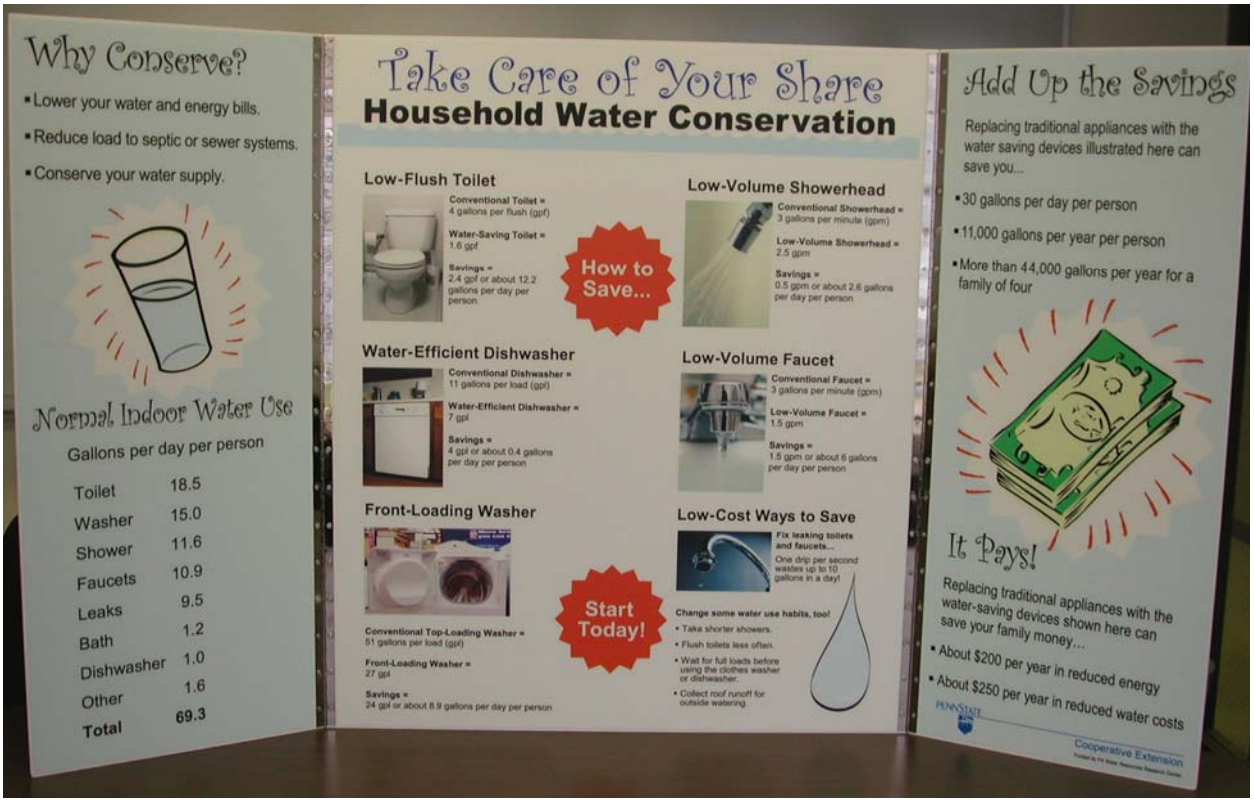
Evaluations were completed by the 25 Extension educators that attended the October workshop. Thirty-two percent rated the program as "excellent", 63% rated it as 'very good" and 5% rated it as "good". No attendees rated the program below "good". The water conservation publications and table-top displays have only recently been delivered to the attendees so impacts to the citizens of Pennsylvania will begin to occur during 2004. So far in 2004, over 400 visits have been documented to the water conservation web site.

Students Supported:

Three graduate students were supported by this project including:

1. Jennifer Sidleck, M.S., Environmental Pollution Control
2. Lindsey Donaldson, M.S., Forestry
3. Kate Schmidt, M.F.R., Forestry

Presentations and Other Information Transfer Activities:
A picture of one of the ten table-top displays distributed to the Cooperative Extension regions is shown below.


Why Conserve?
▪ Lower your water and energy bills.
▪ Reduce load to septic or sewer systems.
▪ Conserve your water supply.
Normal Indoor Water Use
Gallons per day per person
Toilet 18.5
Washer 15.0
Shower 11.6
Faucets 10.9
Leaks 9.5
Bath 1.2
Dishwasher 1.0
Other 1.6
Total 69.3
Take Care of Your Share
Household Water Conservation
Low-Flush Toilet
Conventional Toilet = 4 gallons per flush (gpf)
Water-Saving Toilet = 1.6 gpf
Savings = 2.4 gpf or about 12.2 gallons per day per person
How to Save...
Low-Volume Showerhead
Conventional Showerhead = 3 gallons per minute (gpm)
Low-Volume Showerhead = 2.5 gpm
Savings = 0.5 gpm or about 2.6 gallons per day per person
Water-Efficient Dishwasher
Conventional Dishwasher = 11 gallons per load (gpl)
Water-Efficient Dishwasher = 7 gpl
Savings = 4 gpl or about 0.4 gallons per day per person
Low-Volume Faucet
Conventional Faucet = 3 gallons per minute (gpm)
Low-Volume Faucet = 1.5 gpm
Savings = 1.5 gpm or about 6 gallons per day per person
Front-Loading Washer
Conventional Top-Loading Washer = 51 gallons per load (gpl)
Front-Loading Washer = 27 gpl
Savings = 24 gpl or about 8.9 gallons per day per person
Start Today!
Low-Cost Ways to Save
Fix leaking toilets and faucets... One drip per second wastes up to 10 gallons in a day!
Change some water use habits, too!
• Take shorter showers.
• Flush toilets less often.
• Wait for full loads before using the clothes washer or dishwasher.
• Collect roof runoff for outside watering.
Add Up the Savings
Replacing traditional appliances with the water saving devices illustrated here can save you...
▪ 30 gallons per day per person
▪ 11,000 gallons per year per person
▪ More than 44,000 gallons per year for a family of four
It Pays!
Replacing traditional appliances with the water-saving devices shown here can save your family money...
▪ About $200 per year in reduced energy
▪ About $250 per year in reduced water costs
PENNSTATE
Cooperative Extension

Awards:
None

Student Support

Student Support					
Category	**Section 104 Base Grant**	**Section 104 RCGP Award**	**NIWR-USGS Internship**	**Supplemental Awards**	**Total**
Undergraduate	8	0	0	0	8
Masters	13	3	0	0	16
Ph.D.	1	0	0	0	1
Post-Doc.	0	0	0	0	0
Total	22	3	0	0	25

Notable Awards and Achievements

Several of the projects supported during FY 2003 resulted in noteworthy achievements.

Dr. John Stolz with Duchesne University co-authored a paper with R. S. Oremland entitled Ecology of Arsenic that was published in Science in 2003 (Volume 300: 939-944).

Dr. John Stolz gave an invited paper on The Respiratory Arsenate Reductase from the Haloalkaliphile Bacillus selenitireducens: biochemical and molecular aspects at the Gordon Research Conference, Kimball Union Academy, July 3, 2003.

Dr. Fred Brenner, Margaret Dunn and Shaun Busler with Grove City College and Stream Restoration Inc. found in comparisons among a wide range of passive treatment systems for acid mine drainage that manganese removal was only achieved when horizontal flow limestone beds were used as the final treatment system.

Dr.Yeufeng Xie with Penn State, Capital Campus showed that crumb-rubber from waste rubber tires can be used as an effective, low-cost, light-weight filter medium to treat ballast water aboard ships.

Dr. William Sharpe and Bryan Swistock with Penn State, University Park Campus developed an 8-page Cooperative Extension publication Household Water Conservation for use by educators.

Publications from Prior Projects