Water Resources Research Center

Annual Technical Report

FY 1999

Introduction

WATER PROBLEMS AND ISSUES OF MISSOURI The water problems and issues in the State of Missouri can be separated into three general areas: 1) water quality, 2) water quantity, and 3) water policy. Each of Missouri's specific problems usually requires knowledge in these three areas. Water Quality: Recent news media attention to the occurrence of pesticides in drinking water in the Midwest has raised a serious public concern over the quality of Missouri drinking water and how it can be protected. With the large agricultural activity in the state, non-point source pollution is of major interest. Because of several hazardous waste super-fund sites, hazardous waste is still of a concern to the public. The Center's research has been to evaluate the quality of current waste sources and improve the methods to protect them. Areas of research for the past ten years have included (but are not limited to): erosion, non-point pollution, reclamation of strip mine areas, hazardous waste disposal, acid precipitation, anthropogenic effects on aquatic ecosystems and wetlands. Water Quantity: Missouri has a history of either inadequate amounts of rainfall, or spring floods. Because of the 1987-1989 drought years, and the flood of '93 and '95, water quantity has become a major topic of concern. Research is needed to better understand droughts and flood conditions. Water Policy: Policies and programs need to be formulated that will ensure continued availability of water, as new demands are place on Missouri water. The social and economic costs may no longer be held at acceptable levels if water becomes a major issue in cities and rural areas. Past droughts and the possible lowering of the Missouri River have raised serious questions over states' rights to water and priority uses. Research areas in this program have included drought planning, legal aspects, perception and values, economic analysis, recreation, land/water use policy and legislation, and long-term effects of policy decision. PROGRAM GOALS AND PRIORITIES The Missouri Water Resources Research Center's goals are: 1) establish active research programs to aid in understanding and solving Missouri's and the nation's water problems; 2) provide educational opportunities in research for students with an interest in water resources and related fields; and 3) be actively dedicated to the dissemination of water related information, using all aspects of the media. With these goals, the Center is able to mobilize the best faculty expertise in the state to examine specific water resources problems. The Center is familiar with research needs and activities, and it's goals are to help researchers avoid duplicate efforts and to serve as a link between the research community and potential users of research results – such as industries, planning commissions, and state agencies. Because of Missouri's economy revolves around its water resources, the director and principal investigators have worked closely with the state in addressing their problems by providing research data which are necessary in order to solve present and future water problems. Each of the research projects forwarded for regional competition has undergone a thorough evaluation process by the Water Center's Advisory Committee to determine its importance in solving Missouri's and the nation's water problems. COOPERATIVE ARRANGEMENTS The following individuals have participated in the selection and development of our 1999 research program. They have been active advisory committee members, participating in research meetings and assisting with their expertise in the area of water research, and at Center research meetings. Five proposals were submitted for regional competition. Of those five, three were funded; one of which was a continuation from last year. UNIVERSITY OF MISSOURI FACULTY ADVISORY COMMITTEE David Yourtee Associate Professor Pharmacy and Medicine MED M3-115 Kansas City Campus David Garin 227 Benton Chemistry St. Louis Campus Albert Vogt Director, School of Natural Resources 1-29 Agriculture Bldg. Columbia Campus John H. Atkinson III

Engineering Extension Columbia Campus Allen Hathway Professor Civil Engineering 332 McNutt Rolla Campus STATE OF MISSOURI ADVISORY COMMITTEE MEMBERS Dr. Russell Rhodes Southwest Missouri State University 901 South National Springfield, MO 65802 Dr. Patrick Osbourne University of Missouri – St. Louis 224 Research Building St. Louis, MO 63121 David Yourtee University of Missouri – Kansas City MED M3-115 Kansas City, MO 64110 Allen Hatheway University of Missouri – Rolla 334 McNutt Rolla, MO 65401 Jim Barks U.S. Geological Survey 1400 Independence Road Rolla, MO 65401 Steve McIntosh Water Resources Program Department of Natural Resources PO Box 176 Jefferson City, MO 65102 Mike Chippendale University of Missouri – Columbia 1-29 Agriculture Building Columbia, MO 65211 Steve Weithman Department of Conservation PO Box 180 Jefferson City, MO 65102 Michael Bart U.S. Corps of Engineering 601 East 12th Street Kansas City, MO 64106 Scott Totten Missouri Department of Natural Resources PO Box 176 Jefferson City, MO 65102

Research Program

Basic Project Information

Basic Project Information	
Category	**Data**
Title	Influence of Solids on Hydraulic and Treatment Properties of Submerged Flow Wetlands
Project Number	USDI-1434-HQ-96GR-02680-02
Start Date	09/01/1996
End Date	03/01/2000
Research Category	Water Quality
Focus Category #1	Waste Water
Focus Category #2	Wetlands
Focus Category #3	Water Quality
Lead Institution	Water Resources Research Center

Principal Investigators

Principal Investigators			
Name	**Title During Project Period**	**Affiliated Organization**	**Order**
Allen L. Thompson	Associate Professor	University of Missouri	01
Dennis Morline Sievers	Professor	University of Missouri	02

Problem and Research Objectives

Need for Research: Many small, rural communities in the Midwestern U.S. are finding it difficult to

provide adequate wastewater treatment. Elimination of federal construction grants, low tax bases, lack of properly trained technical workers and high operating costs for sophisticated mechanical treatment plants have all contributed to the problem. In response, many of these small communities have turned to natural treatment systems, including constructing wetlands, to provide wastewater treatment. Wetlands offer the potential of good treatment capability with lower construction and operating costs (WEF, 1990). Many small communities chose the submerged flow (SF) wetland design due to its ability to prevent mosquito breeding and smaller surface area requirement compared to free surface water (FWS) wetlands. However, inadequate design of hydraulics caused many of the early SF designs to fail due to surface flooding (Reed, 1993). The SF technology appears to be sound and offers small communities a viable and economic alternative to wastewater treatment, but hydraulic design needs to be improved in order for the technology to enjoy a wider acceptance. Appropriate sizing of these systems is difficult because of the lack of an acceptable method to explicitly account for site specific affects. This study will provide basic information on relationship between solids removal and wetland hydraulics, and lead to a more reliable design method for SF wetlands. Objectives of Research: 1) Quantify the influence of solids on hydraulics of SF wetlands, including relationships between media diameter and porosity for use in Ergun's equation. 2) Monitor the particle size, spatial distribution, and chemical nature of each particle fraction within an SF wetland to determine the influence of solids accumulation and degradation; to permit development and validation of an improved design model for determining the surface area needed for treatment. 3) Based on results from the above objectives, develop guidelines for SF wetland dimensions to maintain adequate BOD5 treatment under the restrictions of total flow capacity. This would permit evaluation of the interation between wetland hydraulics and treatment requirements, providing the basis for accurately designing functioning SF systems.

Methodology

In this study, tests will be conducted to examine the influence of solids on wetland flow hydraulics, and the spatial response to BOD5 and SS in a SF wetland. This information will be used to quantify the size of wetland required for adequate treatment, and provide hydraulic information needed to ensure that treatment can be achieved without exceeding system flow capacity of the system, resulting in overland flow or insufficient resident times. Hydraulic flow tests will be conducted using two large flumes (7.62 m X 0.31 m X 0.46 m) available in the Biological and Agricultural Engineering laboratories. Hydraulic flow tests using sawdust and rock mixtures have previously been conducted as an indicator of the influence of organic materials (Sun et al., 1996). Two sizes of rock media will be selected: smooth rock between 12 and 19 mm diameter, and pea gravel in diameters up to 8 mm. Water head will be varied to maintain flow rates ranging from less thatn 1 L/min to 15 L/min. Flow tests conducted with organic solids mixed into rock pore spaces over the entire bed length and within the first 10 to 30% of the total length will be made. The differential loading is important to examine because systems that fail typically fail due to plugging near the inlet section rather than plugging due to an even distribution of solids throughout the bed. No design procedure to date has been developed to address this problem or to determine its signficance. Measurements will include all parameters necessary to solve Ergun's equation. The flumes are equipped with 31 glass site tubes to measure slope of the free water surface with lateral position along the length of the flume. Resolution for individual readings is 0.5 mm. Data and procedures developed here will be incorporated into the design method demonstrated by Macmanus et al. (1992). Data are also available from a previous study where the influence of plant roots on the flow regime were quantified (DeShon et al., 1995) and a methodology developed to incorporate changes in bed porosity with plant root accumulation. Those tests were conducted with clean rock beds and with rock beds containing plants, but no solids. No measurements were taken to quantify the influence of organics from wastewater flow on system clogging and the resulting effect on head versus flow rate

requirements. For a properly operated SF wetland system, organic solid entry should be kept to a minimum. However, because of the wide variation in primary treatment systems and poor scheduling of routine maintenance, solids typically do enter the SF wetland. Therefore, the effective magnitude of plant roots and solids can be compared, with an improved understanding of the influence of each and how system performance is affected. To evaluate the effect of water treatment, six separate flumes (3 m x 1.0 m x 0.46 m) will be constructed for studying BOD5 and SS treatment with lateral position. All flumes will receive the same waste (primary treated wastewater from the Columbia, Missouri Wastewater Treatment Plant, WWTP). These flumes will be placed in a greenhouse on the University of Missouri,Columbia campus, to provide continuous treatment of wastewater throughout the year, an important feature of this study, since current methods relate treatment to temperature. These flumes will be constructed during the fall of the first year of the study. The same size rock media (pea gravel, with diameters up to 8 mm) will be placed in the flumes. Each flume will have a series of removable sections of rock so solids can be obtained for analysis. The removable sections will consist of rock enclosed in a wire mesh (approximately 0.2m x 0.2 m x 0.3 m) which can be removed from the wetland without disturbing the remainder of the bed. Samples will be removed after 6, 12, 18 and 24 months of continuous water treatment, and analyzed for particle size distribution. The oxygen demand of each fraction as well as the organic fraction will be determined. Water quality measurements will be determined using facilities in the Biological and Agricultural Engineering department. These data will help provide an indepth knowledge of the nature of the solids being trapped and how they are attenuated. These removable samples will be equally spaced in for rows across the 1 m width of each flume. Rows will be spaced along the flume length at 0.6 m intervals starting 0.6 m from the inlet. In this way, the same lateral positions along the flumes will be sampled each time to quantify the effect of time and position on the buildup of solids within in the beds. The same procedure will be used in the other three flumes, except that plants (Scripus validus) will be placed in the rock beds of three flumes, so that both the treatment influence and organic volume of solids and plant roots can be evaluated. Glass site tupbes will be installed along the length of these flumes to quantify the change in bed porosity over time. These data will also be used for model simulation of the head versus flow relationships developed based on measurement with the large flumes. Together, these will provide a comprehensive data base from which to evaluate the influence of treatment efficiency and hydraulic flow considerations.

Principal Findings and Significance

The majority of presently constructed submerged-flow wetlands (SF) have experiences water mounding problems that are caused by clogging of pore spaces with solids, biofilm, and plant root growth. Solids buildup is larger near the inlet of the bed, causing the potential for bed clogging. In an attempt to quantify solids buildup, an experimental design was set up combining three vegetated and three non-vegetated submerged-flow wetland beds (3m x 1m x 0.5m) operated in a semi-continuous-flow mode, which were fed every eight hours, with a 5-day residence time. BOD5 and TSS removals throughout the two-year operating period were high 9up to 98%) for both vegetated and non-vegetated wetlands and tended to follow seasonal variations. The presence of vegetation showed a small degree of process enhancement for the reduction of biodegradable organic matter and suspended solids within the SF environment. The annual average mass removal for ammonia nitrogen in vegetated wetlands beds was 3.3 kg ha-1d-1 (up to 95%). The nitrification process in vegetated wetlands beds was much more pronounced than non-vegetated beds (only up to38% removal). The lack of measurable dissolved oxygen in the non-vegetated wetlands likely restricted the nitrification process. The soluble phosphorus reduction varied from month to month, ranging from 27% to 100% in vegetated wetland beds and from no removal to 66% in non-vegetated beds. The soluble phosphorus reduction in vegetated beds was much higher than non-vegetated beds depending upon seasonal variations of plant growth. The particle size spectrum at the inlet always peaked before the outlet, showing a net longitudinal size spectrum

shift, which generally tended to decrease over time in response to the continuous solids loadings and internal processes of solids decomposition and regeneration within the plant-rooting media. The process of solids deposition in the SF substrate matrix was characterized by a rapid decline of particulate mass over the length of the bed, with more than 75% of the pore solids attenuated within the first of the bed length for both vegetated and non-vegetated wetland beds after two years of operation. The total solids depositions in the vegetated and non-vegetated wetland media were determined to be, respectively, 2.39 kg m-2 and 2.78 kg m-2 after two years of system operation. Net accumulations of pore solids were significantly higher ($P < 0.05$) in the non-vegetated wetland beds than in the vegetated wetland beds. A solids buildup model was developed using the mass balance approach to predict the dynamics of solids depositions and void blockages in the wetland media. Internal generation dominated the system over decomposition throughout the operation in both vegetated and non-vegetated wetlands. This led to an overall solids buildup in the wetland media. The estimated average operating value for G-D (generation – decomposition) for total pore solids depositions was 2.66 mm yr-1 for vegetated wetland media and 4.44 mm yr-1 for non- vegetated wetlands. From the increasing trend of G-D over time for pore organic accumulations, it was revealed that the system experienced the domination of internally and externally generated organics coupled with less biological decomposition, potentially leading to logging of the wetland media due to excessive solids buildup. It is concluded that in a long run, the solids buildup would significantly influence the system hydraulics as the majority of solids are deposited within the first 1/3 bed length.

Descriptors

Wetlands Wastewater Treatment Hydraulics Mathematical Models

Articles in Refereed Scientific Journals

Book Chapters

Dissertations

Water Resources Research Institute Reports

Conference Proceedings

Other Publications

Basic Project Information

Basic Project Information	
Category	**Data**
Title	Developing a Model for Watershed Management through Determining Watershed Conditions Required by the Endangered Topeka Shiner
Project Number	USDI-1434-HQ-96GR-02680-04
Start Date	09/01/1998
End Date	06/01/2000
Research	

Category	Water Quality
Focus Category #1	Conservation
Focus Category #2	Wetlands
Focus Category #3	Ecology
Lead Institution	Water Resources Research Center

Principal Investigators

Principal Investigators			
Name	**Title During Project Period**	**Affiliated Organization**	**Order**
Doug Noltie	Associate Professor	University of Missouri	01
Charles H. Nilon	Associate Professor	University of Missouri	02

Problem and Research Objectives

Runoff from agriculture and urban land-use causes significant harm to stream ecosystems and fish communities. The identification of specific characteristics of these impacts (such as quantities and toxicity) on stream ecosystems is necessary for stream water and habitat quality management. Degrading habitat and water quality are thought to be the main factors leading to extirpation of Topeka Shiner (Notropis topeka) populations, which led the U.S. Fish and Wildlife Service to list the species as federally endangered in January, 1999. Managing for the specific causes of these extipration sdepends on understanding the relationship between land-use and water quality upstream from the extipration sites. It also requires understanding the attitudes of landowners upstream from extirpation sites regarding potential land-use regulations that could remedy Topeka Shiner extirpations. We are currently constructing a regionally applicable model that quantifies the relationship between land-use and water quality relative to Topeka Shiner populations. The Study Site: The Bonne Femme Creek Watershed, including Turkey and Bass Creeks, is typical of many Midwestern drainages. The watershed includes preserved natural areas, but it is predominately agricultural and is experiencing increasing urbanization. Similar patterns of urban degradation and corresponding declines in fish species have been found throughout the Midwest. We chose the Bonne Femme watershed to identify land-use changes that are either: · sufficiently benign as to be compatible with Topeka Shiner population maintenance, or · sufficiently detrimental that extirpations have occurred. Objectives: 1) Collect and analyze historical and current land-use and current habitat information at sites in the Bonne Femme Creek watershed where Topeka Shiner populations have been extirpated (Turkey Creek) or still exist (Bass Creek). 2) Determine whether historical changes in land-use, land cover, and riparian zone characteristics are correlated with the presence or absence of Topeka Shiner populations in these watersheds. 3) Conduct an assessment of landowner attitudes toward participation in specific land recovery (as outlined by the Missouri Department of Conservation's Topeka Shiner Action Plan). 4) Develop conceptual and analytical models for these watersheds that describe and define correlations between land cover, water quantity and quality, and Topeka Shiner presence and absence.

Methodology

Principal Findings and Significance

Preliminary Results: Thus far we have concentrated on creating our land cover and land-use databases. Even so, some interesting contradictions between predicted land cover associations and Topeka Shiner presence and absence has already come to light: 1) Even though agriculture is considered to have a negative impact on Topeka Shiners, agricultural land cover has decreased in both watersheds. 2) Even though an increase in forest cover is considered to improve Topeka Shiner habitat, both of our watersheds have become increasingly forested. 3) The watershed with the greatest amount of urbanization is the one which Topeka Shiners are present (Bass Creek). 4) The watershed with the greatest number of roads is also the one in which Topeka Shiners are present. Yet as predicted, the watershed with the most rapid urban development and with the greater increase in the number of ponds is the one in which its Topeka Shiner populations have been extirpated (Turkey Creek).

Descriptors

Watershed Topeka Shiner Land-use Bonne Femme Creek Turkey Creek Bass Creek Cyprinid Agriculture

Articles in Refereed Scientific Journals

Book Chapters

Dissertations

Water Resources Research Institute Reports

Conference Proceedings

Other Publications

Basic Project Information

Basic Project Information	
Category	**Data**
Title	Development of a Simple Combustion Process for Disposal of Waste from Livestock Operations
Project Number	USDI-1434-HQ-96GR-02680-04
Start Date	03/01/1999
End Date	02/28/2001
Research Category	Water Quality
Focus Category #1	Treatment
Focus Category #2	Non Point Pollution

Focus Category #3	Agriculture
Lead Institution	Water Resources Research Center

Principal Investigators

Principal Investigators			
Name	**Title During Project Period**	**Affiliated Organization**	**Order**
Virgil Flanigan	Professor	University of Missouri	01
Shubhender Kapila	Professor	University of Missouri	02

Problem and Research Objectives

The recent statements emanating from Federal and Sate environmental agencies show a growing concern for the adverse effects of run-off from large livestock operations, [1] particularly in the midwestern states. There is ample data to show that unprotected waste from livestock operations washes into streams as non-point source (NPS) pollution. The United States Environmental Protection Agency (US EPA) estimates that 41 percent of the total NPS pollution results from agricultural sources, and a third of that is attributable to livestock operations [2]. In addition, water quality concerns such as noxious odors and the spread of pathogens and weeds are becoming serious. Clearly, alternative treatment technologies for livestock waste are required to: ? Reduce the volume of the waste ? Reduce the odor ? Destroy pathogens and other undesired components

Methodology

The objective of the proposed research is to develop and evaluate a simple combustion technique for disposal of livestock production waste streams. The proposed research will use an integrated high-efficiency fluidized combustion/drive system. The system will be conceptually similar to a process that our group has developed for treatment of industrial waste streams. The combustion system will integrate the following tasks: -- Concentrate the solid content -- Combust a portion of the dry material -- Oxidize odiferous volatiles The efficiency of the proposed system will first be tested with a bench scale set-up. Parameters of interest include the following: -- BTU value of the livestock waste -- Pathogen concentration of waste -- Pathogen concentration of treated solids -- Volatile sulfurous organics in livestock waste -- Volatile sulfurous organic concentration of the off gas -- Concentration of nutrients in the treated solids The Center for Environmental Science & Technology (CEST) and associated laboratories, occupying approximately 20,000 square feet of laboratory space, are well equipped to determine these parameters.

Principal Findings and Significance

The project was initiated in March 1999. The first four months were spent on design and fabrication of the combustor. The combustor has now been built and installed at the Center for Environmental Science and Technology at the University of Missouri-Rolla. The optimization and evaluation of the burner configuration will be carried out in the next three months. This will be followed up with trial degradation of simulated waste. These evaluations will include chemical and biological measurements to ascertain degradation efficiencies of odiferous chemicals, pathogens, pesticides, residual antibiotics and metabolites. The analytical methodologies required for the measurements are being validated. We are

planning to carry out a trial with farm waste streams during the summer and fall 2000. A complete project report will be submitted by March 2001.

Descriptors

Animal Waste Wastewater Agriculture Waste Disposal Pollution Control Treatment

Articles in Refereed Scientific Journals

Book Chapters

Dissertations

Water Resources Research Institute Reports

Conference Proceedings

Other Publications

Basic Project Information

Basic Project Information	
Category	**Data**
Title	Measurement of Waterborne Pathogens in Wetlands and Wetland Treatment Systems
Project Number	USDI-1434-HQ-96GR-02680-03
Start Date	03/01/1999
End Date	06/01/2000
Research Category	Water Quality
Focus Category #1	Wetlands
Focus Category #2	Water Quality
Focus Category #3	Methods
Lead Institution	Water Resources Research Center

Principal Investigators

Principal Investigators			
Name	**Title During Project Period**	**Affiliated Organization**	**Order**
John Richard Jones	Professor	University of Missouri	01
Jeanne Marie Erickson	Associate Professor	University of Missouri	02

Problem and Research Objectives

Waterborne diseases of significant public-health impact over the past decade have been caused by several microorganisms, including toxin-producing strains of the fecal bacteria E. coli (Craun et al., 1997), and intestinal protozoan parasites such as Giardia and Cryptosporidium (Kramer et al., 1993; Rose, 1997). Such protozoans are readily spread through fecal contamination of water by the protozoan cysts or oocysts. Moreover, cysts and oocysts excreted by animals can infect humans. Any comprehensive examination of water quality should include monitoring for microbial flora, with an emphasis on the pathogens of greatest health concern. Current reservoir research at the University of Missouri-Columbia has the overal objective of determining reservoir water quality within the state (as measure by nutrients, water clarity and algal biomass), and quantifying the major factors controlling present conditions (land use and climate). We propose to expand this research to include measurements of pathogenic microorganisms in surface waters. Objectives: We plan to develop efficient and cost effective molecular techniques to measure the presence of coliform bacteria and parasites in wetlands. One of these techniques is based on the polymerase chain reaction (PCR), an extremely powerful enzymatic reaction used to produce relatively large amounts of a specific DNA fragment from vanishingly small amounts of initial genetic material. This molecular technique is commonly used for the identification of organisms through "DNA fingerprinting," and is often employed in forensics and medical diagnostics. We will develop a sensitive, specific, reproducible and efficient PCR-based assay for the detection and eventual quantification of fecal coliforms, including toxic strains of Escherichi coli. The sensitivity of the PCR-based approach will provide greatly enhanced opportunities for the rapid identification of specific strains of microorganisms from environmental water samples. Moreover, this technique will facilitate studies by allowing for the long-term archiving of microorganisms collected from water samples onto membrane filters and available for future molecular assays in the laboratory. These studies will allow us to determine whether E. coli is a reliable water quality surrogate for the parasitic protozoans Giardia and Cryptosporidium in Missouri wetlands and reservoirs. They will also allow us to determine the relationship between the presence of fecal and pathogenic microorganisms, and other standard limnological parameters of water quality. As such, this project is a natural extension of our study of factors influencing lake fertility. Simply stated, our working hypothesis is that factors favoring the growth of algae in lakes (nutrient input from farmland and urban/suburban areas in the catchments, and certain climate patterns) may likewise correlate with the presence (abundance) of pathogenic organisms. Our molecular biomonitoring program will focus on the Eagle Bluffs Conservation Area in mid-Missouri, including the adjacent wetland treatment system for city wastewater. This large area (>4000 acres) is located 6 miles southest of Columbia, Missouri, in the flood plain of the Missouri River. Water samples will be collected weekly at multiple sites in the treatment and conservation wetlands, and samples will be assayed for microorganisms and other aspects of water quality. In summary, during the 12 months of this proposed project, we will collect water samples from conservation wetlands and adjacent wetland treatment system, and measure a variety of water quality parameters, as well as determine the incidence of total coliforms, fecal coliforms, E. coli, and the protozoan pathogens Giardia and Cryptosporidium. These data will allow us to determine the quality of water in the wetlands, the correlation between microbiological and standard limnological parameters, and the efficacy of using fecal E. coli as an indicator of the protozoan pathogens. Ultimately, we expect to incorporate the techniques developed in this project into our studies of water quality in Missouri reservoirs. A review of liturature suggests this expended effort will be one of the first large-scale, comparative lake studies to include measurement of waterborne pathogens in a routine, water quality monitoring.

Methodology

During the course of this project, we expect to collect water samples at weekly intervals from several

sites in the Eagles Bluff conservation area wetlands and the adjacent city wastewater treatment wetlands. Samples will be collected in sterile bottles using appropriate sampling techniques. These samples will be subjected to the molecular techniques described below, for the measurement and characterization of microorganisms. Fecal coliforms will also be characterized using standard bacteriological methods (Standard Methods for the Examination of Water and Wastewater). Standard limnological parameters of water quality (nutrients, etc.) will also be measured. We will compare the current standard methods for identification and quantitation of total coliforms, fecal coliforms, E. Coli, Giardia cysts, and Cryptosporidium oocysts with new molecular assays we propose to develop in this project, as described below. Polymerase Chain Reaction (PCR)-based Assays: The polymerase chain reaction allows for the enzymatic synthesis of large amounts of DNA, copied from a small amount of initial DNA (template DNA). The DNA from a single cell can serve as the template DNA, resulting in the amplification of detectable amounts of specific DNA fragments. In this technique, specific pairs of DNA oligonucleotides (primers) that bind to specific regions of the genomic DNA determine the size and identity of the genomic DNA fragment(s) that will be amplified by the DNA polymerase enzyme. The specificity of the primer pairs allows one to identify and distinguish DNA from bacteria such as E. coli, from Giardia cysts and from Cryptosporidium oocysts without the need to obtain separate, clonal isolates of each microorganism. We will adapt the PCR technique to provide 'fingerprints' of different strains of E. coli. We will determine if it is possible to analyze the PCR products from a mixture of E. coli from different sources without having to obtain pure colonies of each type of E. coli. If this is possible, it will allow a significant decrease in time and expense, compared to other methods, for the eventual determination of the source of fecal microbial contaminants. All appropriate methods and controls will be used in our protocols to ensure against contamination of the water samples and the PCR reactions with other sources of DNA template or DNA fragments.

Principal Findings and Significance

Results: In order to reduce costs in the development stage of our assay, we focused solely on coliforms, and used CsCl-purified E. coli DNA template to work out the PCR assays in which lacZ and uidA DNA fragments were amplified to identify and quantify total and fecal coliforms, respectively. Successful development of this PCR assay included the following elements: ? Concentration of microorganisms from environmental water samples in a way that allows archiving of microbes for further molecular characterization in the future. ? Purification of DNA templates from concentrated microbes, to the degree that inhibitors of PCR are removed and PCR amplification can proceed. ? Design of specific DNA primer pairs that amplify specific DNA fragments indicative of total or fecal coliforms. ? Optimization of the PCR conditions such that a unique PCR product is formed, and the products are ~ proportional to the initial template concentration over ~ 1-2 orders magnitude difference in the initial template copy number. ? Sensitive visualization of the PCR product using agarose gel electrophoresis and estimation of the concentration of coliforms in the environmental water sample. The assay is highly sensitive. We can detect as few as one copy (one genome equivalent) of E. coli DNA present in the initial PCR reaction, and can visualize the product on ethidium-bromide-stained gels without further amplification of signal. We routinely distinguish standards that include 4, 40, or 400 copies of initial E. coli template DNA. Purification of DNA templates from environmental water samples has been the most challenging part of the assay development. The methods must be fairly rapid, allow for quantitative recovery of template, and eliminate inhibitor to the extent that appropriate volumes, needed to see a positive signal, can be assayed. The most effective method provided a 10 x to 100 x purification with respect to inhibitor, depending on the source of water and the levels of inhibitors present. New Developments: In January 2000, we purchased a new instrument (LightCycler, Roche Diagnostics Inc.) capable of monitoring fluorescence signals from each PCR reaction at the end of each cycle of PCR amplification. This allows us to monitor the kinetics of accumulation of PCR product, and hence

provides a much more accurate and reproducible quantification of initial template copy number. Preliminary experiments suggest this instrument is sensitive enough to detect small copy number, and is quite accurate. We expect use of the LightCycler to decrease the assay time, and improve data acquisition and analysis.

Descriptors

Waterborne Pathogens Bacteria E. coli Giardia Cryptosporidium Biomonitoring Land-Water Interactions Model Studies Wastewater Wetlands Water Quality Water Quality Monitoring

Articles in Refereed Scientific Journals

Book Chapters

Dissertations

Water Resources Research Institute Reports

Conference Proceedings

Other Publications

Information Transfer Program

USGS Internship Program

Student Support

Student Support					
Category	**Section 104 Base Grant**	**Section 104 RCGP Award**	**NIWR-USGS Internship**	**Supplemental Awards**	**Total**
Undergraduate	2	N/A	N/A	N/A	2
Masters	3	1	N/A	N/A	4
Ph.D.	N/A	1	N/A	1	2
Post-Doc.	N/A	N/A	N/A	N/A	N/A
Total	N/A	N/A	N/A	N/A	N/A

Awards & Achievements

Publications from Prior Projects

Articles in Refereed Scientific Journals

Book Chapters

Dissertations

Water Resources Research Institute Reports

Conference Proceedings

Regmi, T., A.L. Thompson, and D. M. Sievers. 1999. Influence of solids on treatment Properties of submerged flow wetlands. ASAE Mid-Central Conference, MC99-117, St. Joseph, Missouri.

Other Publications

Martin, D., D. B. Noltie, C.H. Nilon, and J.D. Hipple. 1999. Developing a model for watershed management through determining land-use effects on the endangered Topeka Shiner (Notropis topeka). Fourth International Urban Wildlife Conservation Symposium. Tucson, Arizona (May 1-5). Poster and abstract. Martin, D., D. B. Noltie, C.H. Nilon, and J.D. Hipple. 1999. Developing a model for watershed management through determining land-use effects on the endangered Topeka Shiner (Notropis topeka). American Society for Photogrammetry and Remote Sensing Annual Conference. Portland, Oregon (May 17-21). Poster and abstract.