Texas Water Resources Institute
Annual Technical Report
FY 2002

Introduction

Research Program

During 2002-03, TWRI supported 10 research projects to graduate students at 6 universities in Texas, including Texas A&M University, Baylor University, Rice University, West Texas A&M University, the University of Texas at Austin, and Texas Tech University.

In broad terms, these studies focused on such broad issues as irrigation (1 study), water quality (5 studies), wastewater (3 studies), aquatic biology (1 study), flooding and runoff (2 studies), computer modeling (3 studies), bays (1 study), wetlands (1 study) and human health (1 study).

Nyland Falkenberg of Texas A&M University carried out field research in South Texas (Uvalde) to develop improved technologies to manage irrigation needs and control biological stressors facing agricultural crops. Jordan Furnans of the University of Texas at Austin utilized complex computer models to simulate the development of algal blooms off the Texas coast. Jennifer Hadley developed a computer simulation model that will provide real-time estimates of runoff throughout Texas over the World Wide Web. June Wolfe at Baylor is exploring the role of periphyton in processing and removing phosphorus from streams using laboratory studies. Jude Benavides of Rice created a high-tech website that incorporates the latest in computer simulation modeling to provide real-time estimates of flooding in downtown Houston. Judy Vader of Texas A&M University investigated the fate of atrazine in lake sediments at selected sites throughout Texas. Kevin Heflin of West Texas A&M University tested whether the use of cattle feeds with reduced phosphorus concentrations might lessen nutrient runoff. Audra Morse of Texas Tech measured concentrations of antibiotics in wastewater plants and effluent runoff in the Texas High Plains to determine if there might be possible human health effects. Matthew Simmons of Texas A&M University worked to design and restore a wetland in an urbanized portion of the Dallas, TX area. Amanda Bragg of Texas A&M University determined whether the use of a chemical additive (struvite) might reduce phosphorus runoff from dairy wastes.

Enhanced Flood Warnings for the Texas Medical Center: A Second Generation Flood Alert System (FAS2)

Basic Information

Title:	Enhanced Flood Warnings for the Texas Medical Center: A Second Generation Flood Alert System (FAS2)
Project Number:	2002TX47B
Start Date:	3/1/2002
End Date:	2/1/2003
Funding Source:	104B
Congressional District:	7th
Research Category:	None
Focus Category:	Floods, Models, Hydrology
Descriptors:	None
Principal Investigators:	Jude A. Benevides, Philip B. Bedient

Publication

1. Benavides, Jude. Enhanced Flood Warnings for the Texas Medical Center: A Second Generation Flood Alert System (FAS2). Texas Water Resources Institute SR 2003-017.

Title: Enhanced Flood Warnings for the Texas Medical Center: A Second Generation Flood Alert System (FAS2)

Keywords: Flood warning; Flood alert; NEXRAD; Flood protection; Brays Bayou, Texas Medical Center.

Duration: March 2002 – Feb 2003
Federal Funds Requested: $5,000.00
Non-Federal (Matching) Funds Pledged: $10,980.00

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Co-Principal Investigator: Philip B. Bedient, Ph.D., P.E., Hermann Brown Professor of Engineering, Dept. of Civil and Environmental Engineering, Rice Univ., E-mail: **bedient@rice.edu**
Congressional District: U.S. Congressional District # 2670

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Results and Progress to Date:

Significant progress has been made over the last year with respect to developing an enhanced flood warning system for Brays Bayou and the Texas Medical Center in Houston, Texas. Research has been made possible by a wide range of funding sources in addition to the TWRI, including the Federal Emergency Management Agency (FEMA), the Texas Medical Center (TMC), and Rice University. The research funds provided by TWRI were specifically used to upgrade computer hardware capabilities to permit the wide ranging and intense computational analyses performed as part of this research.

The second generation Rice University / Texas Medical Center Flood Alert System (FAS2) has upgraded the capabilities of the current FAS by incorporating recent advances in NEXRAD technology, weather prediction tools and GIS-based distributed hydrologic models. This section briefly presents results and progress to date in each of these areas.

Next-Generation Radar and Quantitative Precipitation Forecasts

The lead-time afforded by the first generation FAS is being improved by the incorporation of a Quantitative Precipitation (QPF) algorithm in its rainfall analysis process. The QPF algorithm selected for analysis and application to a hydrologic model was based on the Growth and Decay Storm Tracker (GDST) developed at MIT (Wolfson, Forman et al. 1999) . The GDST provides forecasts of 16-level precipitation at grid scales as small as 1 km^2. GDST-based data has been obtained through Vieux and Associates, Inc. (VAI). The data product provided by VAI, called PreVieux, provides up to 60-minute forecasts (or extrapolations based on radar images) for each radar volume scan. Forecasts are provided in 5 minute bins; therefore, each radar scan has 12 associated forecast images or datasets beginning with the t+5 minute scan and continuing with t+10, t+15 and so forth up to t+60 minutes. The algorithm currently uses 16-level, base reflectivity, lowest radar tilt data.

The goal of this portion of the research was to evaluate the performance of the GDST from a hydrologic perspective, first from a rainfall intensity perspective and then later incorporate the data into a hydrologic model. The impetus for this research was based on the previous use and performance of the original FAS. It was observed that while the FAS provided about 2 hours of lead time from a strictly hydrologic perspective, system users were deriving qualitative estimates of rainfall in the future from observed storm motion in the radar image loops. Any method to quantify the future position and intensity of existing storms would greatly reduce the error associated with these qualitative estimates. Figure 1 provides an example of the

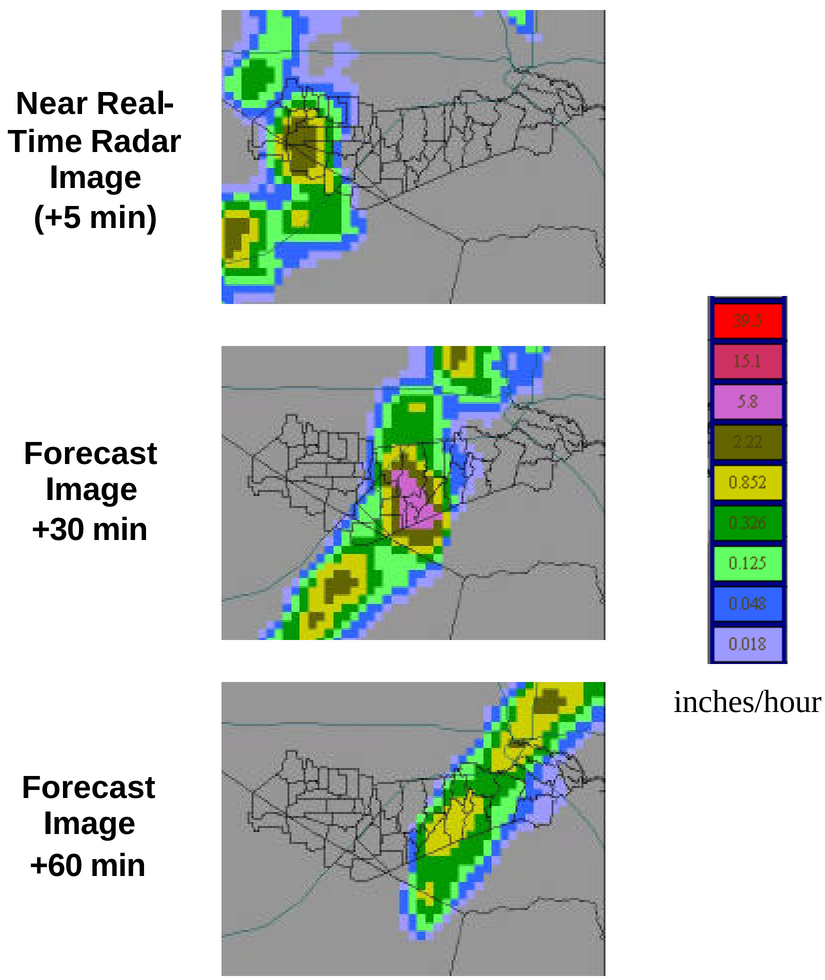

Figure 1 : GDST (PreVieux™) data in gridded format over Brays Bayou

GDST data as provided by VAI. The figure shows the progression of a frontal storm as predicted by the algorithm. The grid values are intensities in inches/hour and are superimposed on the subwatersheds of Brays Bayou.

QPF data based on the GDST algorithm was obtained through VAI for the period May 2002 through December 2002. Twenty-seven separate rainfall events have been identified and collected over that period. Although the data is available in gridded format as seen previously, for the purposes of this study, the data was provided in subbasin averaged rainfall format. Figure 2 shows an example of this basin averaged data for a storm event on April 7th, 2003, during

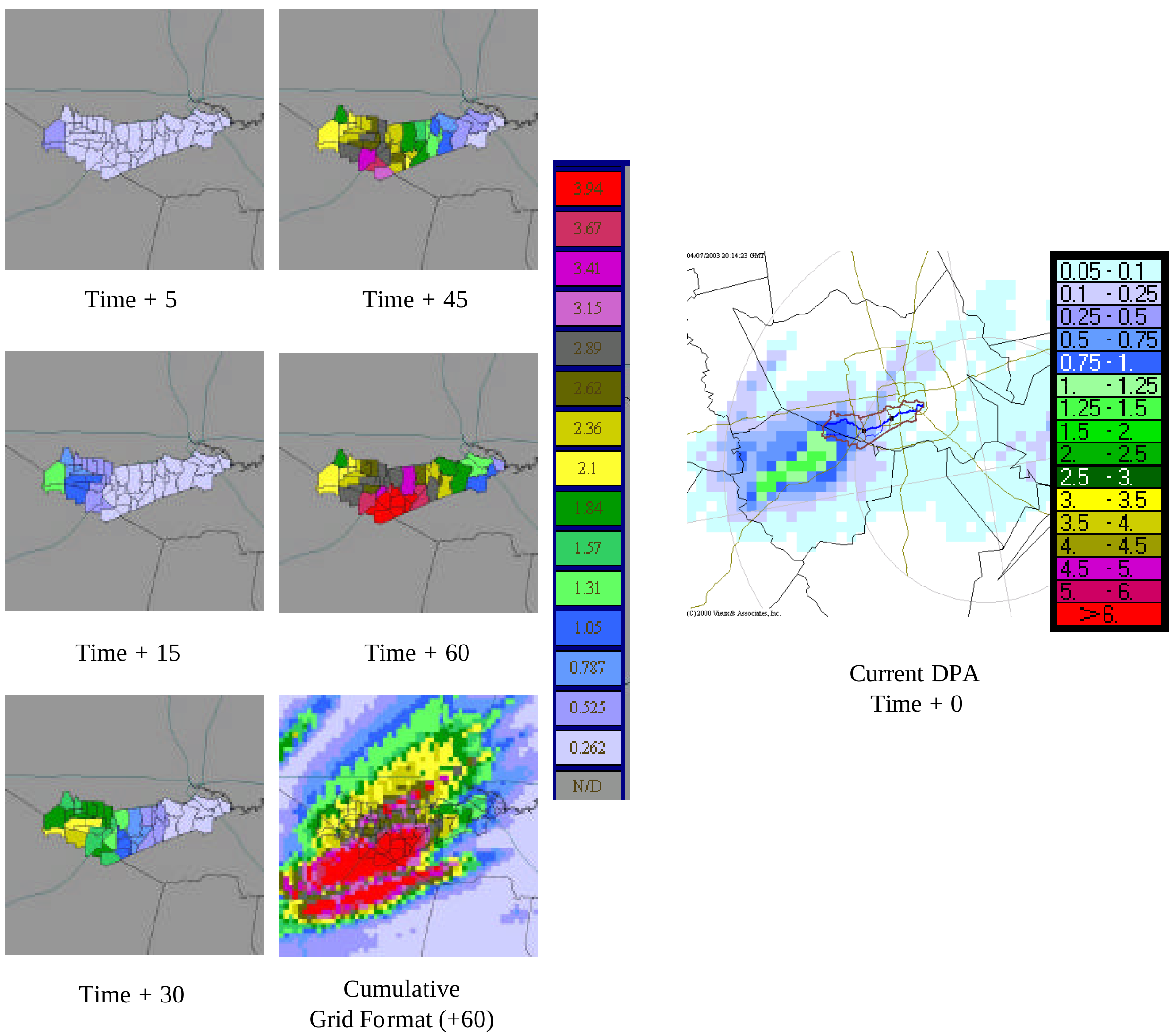

Figure 2 : QPF (PreVieux TM) and DPA data for a storm cell moving west to east across Brays Bayou on April 7th, 2003 (Color schemes for each legend are different)

which an isolated storm cell moved from west to east across Brays Bayou. The images on the left are a PreVieux™ product operating in real-time and show the cumulative predicted rainfall expected over a 60 minute period in inches. Snapshots of the basin averaged values were taken at 15 minute intervals. The image in the lower right corner shows the same data accumulated over 60 minutes but in the 1 km^2 grid format. The image on the right is the radar image displayed on the current FAS website, which shows the Digital Precipitation Array product. The DPA exhibits rainfall (in inches) that has fallen over the previous 60 minutes in a 4 km^2 grid format.

On-going research is focusing on comparing the QPF data at various forecast time intervals (+15, +30, +45, and +60) to the actual radar data and then rain gages to determine the feasibility of incorporating it with a hydrologic model. Preliminary results are indicating that the algorithm performs acceptably well for line storms (well-organized frontal systems) up to the +45 to +60-minute forecast interval. While the algorithm does not perform as well for convective systems, exhibiting the approximately the same skill for frontal storms at only the +30 min forecast interval, additional research must be performed to confirm the results. Additionally, the QPF algorithm's performance remains to be evaluated once coupled with and used as input to a hydrologic model.

Development of Real-Time Hydrologic Models

The second major improvement to the original FAS completed as part of this research is the creation of real-time hydrologic models that make the best use of radar data, QPFs, and the information dissemination capabilities of the internet. Two real-time models have been developed and are scheduled to eventually replace the "nomograph" approach used in the current system. Two models were developed, one a distributed hydrologic model and the other a lumped parameter hydrologic model, to enable the system to draw on the strengths of each modeling approach.

The distributed model being used in this study was created using a proprietary software package called Vflo™, developed by VAI. The Brays Bayou Vflo™ model was developed by Eric Stewart and has been calibrated and validated against historical storms. A real-time operational structure for this particular model has been developed by VAI and will be incorporated in the new system shortly. Figures 3 and 4 show the Vflo interface and two different scale views of the Brays Bayou model.

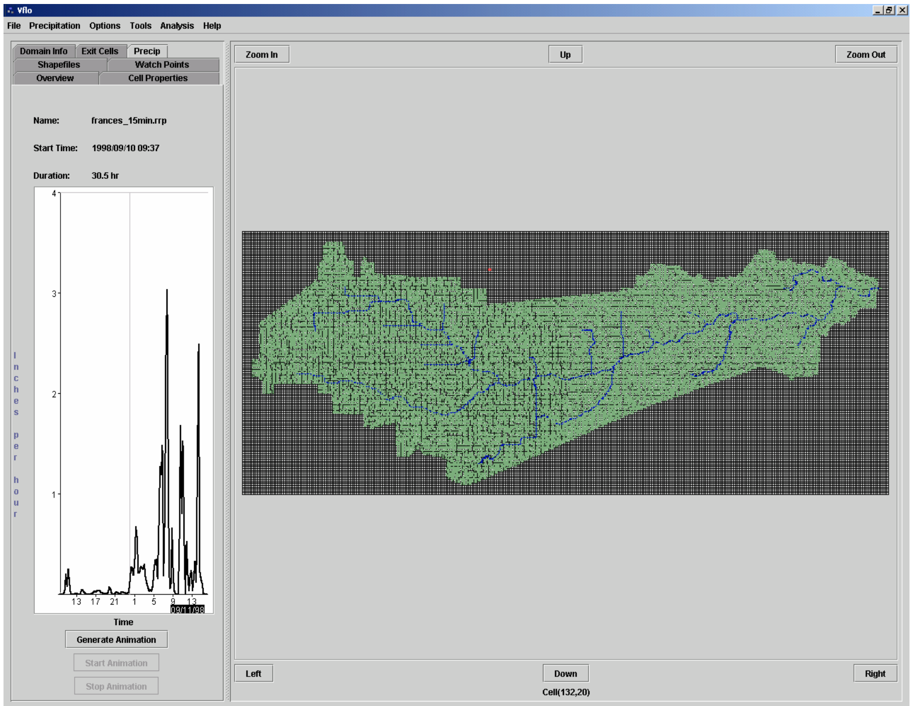

Figure 3 : Screenshot of the newly developed Brays Bayou Vflo^{TM} Distributed Hydrologic Model

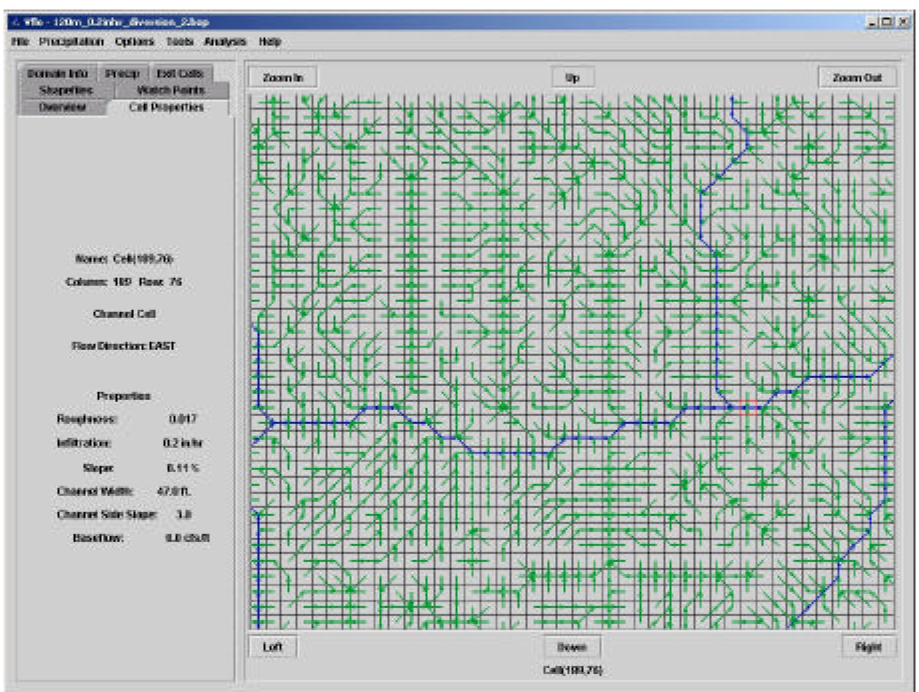

Figure 4 : Close-up screenshot of the Brays Bayou Vflo^{TM} model showing both overland and stream flow connectivity

The lumped parameter model created for use in this study was developed using the standard HEC-1 / HEC-HMS hydrologic modeling programs used in flood studies throughout the United States. However, the Brays Bayou HEC-1 model has been upgraded with a novel real-time interface, permitting both the incorporation of real-time rainfall data and the dissemination of real-time flow hydrographs for Brays Bayou. The interface has been tested for several small storm events in early 2003. The Real-Time HEC-1 Brays Bayou Model (RT HEC-1) remains to be calibrated and validated against both historical and real-time storms. It is hoped that this will be completed by late Summer / early Fall 2003. Figure 5 illustrates the RT HEC-1 real-time output for a small storm event over Brays Bayou on March 3rd, 2003. The graphs show the

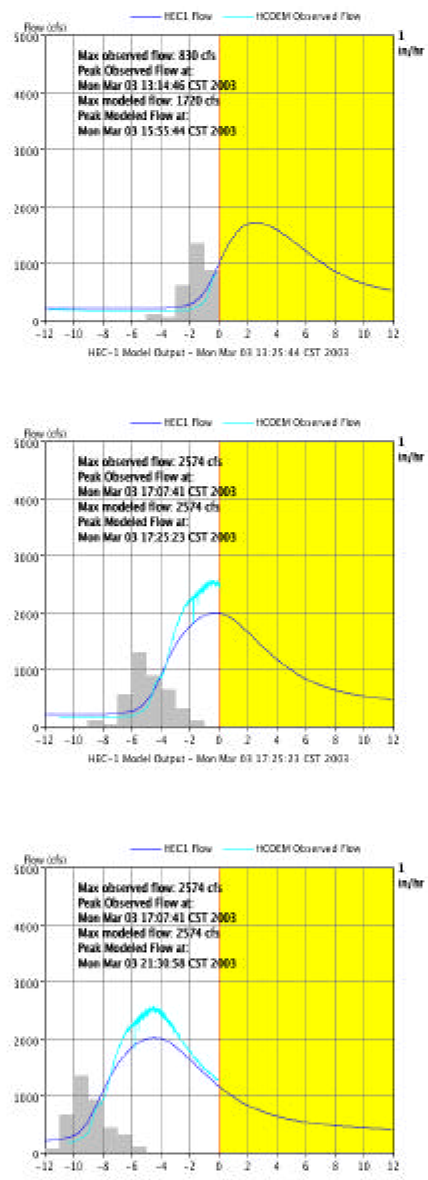

Figure 5 : Real-Time HEC-1 model results for a small storm over Brays Bayou (uncalibrated)

progression of the flood wave past Main St. The vertical red line in the center represents the "now-line" or time of current observation. The light blue line is the observed stream flow data as recorded by the Harris County Office of Emergency Management (HCOEM). The dark blue line represents the modeled hydrograph based on HEC-1 runs using Digital Precipitation Array (DPA) NEXRAD radar as rainfall input. The rainfall intensities are illustrated with gray hyetographs in each figure. The differences between the observed and modeled hydrographs are attributed to the fact that the model is currently uncalibrated and the fact that the storm event was quite small.

System Redundancy and Web-based Improvements

A wide range of operational system improvements have been completed. These include the securing of a second radar rainfall feed from the KGRK NEXRAD installation located in central Texas. This second feed is in addition to the currently used KHGX NEXRAD feed located in Dickinson, Texas. The need for radar feed redundancy was highlighted in the summer of 2002 when the KHGX installation was out of service for approximately 2 weeks after it suffered multiple lightning strikes. The system now has the capability to illustrate radar images and process radar rainfall data from each installation.

Additional system servers are currently being installed for a total of three server locations: Rice Univeristy, the Texas Medical Center, and the University of Oklahoma. The multiple server locations will allow the alert system to continue to process information and issue warnings and flood updates even in the event of a local loss of electrical power. Additional methods of communicating these alerts are being implemented to include automated email, pager, and cell phone alerts.

A number of improvements have been made to the current website including improving the efficiency of the web page by developing custom JAVA scripts, enabling the system to withstand a larger number of "hits" during critical times of operation.

Improved Alert Level Information for Harris Gully and the Texas Medical Center (TMC)

A detailed study has been completed of historical rainfall and stream flow levels at the Harris Gully / Brays Bayou confluence in order to determine a new set of alert level data for the Texas Medical Center. The updated alert levels are still in the process of being evaluated and verified, although initial results are indicating that the action levels might become less stringent –

effectively reducing the number of false alarms and thus, reducing inefficiencies and costs to the overall operation of the TMC. These new alert levels are being developed in close cooperation with TMC emergency response personnel and other consultant agencies currently working on the flood proofing/flood protection measures in the “tunnel system” of the TMC.

Reduced Phosphorus Pollution from Dairies by Removal of Phosphorus from Wastewater through Precipitation of Struvite

Basic Information

Title:	Reduced Phosphorus Pollution from Dairies by Removal of Phosphorus from Wastewater through Precipitation of Struvite
Project Number:	2002TX49B
Start Date:	3/1/2002
End Date:	2/1/2003
Funding Source:	104B
Congressional District:	8th
Research Category:	None
Focus Category:	Agriculture, Water Quality, Treatment
Descriptors:	None
Principal Investigators:	Amanda Bragg, Kevin McInnes

Publication

1. Bragg, Amanda. Reducing Phosphorus in Dairy Effluent Wastewater through Flocculation and Precipitation. Texas Water Resources Institute SR 2003-009.

Reducing Phosphorus in Dairy Effluent Wastewater through Flocculation and Precipitation

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Objective

The objective of my research is to find methods to reduce the phosphorus concentration in dairy effluent wastewater through removal of suspended solids and precipitation of calcium or magnesium-ammonium phosphates.

Hypothesis

The majority of phosphorus in fresh dairy effluent is associated with suspended solids. Removal of solids before wastewater enters the holding lagoons would considerably reduce phosphorus content of water that is held in the lagoons, and reduce phosphorus applied to land when the water is used for irrigation. In addition, based on chemical solubilities, it should be possible to precipitate soluble phosphorus remaining in wastewater as calcium and magnesium-ammonium phosphates if the pH of the wastewater were raised with an addition of ammonium hydroxide. Combined with flocculation of solids, precipitation of soluble phosphorus could leave wastewater applied to fields with agronomically manageable levels of phosphorus.

Materials and Methods

Fresh dairy effluent samples were obtained from a 2000-head dairy in Comanche, Texas. Samples were collected before the wastewater entered the lagoons and stored at room temperature in 50-gallon plastic drums. The drums were open to the room air through a small hole in the barrels' bung. Solids were re-suspended once when the barrels were placed in the laboratory and then allowed to settle with time. Subsamples were withdrawn from the barrels at the time of resuspension and at weekly intervals thereafter.

Flocculation

Suspended solids in the subsamples were flocculated with a mixture of diallyl-dimethyl ammonium chloride (DADMAC) and a medium charge density, high molecular weight, cationic polyacrylamide (PAM). The flocculant was mixed with 40 mL of effluent and allowed to settle. After the floccules settled, clear solution was decanted and analyzed for phosphorus, sodium, ammonium, calcium, magnesium, zinc, manganese, copper, iron, and potassium. Concentrations in flocculated samples were compared to untreated samples.

Precipitation

Studies were conducted to determine when and how high the pH should be raised to precipitate phosphorus. These studies involved filtering the solution after flocculation and then adding ammonium hydroxide solution to 40 mL of the effluent to produce pHs from 8.8 to 9.3. Other studies focused on the effect that flocculated material had on precipitation and that concentration of flocculant used to remove the suspended solids had on precipitation.

Results

Flocculation

After adding the DADMAC/PAM treatment and mixing the effluent, flocculation occurred in a short time (Figure 1). Within minutes, floccules, aggregates of suspended solids, formed and either floated to the top or sank to the bottom of the column. Whether the floccules floated or sank appeared to be related to the amount of air entrapment in the aggregated masses.

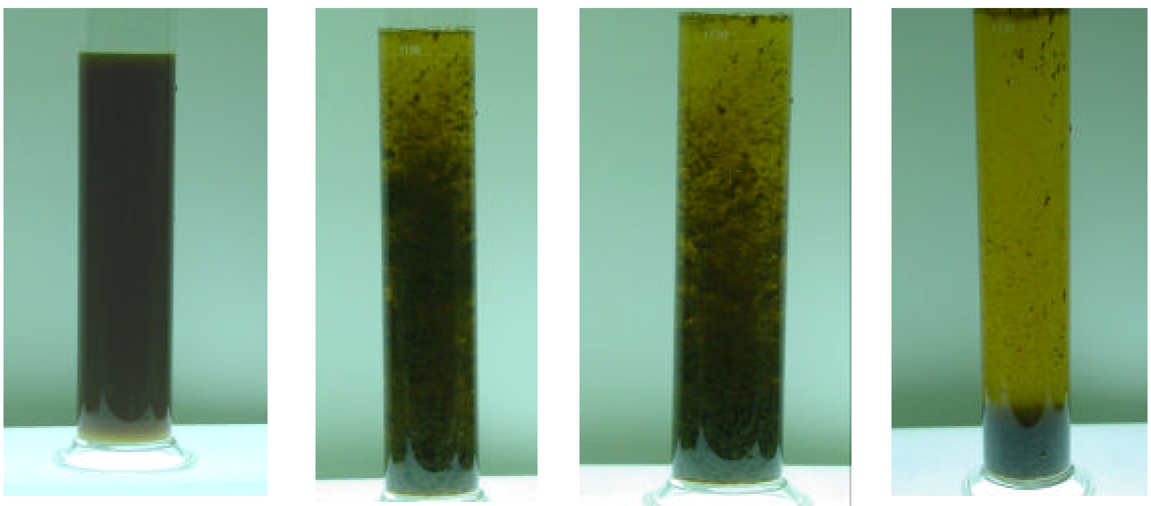

Figure 1: Flocculation of suspended solids in effluent with DADMAC/PAM flocculant. Left to right: untreated effluent, treated effluent 30 seconds after, 1 minute after, and 10 minutes after addition of flocculant.

During storage, solids settled from the solution and total phosphorus concentration in the suspension decreased (Table 1). This mechanism of separation is slow and accounts for an accumulation of phosphorus at the bottom of a lagoon. This phosphorus in the bottom of the lagoon then has the potential to mineralize and form soluble phosphorus. Best management practices suggest if the solids were kept out of lagoons by a fast-acting flocculation processes such as shown using the DADMAC/PAM combination decreased costs of dredging and extended lagoon life would be realized. Additionally, recent studies indicate that the majority of the solids that enter lagoons are converted to methane by microbes and lost to the atmosphere. Methane is a greenhouse gas targeted for reduced emissions.

Table 1: Average % of Phosphorus removed over treatments and time

TREATMENT CONCENTRATION (MG/L)	DAY 1	DAY 8	DAY 15	DAY 30
0	0	39.76	48.83	64.33
0.13	8.41	54.25	51.39	70.06
0.42	11.05	60.06	53.81	68.30
1.3	37.03	62.84	57.86	75.34
3.73	65.21	70.85	60.63	79.30

Larger doses of flocculant were less efficient in reducing phosphorus concentration with time. The decreased efficiency of efficiency was most likely because there were fewer solids in suspension to be flocculated.

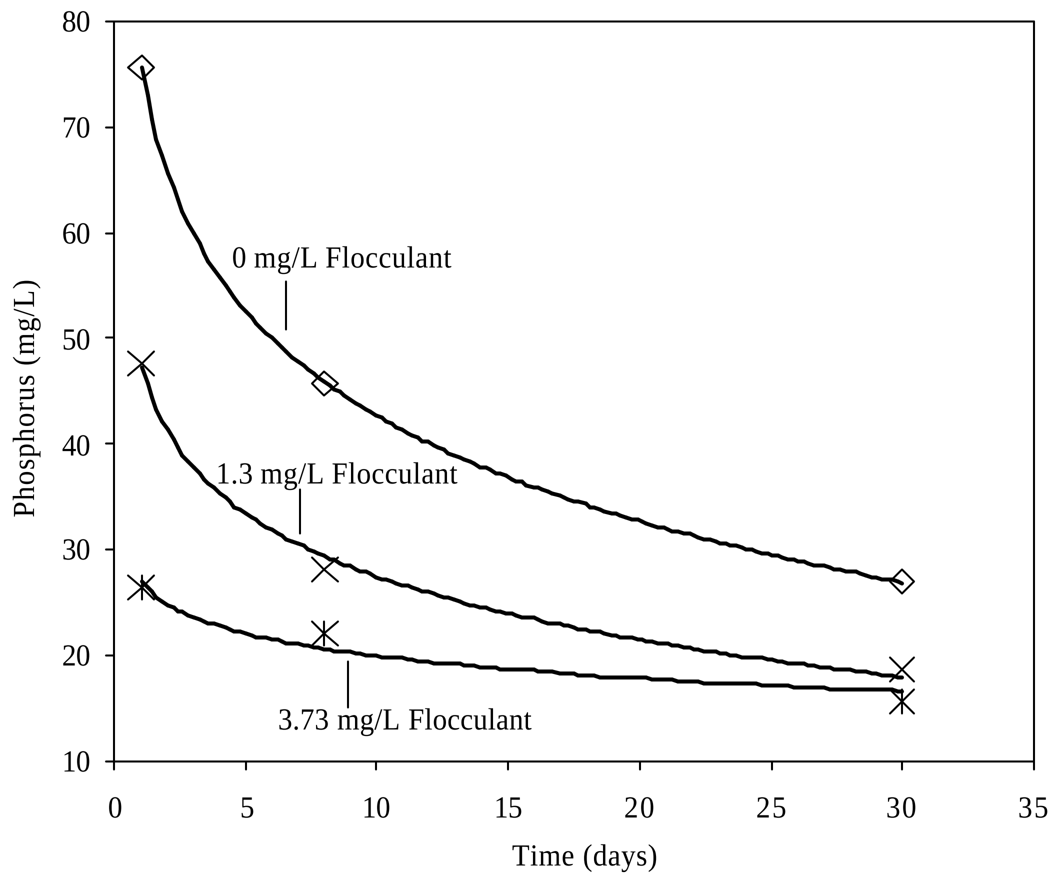

Figure 2: Total P remaining in solution or suspension as a function of time and concentration of flocculant.

Precipitation

When effluent pH was raised above 9 with addition of NH_4OH, soluble phosphorus and calcium declined considerably (Table 2). The concentrations of magnesium did not show a significant change after the pH was raised so the reduction in phosphorus was probably as one or a combination of numerous possible calcium phosphates compounds. The phosphorus which was removed by raising the pH was not precipitated out as struvite, an ammonium-magnesium phosphate. Struvite forms readily in effluent from swine operations, but not from dairy operations.

Table 2: Average Phosphorus and Calciumreduction after raising the pH to 9.1 with NH_4OH at 30 days after suspension.

FLOCCULANT CONC.	P BEFORE	P AFTER		CA BEFORE	CA AFTER	
mg/L	mg/L	mg/L	% reduction	mg/L	mg/L	% reduction
0	25	26	0	217	217	0
0.13	27	9	65	250	31	87
0.42	26	7	71	274	29	89
1.3	18	3	81	274	16	94
3.73	16	2	90	260	14	94

Conclusion

Phosphorus concentrations in dairy effluent can be reduced considerably by treating the effluent with flocculates to remove suspended solids and then with a base such as ammonium hydroxide to precipitate soluble phosphates.

Increase Water Use Efficiency: Implementation of Limited Irrigation for Crop Biotic and Abiotic Stress Management

Basic Information

Title:	Increase Water Use Efficiency: Implementation of Limited Irrigation for Crop Biotic and Abiotic Stress Management
Project Number:	2002TX50B
Start Date:	3/1/2002
End Date:	2/1/2003
Funding Source:	104B
Congressional District:	23rd
Research Category:	None
Focus Category:	Agriculture, Irrigation, Water Use
Descriptors:	None
Principal Investigators:	Nyland R. Falkenberg, Giovanni Piccinna

Publication

1. Falkenberg, Nyland. Site Specific Management of Plant Stress Using Infrared Thermometers and Accu-Pulse. 2002 American Society of Agronomy Meeting (ASA), Indianapolis, IN.
2. Falkenberg, Nyland. Remote Sensing for Site Specific Management of Biotic and Abiotic Stress in Cotton. 2003 Beltwide Cotton Conference, Nashville, TN.
3. Falkenberg, Nyland. Will be presenting again at the Beltwide Cotton Conference in San Antonio, TX and in Denver, CO at the American Society of Agronomy Meeting for the 2003-04 meetings.
4. Falkenberg, N. R., G. Piccinni, M. K. Owens, and J. T. Cothren. Increased Water Use Efficiency-Limited Irrigation to Manage Crop Stress: A Remote Sensing Study. Texas Water Resources Institute SR 2003-003.

REMOTE SENSING FOR SITE-SPECIFIC MANAGEMENT OF BIOTIC AND ABIOTIC STRESS IN COTTON

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M.K. Owens[1], and Dr. Tom Cothren[2]
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[2]Texas Agricultural Experiment Station
College Station, TX

Abstract

This study evaluated the applicability of remote sensing instrumentation for site-specific management of abiotic and biotic stress on cotton grown under a center pivot. Three different irrigation regimes (100%, 75%, and 50% ETc) were imposed in the cotton field to: 1) monitor canopy temperatures of cotton with infrared thermometers (IRTs) to pinpoint areas of biotic and abiotic stresses, 2) compare aerial infrared photography to IRTs mounted on center pivots to correlate areas of biotic and abiotic stresses, and 3) to relate yield and yield parameters relative to canopy temperatures. Pivot mounted IRTs and IR cameras were able to differentiate water stress between the irrigation regimes. However, only the IR cameras were effectively able to distinguish between biotic (cotton root rot) and abiotic (drought) stresses with the assistance of ground-truthing. Cooler canopy temperatures were reflected in higher lint yields. The 50% ETc regime had significantly higher canopy temperatures, which were reflected in significantly lower lint yields when compared to the 75 and 100% ETc regimes. Deficit irrigation up to 75% ETc had no impact on yield, indicating that for this year water savings were possible without yield depletion. Canopy temperatures were effective in monitoring plant stress during the canopy development.

Introduction

In 1993, the Texas Legislature placed water restrictions on the farming industry by limiting growers to a maximum use of 2 acre-foot of water per year in the Edwards Aquifer Region. Since then, maximization of agricultural production efficiency has become a high priority for numerous studies in the Winter Garden Area of Texas. Recent investigations have proposed Site-Specific Management (SSM) as an alternative to address this problem. SSM involves satellite-based remote sensing technology and mapping systems to detect specific areas suffering from stress within a field (i.e. water, insect, and disease stress). Crop canopy temperature has been found to be an effective indicator of plant water stress (Moran, 1994). Coupled with remote sensing technology, this concept allows collection and analysis of temperature data from crops using infrared thermometers (IRTs). IRTs mounted on irrigation systems or operated from aircraft can detect water stress by recording changes in leaf temperature caused by the alteration of the soil-plant water flow continuum (Hatfield and Pinter, 1993; Michels et al., 1999). Therefore, remote sensing equipment and mapping systems provide an excellent potential for producers to grow crops under high water use efficiency, by treating only the areas where treatment is needed (i.e. irrigation).

Objectives

The overall objectives of this project are as follow: 1) use remote sensing instrumentation for locating areas showing biotic and abiotic stress signs and/or symptoms in a cotton field, 2) evaluate canopy temperature changes in cotton with the use of IRTs, 3) and assess yield and yield parameters relative to the canopy temperatures.

Materials and Methods

The experiment was conducted at the Texas A&M Agricultural Research and Extension Center in Uvalde, Texas. Cotton variety Stoneville 4892B/Round-up Ready was planted in a circle at 50,000 ppa on 40-inch row spacing and grown under a center-pivot LESA (Low Elevation Sprinkler Application) irrigation system. Furrow dikes were placed between beds to increase water capture and minimize run-off. The soil type is a Knippa clay soil (fine-silty, mixed, hyperthermic Aridic Calciustolls) with a pH of 8.1. Three irrigation regimes (100%ETc, 75%ETc, and 50% ETc) were replicated twice in a randomized block design. A 90-degree wedge was divided equally into six 15-degree regimes, which were maintained at the above mentioned (ETc) values. Thirty Exergen (Irt/c.01-T80F/27C) infrared thermometers (IRTs) were mounted at approximately 15-foot spacing along the pivot length to scan the canopy temperature as the pivot moved. The IRTs recorded canopy temperatures every 10 seconds, and average temperature values every 60 seconds, on a 21X Campbell Scientific datalogger. In addition, canopy temperature differences were determined among treatments using a helicopter equipped with a Mikron 7200 LWIR (Long Wave-length Infrared) infrared camera with infrared band of 8-14 microns. Physiological parameters (i.e., leaf water and osmotic potential) were taken from leaves to determine the level of stress imposed by the different irrigation regimes and the presence of disease. Temperature data were statistically analyzed by ANOVA and separated by Fisher's LSD at $\alpha = 0.05$. Aerial infrared temperature readings were analyzed by using the program Mikroscan 2.6.

Results and Discussion

Environmental conditions for the 2002 cotton season showed that the minimum and maximum temperatures were normal for the area, but excessive rainfall in the month of July prevented the imposition of differential irrigation regimes. Significant differences in canopy temperature were detected in all three irrigation regimes, with a linear increase in canopy temperature resulting from a decrease in plant water availability. Extreme temperatures detected early in crop development were related to the detection of bare soil and moisture availability in the soil by the IRTs. Pivot mounted IRTs were effective in detecting crop canopy temperature differences between the 3 irrigation regimes. Early in the season there were significant differences between all three irrigation regimes; however, at the end of the growing season no significant differences were found between the 100 and 75% ETc regimes. These results are also best explained by yield differences. No significant differences in lint yield were found between the 75% and 100% ETc regimes. Yield from the 50% regime were significantly less than the 75% and 100% ETc regimes. This yield reduction is associated with increased canopy temperatures of this regime. Yields were 1160 lb/acre, 1420 lb/acre, and 1600 lb/acre for the 50%, 75%, and 100% ETc treatments, respectively. Abiotic and biotic stress can be differentiated better by the Mikron 7200 than the pivot mounted IRTs because of its increased image scanning resolution. The IR camera was able to detect distinct canopy temperature differences between all 3 irrigation regimes. Biotic stress (root rot) was detected by using the camera before symptoms could be detected visually. Pivot mounted IRTs and IR cameras were able to differentiate water stress between the irrigation regimes, but only IR cameras were able to distinguish between abiotic and biotic stress. There was an excellent correlation between canopy temperature and lint yield. Deficit irrigation up to 75% ETc had no impact on yield, indicating that water savings are possible without yield depletion. Also, canopy temperature can be an excellent tool to monitor plant stress.

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Acknowledgements

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Higher-Order Statisticts in Transport and Evolution of Algae Blooms

Basic Information

Title:	Higher-Order Statisticts in Transport and Evolution of Algae Blooms
Project Number:	2002TX51B
Start Date:	3/1/2002
End Date:	2/1/2003
Funding Source:	104B
Congressional District:	10th
Research Category:	None
Focus Category:	Water Quality, Nutrients, Ecology
Descriptors:	None
Principal Investigators:	Jordan E. Furnans, Ben R. Hodges

Publication

1. Furnans, Jordan, David Maidment, and Ben Hodges. An Integrated Geospatial Database for Total Maximum Daily Load Modeling of the Lavaca Bay - Matagorda Bay Coastal Area. Texas Water Resources Institute SR 2002-018.

Higher-Order Statistics in Transport and Evolution of Algae Blooms

By Jordan Furnans

The purpose of this project was to determine the capacity of numerical models for transporting distributive information needed for accurate modeling of algal blooms. The first phase of this project involved a numerical analysis of the feasibility of modifying the standard transport equation for quantities more accurately described by higher order statistics rather than just by mean values. This numerical exercise demonstrated reasonable results are obtainable as by applying the transport equation to local mean values across the distribution of the quantity transported by the flow. It also demonstrated the need to develop "particle tracking" capabilities in the numerical models in order to accurately describe the energy flux path the particles & transported objects follow in the flow. This path determines the environmental conditions to which the transported substance is subjected, and therefore aides in determining the affects of the conditions on the time history of the transported substance. In reference to algal blooms, the energy flux path is vital in determining the life history of the algae particles contributing to a bloom.

The second phase of this project involved the development of sub-grid scale particle tracking capabilities within the 3D hydrodynamic model ELCOM. This work was conducted while I was researching at the University of Western Australia on a US Fulbright Fellowship. The particle tracking model that was developed has been checked for accuracy against field measurements of drifter movement in Lake Kinneret (Israel) as well as in the Marmion Marine Park in Western Australia. Further analysis is being conducted, but the preliminary results are that the particle tracking model follows directly from the results of the hydrodynamic model, and variations between field and numerical drifter results are predominantly indicators of the overall inaccuracy of the hydrodynamic model given the boundary conditions imposed.

The third phase of this work will involve the quantification of horizontal dispersion/diffusion coefficients detemined from field and numerical drifters. This work will form the final portion of my Ph.D. research, which will be completed by May, 2004.

The papers that are currently under development as a result of the TWRI grant are:

1. On Horizontal Dispersion in the Coastal Boundary Layer
2. Numerical Modeling of Lagrangian Drifters

These working titles are likely to be changed. The first paper focuses on the calculation of horizontal dispersion coefficients in the costal zone using field and numerical drifters in Marmion Marine Park. The second paper details the working numerics and the accuracy of the particle tracking routine within the ELCOM model, as verified against an analytically derived velocity field. Each of these topics will be addressed in the final report submitted to TWRI in June, 2003.

Real-Time Distributed Runoff Estimation Using NEXRAD Precipitation Data

Basic Information

Title:	Real-Time Distributed Runoff Estimation Using NEXRAD Precipitation Data
Project Number:	2002TX58B
Start Date:	3/1/2002
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Funding Source:	104B
Congressional District:	8th
Research Category:	None
Focus Category:	Models, Floods, Hydrology
Descriptors:	None
Principal Investigators:	Jennifer Hadley, Raghavan Srinivasan

Publication

1. Hadley, Jennifer. Near Real-Time Runoff Estimation Using Spatially Distributed Radar Rainfall Data. Texas Water Resources Institute SR-2003-015.

Real-Time Distributed Runoff Estimation Using NEXRAD Precipitation Data
Progress Report by
Jennifer Hadley, Forest Science Department, TAMU

The objective of this study is to develop near real-time runoff estimation for Texas using precipitation data from the Next Generation Weather Radar (NEXRAD) network. This will provide information useful for flood mitigation, reservoir operation, and watershed and water resource management practices.

Materials and Methods

The datasets used in this analysis were the USGS Multi-Resolution Land Characteristic (MRLC) dataset, the USDA-NRCS State Soil Geographic Database (STATSGO), and the Next Generation Weather Radar (NEXRAD) data. The MRLC dataset served as the land cover information and the STATSGO database was used to determine the hydrologic soil group for the analysis areas. Corrected NEXRAD data was used for daily precipitation information.

The runoff estimates for each grid cell were calculated using the Soil Conservation Service (SCS) Curve Number Method, which provides a means of estimating runoff based on land uses, soil types, and precipitation. This calculation is based on the retention parameter, S, initial abstractions, I_a (surface storage, interception, and infiltration prior to runoff), and the rainfall depth for the day, R_{day}, (all in mm H_2O).

The retention parameter is variable due to changes in soil type, land use, and soil moisture, and is defined as:

$$S = (1000 / CN - 10), \text{ where } CN \text{ is the assigned SCS curve number}$$

For the runoff calculations, initial abstractions were approximated as $0.2S$, and NEXRAD rainfall maps were used to identify R_{day}. The runoff equation becomes:

$$Q_{surf} = (R_{day} - 0.2S)^2 / (R_{day} + 0.8\, S)$$

Runoff will occur only when $R_{day} > I_a$ (Neitsch et al., 2001).

A curve number (CN) grid was generated based on the MRLC and STATSGO datasets at a 100m × 100m resolution with the use of ESRI's ArcInfo and the ArcGIS 3.1.2 raster calculator. The CN was assigned based on average soil moisture conditions (Table 1), and then altered to account for the antecedent soil moisture conditions.

Table 1. Curve number assignments based on land use / land cover

Land Use/ Land Cover	Curve Numbers (Soil Hydrologic Group A, B, C, D)
Water	100
Urban	77, 85,90,92
Forest	36, 60, 73, 79
Rangeland	30, 58, 71, 78
Pasture	49, 69, 79, 84
Agriculture	67, 78, 85, 89
Wetland	100

The antecedent soil moisture conditions were defined as dry (wilting point), average, or wet (field capacity), and were based on the previous five-day rainfall totals (Table 2) (Mitchell et al., 1993).

Table 2. Rainfall break points for antecedent soil moisture conditions.

Antecedent Moisture Conditions	Rainfall Range
I - Wilting Point	**< 12 mm**
II – Average	**12-41 mm**
III – Field Capacity	**> 41 mm**

An Arc Macro Language (AML) script and batch file was used with ESRI's ArcInfo software to make the daily calculations for the study period, April 1 - 15, 2002.

First, the rainfall totals for March 27 - 30 were calculated. This information was then used to estimate the antecedent soil moisture conditions for April 1. Through the use of an "if-then" statement, the script then applied the appropriate *CN* grid to the NEXRAD rainfall data to calculate the "true" runoff for the current day. A batch file was then used to create a semi-automated way of processing the data for each consecutive day in the study period.

For comparison purposes, the runoff maps were re-calculated using only average antecedent soil moisture conditions, and summary and difference maps were generated for the two map sets. An additional AML was used to calculate total runoff for the "true" and average runoff datasets (Figures 1& 2). This same AML then subtracted the "true" runoff summary from the average runoff summary to generate a difference map (Figure 3).

"True" runoff ranged from 16.29 – 1,706.56 mm, whereas average runoff ranged from 0 – 355.36 mm. The differences between the "true" and average summaries ranged from -40.29 – 1,706.56 mm.

Figure 1. "True" runoff summary map.

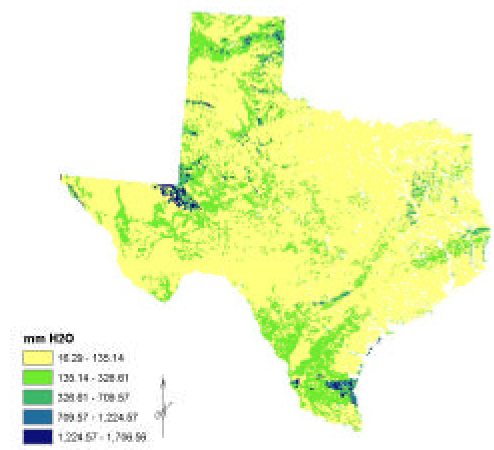
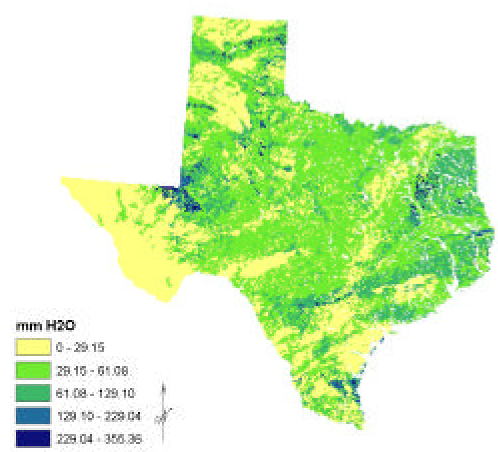
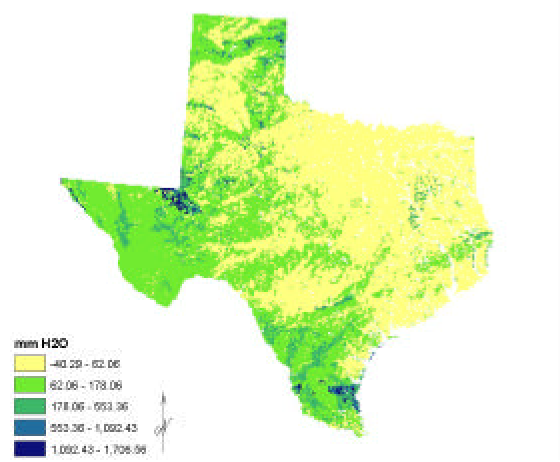


Figure 2. Average runoff summary map.



Results and Discussions

Figure 3. Difference map: "true" – average runoff.



Although the runoff vales estimated in this analysis have not been calibrated they do highlight the potential issues involved in estimating runoff without accounting for the antecedent conditions along with land cover and soil hydrologic group.

In general, the "true" runoff was substantially higher than the average values. In some cases however, the average calculations did generate higher runoff, as evidenced by the negative values in the difference map. This could be attributed to the fact that the antecedent soil moisture conditions were generally wetter than average, generating additional runoff when factored into the calculations, or dryer than average in the case of the over-estimated average runoff values.

Although the runoff values generated here are not indicative of actual runoff values for Texas, they do illustrate the need for accurately estimating antecedent soil moisture conditions in surface runoff calculations.

Future Considerations

The results of this analysis need further calibration and validation to determine the appropriate rainfall break-points for various antecedent soil moisture conditions and to evaluate the accuracy of runoff estimates. The process of generating these maps must also be automated to achieve the ultimate goal of the research, which is a daily surface runoff map of Texas at a resolution of 4km × 4km. Once calibration and validation procedures are complete, these runoff maps would be made available on the World Wide Web (WWW) for use by public and private water resource managers and various government agencies.

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Reduced Phosphorus Concentrations in Feedlot Manure and Runoff

Basic Information

Title:	Reduced Phosphorus Concentrations in Feedlot Manure and Runoff
Project Number:	2002TX59B
Start Date:	3/1/2002
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Funding Source:	104B
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Research Category:	None
Focus Category:	Agriculture, Water Quality, Nutrients
Descriptors:	None
Principal Investigators:	Kevin Heflin, Brent W. Auvermann

Publication

Reduced Phosphorus Concentrations in Feedlot Manure and Runoff

Progress Report
By
Kevin Heflin

A beef cattle feeding trial was conducted at the TAES/ USDA-ARS experimental feedyard, at Bushland, Texas. The feeding trial focused on the reduction of phosphorus in the cattle diet to reduce the concentration of phosphorus in the excreted manure. The 188 cattle were fed 4 different diets with varying levels of phosphorus in 18 feed pens from June-December 2002. Each feed pen measured 6m x 27m (162m2). Surfaces for 12 of the feed pens consisted of compacted fly ash, and the remaining 6 pen surfaces were native soil.

Water samples were collected from 6 different rain events that produced sufficient runoff from the pen surfaces. Water samples were collected with an ISCO 3700 that was activated automatically by runoff waters. Each sampler is capable of collecting 24 samples with each sample containing 1000 ml. Water samples were collected at 5 minute intervals until all 24 bottles were filled or runoff levels were not sufficient to trigger the sampler.

Due to the large volume of water samples (500+) results are still pending.

Fate of a Representative Pharmaceutical in the Environment

Basic Information

Title:	Fate of a Representative Pharmaceutical in the Environment
Project Number:	2002TX60B
Start Date:	3/1/2002
End Date:	2/1/2003
Funding Source:	104B
Congressional District:	19th
Research Category:	None
Focus Category:	Toxic Substances, Ecology, Non Point Pollution
Descriptors:	None
Principal Investigators:	audra.morse.1, Andrew Jackson

Publication

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Fate of a representative pharmaceutical in the environment

Final Report

Submitted to

Texas Water Resources Institute

By:

Audra Morse, Ph.D.
Andrew Jackson, Ph.D., P.E.

May, 2003

TABLE OF CONTENTS

ABSTRACT

The purpose of this research was to determine the fate of amoxicillin in the City of Lubbock's Water Reclamation Plant and to determine the antibiotic resistance patterns in the plant. Amoxicillin was detected in the influent of the plant during one month of the study, but amoxicillin was not detected at any other plant flow streams. The antibiotic resistance patterns of the LWRP varied monthly; heterotrophic bacteria were resistant to most of the antibiotics investigated during the nine month study.

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INTRODUCTION

As existing potable water supplies are depleted and populations continue to grow in arid and semi-arid areas of the country, including West Texas, the need for complete recycling of wastewater for water distribution may become necessary. Already the dilution factor for wastewater effluent continues to decrease with shorter and shorter intervals between release and reuse. Many municipalities are in fact using treated effluent in their primary water source although it may have spent some time in a natural water course. Historically, the concern with recycled wastewater has been the presence of disease-causing organisms called pathogens. However, a more recent concern of reusing wastewater for consumption is the presence of chemical contaminants, including a new category of compounds: personal care products and pharmaceuticals. Pharmaceuticals, including anti-inflammatories, antibiotics, caffeine, hormones, antidepressants, and others have been observed in various water bodies (Ternes et al., 1998; Heberer et al., 1998; Hirsch et al., 1999; Qiting and Xiheng, 1988).

Antibiotics are one especially troubling class of compounds due to the build-up of resistance in microbial populations. Antibiotics enter the environment from a variety of sources including discharges from domestic wastewater treatment plants and pharmaceutical companies, runoff from animal feeding operations, infiltration from aquaculture activities, leachate from landfills, and leachate from compost made of animal manure containing antibiotics (Figure 1). However, antibiotics are not confined to the natural aquatic environment. Detectable concentrations of antibiotics have been observed in tap water (Herberer et al., 1998; Masters, 2001). The startling fact is that these compounds are passing through water treatment processes and contaminating drinking water supplies. The concentrations of these contaminants typically range from nanogram/liter (ng/L) to microgram/liter (µg/L); the consequences of their presence at these concentrations are unknown. The overall potential for antibiotic removal by biological and physiochemical treatment systems and simultaneous risk of antibiotic resistance development has been relatively unexplored.

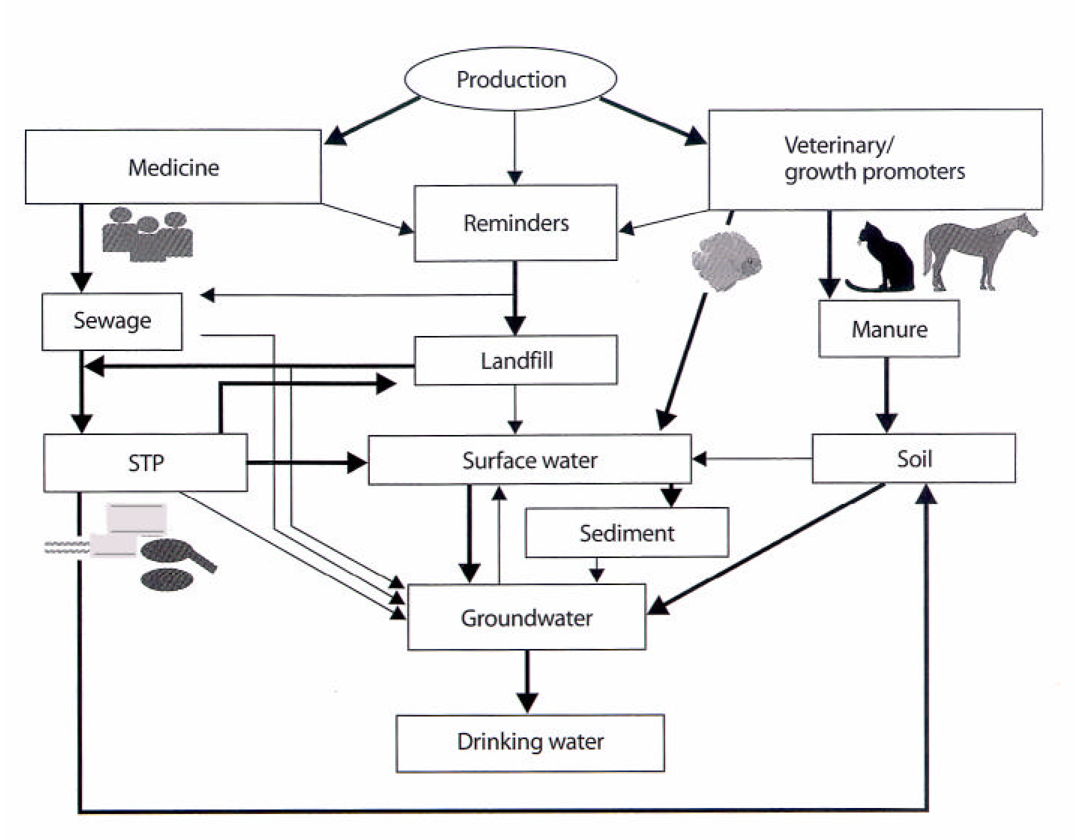

Figure 1. Sources, pathways, and sinks of pharmaceuticals (Kummerer, 2001).

Research has begun to determine the concentrations of antibiotics in the environment, and from this information, the health effects to humans and animals may be estimated by toxicologists. An additional problem that may be created by the presence of antibiotics at low concentrations in the environment is the development of antibiotic resistant bacteria. In recent years, the incidence of antibiotic resistant bacteria has increased and many people believe the increase is due to the use of antibiotics (Walter and Vennes, 1985). The presence of antibiotics can result in selective pressure that favors organisms that possess genes coding for antibiotic resistance. This may pose a serious threat to public health in that more and more infections may no longer be treatable with known antibiotics (Hirsch et al., 1999). In the event that antibiotic resistance is spread from nonpathogenic to pathogenic bacteria, epidemics may result. In fact, bacteria have been observed to transfer their resistance in laboratory settings as well as the natural environment (Kanay, 1983).

The objective of this research was to investigate the effect of a representative pharmaceutical in a biological water reclamation system. The antibiotic evaluated in this study was amoxicillin, which is a semi-synthetic, beta-lactam antibiotic used for a variety of infections. The focus of this particular project is to determine the fate of amoxicillin in the City of Lubbock's Wastewater Reclamation Plant and to determine the antibiotic resistance patterns in the plant.

BACKGROUND

Pharmaceuticals are used in large quantities in human and veterinary medicine or as food additives in animal production (Stan and Heberer, 1997). In animal feeding operations, antibiotics are often prescribed as a preventative measure to keep the animals healthy. The abuse of antibiotics has been rampant since Fleming's discovery of penicillin. Antibiotics were prescribed for the treatment of many illnesses and at doses that may have been inappropriate. There are many forms of antibiotic misuse and abuse. For instance, viral illnesses should not be treated with antibiotics. Also, patients should be educated on compliance issues and the importance of proper use of the antibiotic. Misuse, which includes not completing the prescription, can lead to resistance development (Leiker, 2000a). Preventative measures that may be taken by a clinician to reduce antibiotic resistance development include using the most appropriate spectrum antibiotic for each infection, shortening the duration of antibiotic treatment, knowing local resistance patterns, and limiting antimicrobial prophylaxis if possible (Leiker, 2000a).

Due to the overuse of antibiotics, bacteria have developed resistances to antibiotics. There are three main modes of antibiotic resistance that generally render the antibiotic ineffective, but not all bacteria use the same resistance mechanisms. The first mechanism prevents the antibiotic from binding with and entering the organism, which has been observed in some *P. aeruginosa* (Leiker, 2000b); this form of resistance is related to Multi-Drug Efflux. Other examples are *Steptococcus pnuemoniae* and *Group A Streptococci* penicillin-resistant mutants that have been isolated in the laboratory due to immense and common selective pressure; these mutants contain altered penicillin-binding proteins (Tomasz and Munoz, 1995). The second type of resistance mechanism is the production of an enzyme that inactivates the antibiotic. The classic example of this resistance mechanism is the production of beta-lactamase enzymes in *H. influenze* and *M. catarrhalis*, which destroys the beta-lactam ring of the beta-lactam antibiotic. There are many different enzymes produced by bacteria that are capable of degrading the beta-lactam ring. Fortunately for bacteria, this type of resistance may be spread to other bacteria through a process called "transference" (Leiker, 2000b). The last form of bacterial resistance is the change in the internal binding site of the antibiotic. For example, the site to which the antibiotic binds has been altered so that the antibiotic may no longer bind, which makes the bacteria are resistant to the antibiotic. This process has been observed in penicillin-resistant *S. pneumoniae*.

Antibiotic resistance may spread using various mechanisms, including conjugation, transduction, and transformation. In conjugation, DNA may be transferred from one bacterial cell to another in the form of a plasmid. Plasmids may carry genetic information in addition to the information contained on a chromosome, which bacteria may use under special conditions. For instance, plasmids may carry the genetic information for antibiotic resistance, virulence, bacteriocins, and metabolic activity (Madigan et al., 2000). Transduction is the process in which a part of a donor chromosome is packaged into a phage head and transferred by viruses. If the virus packaging mechanism selects genes that confer antibiotic resistance, then resistance may be spread to bacterial cells infected by the viruses. Transformation is the process in which cells take up free DNA from the environment (Snyder and Champness, 1997). If the DNA contains antibiotic resistance genes, then antibiotic resistance may be conferred to the transformant. Thus the transformant now has the genetic material encoding antibiotic resistance.

Amoxicillin

Amoxicillin is an orally absorbed broad-spectrum antibiotic with a variety of clinical uses including ear, nose, and throat infections and lower respiratory tract infections. As a chemical modification of ampicillin, which is poorly absorbed after oral administration, amoxicillin is better absorbed by the gastrointestinal tract than ampicillin (Sum et al., 1989). Amoxicillin is prescribed for the treatment of infections of beta-lactamase-negative stains, which are bacterial strains that do not possess the ability to produce beta-lactamase enzymes. Figure 2 presents the chemical structures of amoxicillin (R=0H) and penicilloic acid, a transformation product produced during beta-lactam ring cleavage.

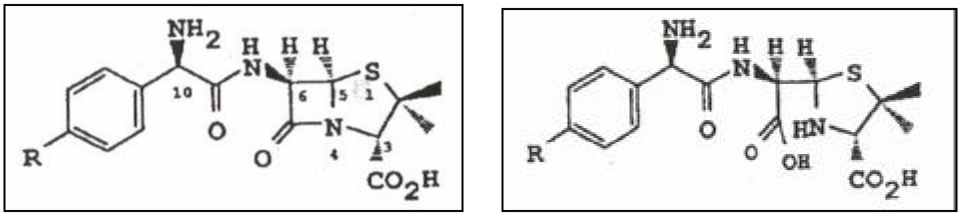

Figure 2. Chemical structure of amoxicillin (left) and penicilloic acid (right) (Connor et al., 1994).

Amoxicillin is a semi-synthetic penicillin obtaining its antimicrobial properties from the presence of a beta-lactam ring. Amoxicillin and other penicillin-like antibiotics target bacterial cell walls. Beta-lactam antibiotics bind to and inhibit the enzymes needed for the synthesis of peptidoglycan, a component of bacterial cell walls. As bacteria multiply and divide, the defective walls cannot protect the organism from bursting in hypotonic environments and cell death occurs.

Many mechanisms exist for resistance to beta-lactam antibiotics. Resistance is considered an increase in the minimal inhibitory concentration (MIC) of the antibiotic, which could be the result of many different mechanisms, whereas tolerance does not alter the bacteria's susceptibility to the drug but improves bacterial survival during treatment. For optimal bactericidal action, the dose must be greater than the organism's MIC. In the case of beta-lactam antibiotics, this dose is approximately four to five times the MIC. When antibiotic concentration is less then the MIC, bacteria recover from the exposure and begin growth (Ronchera, 2001). When the drug is prescribed to a patient with the infection, the dose will be greater than the MIC. However, it is unlikely that wastewater containing urine and feces will have antibiotic concentrations greater than the MIC; therefore, antimicrobial effects will probably not be observed. However, low concentrations of antibiotics encourage the development of antibiotic resistance. Thus, wastewater streams containing urine and feces likely aid in the development of antibiotic resistance. In *S. aureus*, which is a major human pathogen, three mechanisms of beta-lactam resistance have been identified: (1) beta-lactamase-mediation inactivated through hydrolysis of the beta-lactam nucleus, (2) penicillin-binding proteins (PBP)-associate intrinsic resistance due to the lower of the affinity of PBPs or the acquisition of new PBPs, and (3) tolerance of the beta-lactam antibiotic as a result of autolysins inhibition (Georgepapadakou et al.,1988). PBPs are the enzymatic targets of beta-lactam antibiotics. Beta-lactam resistance due to the alteration of PBPs has been detected in many isolates as well as most of the major human invasive pathogens (Tomasz, 1988).

For Gram-negative organisms, such as *E. coli* and nitrifying organisms, another mechanism of resistance to beta-lactam antibiotics, including amoxicillin, is the hindering of diffusion of the antibiotic by the outer membrane, which acts as a permeability barrier (Frere and Joris, 1988). Antibiotics must pass through porins, which are non specific outer membrane channels. The antibiotics ability to pass through porins depends on the size, hydrophobicity, and charge of the antibiotic (Danziger and Pendland, 1995). In addition, the outer membrane prevents the leaking of beta-lactamases into the culture environment (Frere and Joris, 1988). All bacteria may be divided into Gram-positive and Gram-negative organisms. The classification was developed by Gram, which is based on a dye procedure; the color of the dyed bacteria is related to the composition of bacterial cell walls. Gram-positive organisms appear blue following a Gram stain, and they posses a thick layer of peptidoglycan and no outer membrane. Beta-lactam antibiotics easily penetrate the thick layer of peptidoglycan in Gram-positive bacteria (Danziger and Pendland, 1995). Gram-negative organisms have an outer membrane and a thin layer of peptidoglycan inside the periplasmic space and are stained red in a Gram stain. Figure 3 is a drawing of the cell wall structures of Gram-negative and Gram-positive bacteria.

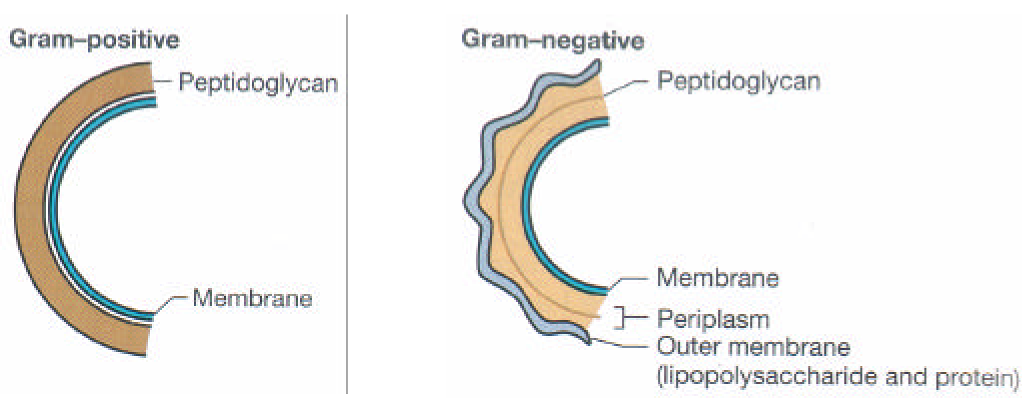

Figure 3. Structure of Gram-positive and Gram-negative bacteria (Madigan et al., 2000).

As mentioned previously, beta-lactamases are enzymes that cleave the beta-lactam ring and render the antibiotic useless. The genetic information for beta-lactamases is contained on either plasmids or chromosomes; however, genes for resistance are usually carried by plasmids. Beta-lactamase production may be either constitutive or inducible. Constitutive production results in a constant level of beta-lactamase production, which is independent of exposure to antibiotics. If beta-lactamase production is inducible, then beta-lactamases are produced following exposure to a signal, such as a beta-lactam antibiotic. Furthermore, production of the beta-lactamases ceases when the bacterium is no longer exposed to the signal (Danziger and Pendland, 1995). Beta-lactamases are classified according to (1) their genetic location (chromosome vs. plasmid), (2) gene expression (inducible vs. constitutive), (3) microorganism, (4) inhibition by beta-lactamase inhibitors, and (5) substrate. Figure 4 presents beta-lactamases and their distribution in nature.

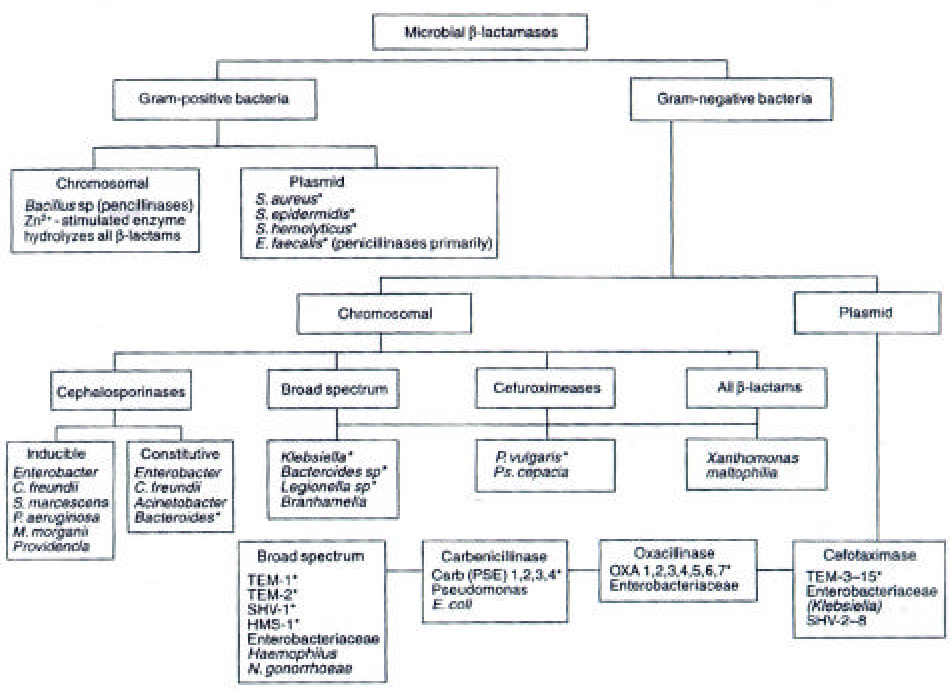

Figure 4. Beta-lactamases and their distribution in nature (Danziger and Pendland, 1995).

To reduce the potential for beta-lactam cleavage, beta-lactamase inhibitors are frequently combined with beta-lactam antibiotics. The purpose of the beta-lactamase inhibitors is to prevent the beta-lactamases from inactivating the antibiotic thereby increasing the effectiveness of the antibiotic. Examples of beta-lactamase inhibitors are sulbactam, clavulanate, and tazobactam (Danziger and Pendland, 1995). In many cases, amoxicillin is combined with clavulanic acid, a beta-lactamase inhibitor.

Antibiotics in the Environment

Drug residues, including antibiotics, have been observed in various aquatic environments including groundwater, surface water, and tap water (Alvero, 1987; Campeau et al., 1996). Sources of antibiotics include the treatment of human infections, veterinary use (e.g., animal feeding operations), aquaculture, and land application of compost containing sludge from wastewater treatment plants. In human uses, which will be the primary focus of this paper, antibiotics enter waste streams through feces and urine. To demonstrate, Hoeverstadt et al. (1986) detected several antibiotics in human feces, including trimethoprim and doxycycline in concentrations ranging from 3 to 40 mg/kg and erythromycin concentrations from 200 to 300 mg/kg. The concentration of antibiotics in urine is dependent on dosage, type of dosing (intravenous, intramuscular, or oral), food and beverage consumption, and elapsed time since dosage (Mastrandrea et al., 1984). In addition, absorption is also a property of the antibiotics. For example, amoxicillin is a chemically modified form of ampicillin and the modifications improve its absorption characteristics.

Extreme difficulties arise in estimating the mass of antibiotics entering the environment. In general, records containing the quantity of antibiotics prescribed annually are incomplete and the data available varies from country to country. Furthermore, it is unknown if the medication is taken as prescribed. Absorption rates vary for each individual further complicating the estimate of antibiotics entering the environment. Therefore, researchers have begun analyzing environmental samples for the presence of antibiotics. Table 1 presents the concentration of antibiotics present in secondary effluent and surface water in Germany.

Table 1. Concentrations of selected antibiotics applied in Germany (Zwiener et al., 2001).

Antibiotic	Prescribed Mass (tons/yr)	Secondary Effluent Concentration (μg/L)	Surface Water Concentration (μg/L)
Clarithromycin	1.3-2.6	0.24	0.26
Erythromycin	3.9-19.8	6.00	1.70
Roxithromycin	3.1-6.2	1.00	0.56
Chloramphenicol	--	0.56	0.06
Sulfamethoxazole	16.6-76	2.00	0.48
Trimethoprim	3.3-15	0.66	0.20

In the United States, the U.S. Geological Survey completed a study that measured the concentrations of 95 organic wastewater contaminants (OWCs) in water samples from 139 streams in thirty states during 1999 and 2000 (Kolpin et al., 2002). OWCs include pharmaceuticals, hormones, and other organic contaminants. The compounds detected represented a wide range of residential, industrial, and agricultural sources. The most frequently detected compounds were coprostanol (fecal steroid), cholesterol (animal and plant steroid), insect repellant (N,N-diethyltoluamide), caffeine, triclosan (antimicrobial disinfectant), fire retardant (tri(2-chloroethyl)phosphate), and a nonionic detergent metabolite (4-nonylphenol). In addition to these compounds, 31 veterinary and human antibiotic and antibiotic metabolites were investigated. Fourteen of the 31 antibiotics were not detected in this study. Table 2 contains the antibiotic, frequency of detection, maximum detected concentration (μg/L), and median detected (μg/L) concentration of the remaining 17 antibiotics.

Table 2. Summary of antibiotics in streams of the U.S. (Kolpin et al., 2002).

Antibiotic	Number of Samples	Reporting Level (μg/L)	Frequency (%)	Max (μg/L)	Median (μg/L)
Chlortetracycline (1)	84	0.10	2.4	0.69	0.42
Ciprofloxacin	115	0.02	2.6	0.03	0.02
Erythromycin-H_20	104	0.05	21.5	1.7	1.0
Lincomycin	104	0.05	19.2	0.73	0.06
Norfloxacin	115	0.02	0.9	0.12	0.12
Oxytetracycline (2)	84	0.10	1.2	0.34	0.34
Roxithromycin	104	0.03	4.8	0.18	0.05
Sulfadimethozine (2)	84	0.05	1.2	0.06	0.06
Sulfamethazine (1)	104	0.05	4.8	0.12	0.02
Sulfamethazine (2)	84	0.05	1.2	0.22	0.22
Sulfamethizole (1)	104	0.05	1.0	0.13	0.13
Sulfamethoxazole (1)	104	0.05	12.5	1.9	0.15
Sulfamethoxazole (3)	84	0.023	19	0.52	0.066
Tetracycline (2)	84	0.10	1.2	0.11	0.11
Trimethoprim (1)	104	0.03	12.5	0.71	0.15
Trimethoprim (3)	84	0.014	27.4	0.30	0.013
Tylosin (1)	104	0.05	13.5	0.28	0.04

Several studies have identified antibiotics in wastewater treatment plant (WWTP) flow streams and in WWTP effluents (Stelzer et al., 1985; Grabow et al., 1976; Bell, 1979; Misra et al., 1979; Radtke and Gist, 1989; Malik and Ahmad, 1994) at concentrations from ng/L to μg/L. Alder et al. (2000) detected up to 0.8 μg/L of cirpofloxacin in a WWTP effluent and 0.01 to 0.29 μg/L in the WWTP influent. Hirsch et al. (1999) found erythromycin concentrations up to 6 μg/L in WWTP effluent. Ciprofloxacin was observed in hospital effluent at concentrations between 3 and 89 μg/L, which is significantly higher than concentrations presented in other studies. Amoxicillin concentrations in wastewater from a German hospital were between 28 and 82.7 μg/L (Henninger et al., 2000). Peniciloly groups were observed at concentrations greater than 25 ng/L and 10 μg/L in river water and potable water, respectively (Halling-Sorensen et al., 1998). Therefore, wastewater treatment plants (WWTPs) are receiving wastes that contain low concentrations of antibiotics. Exposure to small concentrations of antibiotics selects for organisms resistant to antibiotics. Subsequently, WWTPs may be a reservoir of antibiotics as well as antibiotic resistant bacteria.

Antibiotic Resistance

Antibiotic resistance has been observed in various aquatic environments including river and costal areas, domestic sewage, surface water and sediments, lakes, sewage polluted ocean water, and drinking water (Merzioui and Baleux, 1994). These aquatic environments represent a variety of ecosystems and may include a variety of climates. The consequences of antibiotic resistant organisms may be different for each environment.

WWTPs are used to treat domestic and industrial wastewater so that it may be disposed in the natural aquatic environment, including rivers, lakes and streams, with minimal impact on aquatic life. Currently, the WWTP effluent must meet regulatory limits for suspended solids, nutrients, fecal coliforms, total coliforms, and a biological oxygen demand; however, regulatory limits have not been developed for antibiotic agents and the effect of low antibiotic concentrations and antibiotic resistance development receives limited attention. The role of WWTPs on the spread of antibiotic resistance to the natural environment is an important key to the ecological impact of human discharges.

Antibiotic Resistance in WWTP Influent

WWTPs typically accept discharges from hospitals and may receive discharges from pharmaceutical plants. Guardabassi et al. (1998) investigated the antibiotic resistance of *Acintobacter* spp. in sewers receiving waste from a hospital and pharmaceutical plant. The level of susceptibility to six antimicrobial agents was determined in 385 *Acinetobacter* strains isolated from samples collected upstream and downstream from the hospital and pharmaceutical plant. The antimicrobial agents analyzed include amoxicillin, oxytetracycline, chloramphenicol, sulfamethoxazole, gentamicin, and ciprofloxacin. A prevalence of oxytetracycline resistance was observed to increase in the sewer as the result of hospital discharge; however, the level of resistance decreased downstream of the discharge.

Antibiotic Resistance in WWTPs and Their Discharges

The incidence of outbreaks involving waterborne antibiotic-resistant bacteria has led to a serious problem of the death of patients who do not respond to antibiotics. One source of antibiotic-resistant bacteria in the environment is effluent from WWTPs (Hassani et al., 1992). The purpose of the Hassani et al. (1992) study was to evaluate the distribution of *Aeromonas* species present in wastewater treatment ponds to determine the effect of treatment on drug resistance incurred by the species. The importance of evaluating *Aeromonas* species is that they are a broad group of organisms commonly found in aquatic environments. During the course of this 17-month study, the distribution of the *Aeromonas* observed in the system differed between the cold and warm months. The most common species of *Aeromonas* observed were *A. caviae, A. hydrophila,* and *A. sobria*. Seven antibiotics (amoxicillin, cephalothin, streptomycin, trimethoprim-sulfamethoxazole, chloramphenicol, polymyxin B, and nalidixic acid) were tested on 264 isolates in this study. All of the isolates were resistant to amoxicillin and 73 percent exhibited resistance to cephalothin, both of which are beta-lactam antibiotics. The overall frequency of multiple antibiotic resistances among bacterial isolates was 77 percent and the antibiotic resistance index for the total strains was 0.29. Temperature appeared to have an effect on multiple-drug resistance. During the warm months, the level of resistance was greater in the bacteria isolated from the influent than those isolated in the effluent from the pond. Overall, the *A. sobria* were more susceptible to the antibiotics investigated in this study than either *A. caviae* or *A. hydrophila*. In addition, each species exhibited different resistance patterns than the other species. For example, the resistance to cephalothin of *A. caviae*, *A. hydrophila* and *A. sobria* were 91, 96 and 9 percent, respectively.

Another study evaluated the effect of wastewater stabilization ponds on antibiotic resistance on *Aeromonas* (Imzilin et al., 1996). Differences in resistance patterns of *Aeromonas* isolated from the raw sewage and stabilization pond effluent were not observed. All strains possessed multiple resistances, including resistance to ampicillin, amoxicillin, and novobiocin. Approximately 90 percent of the strains of *A. hydrophila* and *A. caviae* were resistant to cephalothin, and almost 80 percent of the *A. sobria* were susceptible. The results of this study are fairly similar to the results obtained from the study by Hassani et al. (1992).

Mezriou and Baleux (1994) investigated the antibiotic resistance of 879 *E. coli* strains isolated from raw domestic sewage and the effluent from aerobic lagoons and activated sludge plants. Both aerobic lagoons and activated sludge plants are used to reduce the BOD_5 leaving the treatment facility. The results of this study indicate that the aerobic lagoons were effective in removing fecal coliforms in the wastewater, but the system selected for antibiotic resistant *E. coli* by selecting for *E. coli.* The number of antibiotic resistant strains of *E. coli* in the effluent increased as compared to the influent. For both the inflow and outflow, the incidence of antibiotic resistance increased as the number of antibiotics was reduced from seven to one. The maximum polyresistance for a strain was seven antibiotics (ampicillin, mezlocillin, gentamicin, netilmicin, tobramycin, doxycyclin, and chloramphenicol). The level of antibiotic resistant *E. coli* strains in the outflow of the activated sludge was not constant and did not appear to develop in a manner similar to the aerated lagoon. In both the activated sludge and aerated lagoon system, resistance to quinolones and aminosides was not observed.

WWTP Discharges and Their Effect on the Natural Environment

To evaluate the impact of urban effluent, including WWTP discharges, Goni-Urriza et al. (2000) investigated antibiotic resistance in bacteria isolated from the Arga River in Spain. River samples were collected upstream and downstream of the water discharged from the city of Pamplona's WWTP. *Enterobacteriaceae*, from human and animal commensal flora, and *Aeromonas* were investigated. Most *Aeromonas* (72 percent) and 20 percent of the *Enterobacteriaceae* were resistant to nalidixic acid, which is a quinolone. The rate at which antibiotic resistances decreased downstream from the discharge was similar for the two groups of bacteria. Genetic analysis indicated that these resistances were mostly chromosomal mediated for *Enterobacteriaceae* and exclusively chromosomally mediated for *Aeromonas*. Other studies have observed less resistance of native and fecal bacteria upstream of urban areas and WWTP discharges, increased resistance immediately downstream of urban areas and WWTP discharges, and decreased resistance farther downstream (Boon and Cattanach, 1999; Pathak et al., 1993; Iwane et al., 2001). Another study by Gonzalo et al. (1989) evaluated antibiotic resistance and virulence factors of 418 *E. coli* strains isolated from river water receiving sewage discharge. The data indicated that bacteria from less contaminated water present less antibiotic resistance and virulence factors than those isolated from highly contaminated water. The results suggest that antibiotic resistance and virulence factors do not survive well in environments without selective pressure.

Natural aquatic environments, including lakes, rivers, and streams are environments in which antibiotic resistance may be developed. Arvanitdou et al. (1997) investigated the transfer of antibiotic resistance among *Salmonella* strains isolated from surface waters in northern Greece. Differences in antibiotic use and climate conditions resulted in geographic variations of antibiotic resistance among bacteria in surface water. The study showed that 24 percent of the *Salmonella* strains tested showed resistance to one or more of the antibiotics tested. Resistance to streptomycin was most common but was not transferable in all cases. However, ampicillin resistance (ampicillin is a beta-lactam antibiotic) was transferable. The authors believed these findings supported the presence of a common plasmid-mediated TEM type beta-lactamase. In one case, ampicillin resistance was cotransferred with resistance to aminoglycosides. Bacteria of non-fecal origin in natural aquatic environments free of natural anthropogenic influence demonstrated antibiotic resistance to one or more antibiotics; resistance may not be plasmid-mediated (Magee and Quinn, 1991).

Antibiotic Resistance Transfer

One of the greatest concerns of antibiotic resistance is the spread of antibiotic resistance from one bacterial species to another, especially in the case of resistance transfer between nonpathogenic to pathogenic bacteria. Antibiotic resistance has been a concern in an institutionalized environment such as a hospital; however, it may also be a concern in aquatic environments such as wastewater treatment systems. Resistance to newer beta-lactam agents was observed between *Klebsiella pneumoniae* and *E. coli* in a hospital in France, as observed by a decreased susceptibility of *E. coli* to cefotaxime. Three beta-lactamases were identified mediating cefotaxime resistance as well as penicllin and other cephalosporin resistance. Therefore, these beta-lactamases were termed extended broad-spectrum beta-lactamases (Jarlier et al., 1988).

Evidence suggests that healthy members of a community may contain a reservoir of bacterial antibiotic resistance genes even in commensal flora (Shanahan et al., 1994). These resistance reservoirs may complicate treatment of infections by invading pathogens who transfer resistance to nonpathogens. In Gram-negative bacteria, resistance is commonly mediated by TEM-1 beta-lactamases, which have been shown to account for up to 80 percent of all plasmid-mediated resistance. In Edinburgh, U.K., antibiotic resistance was observed in healthy human subjects, including resistance to ampicillin. Plasmids containing TEM-1 beta-lactamases encoding information were present throughout the community and were believed to be culprit of many extended-spectrum beta-lactamases.

Summary

These studies indicate that industrial and domestic discharges may affect the antibiotic resistance patterns observed in a WWTP. Furthermore, WWTPs have the ability to alter the antibiotic resistance patterns of bacteria in ecosystems containing the WWTP outfall. As a consequence, environmental bacteria, pathogens, and non-pathogens may confer resistance to currently prescribed antibiotics.

Lubbock Water Reclamation Plant

The Lubbock Water Reclamation Plant (LWRP), located in Lubbock, Texas, served as the test facility for the fate of amoxicillin in a full-scale wastewater treatment plant. The flow rate for the LWRP is approximately 20 MGD. Figure 5 is a flow diagram of the LWRP. Note that there are three process streams for the plant, which have been the result of plant expansions over the many years of operation. Primary treatment of the influent to the plant consists of screening and grit removal. After primary treatment, the flow streams are split before secondary treatment. For secondary biological treatment, the plant employs activated sludge in Plants 3 and 4. Plant 2 uses biotowers for secondary treatment. The facility does not employ tertiary removal. Instead, the effluent is used to irrigate farmland, discharged to Yellow House Canyon, or sent to XCEL. Sludge from secondary treatment is thickened, digested in anaerobic digesters, dewatered and landfilled.

The fate of amoxicillin in the LWRP was determined by measuring the ambient concentrations of amoxicillin at four locations in the plant over nine months. The objective of this experiment is to investigate the fate of amoxicillin in a full-scale wastewater treatment plant. Due to the dilution of urine by other wastewater streams entering a full-scale wastewater treatment plant as well as biotic activity in sewer systems, amoxicillin concentrations were expected to be near the detection limit in the influent and effluent of the plant, respectively.

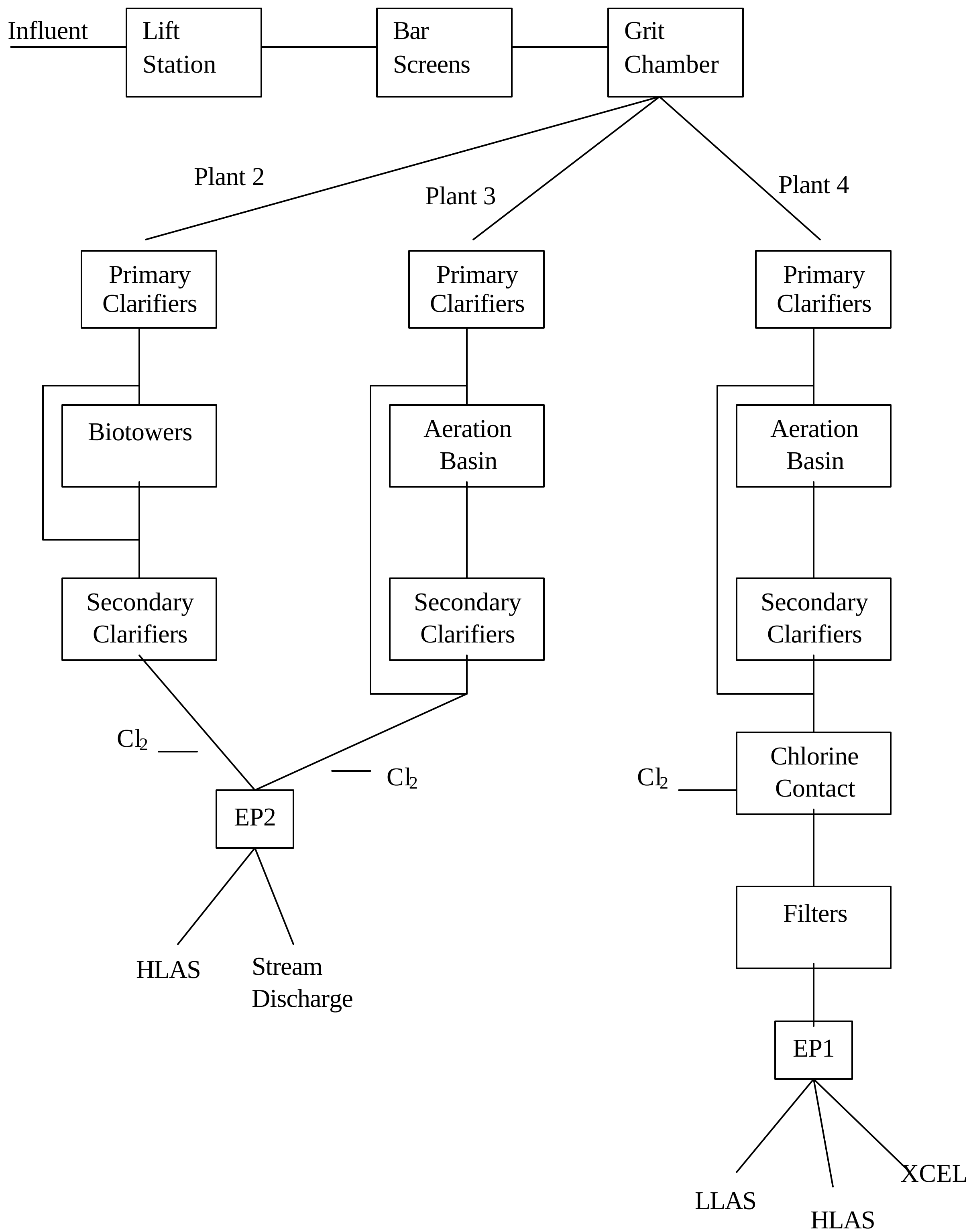

Figure 5. Schematic of the LWRP.

EXPERIMENTAL PROCEDURE

Fate of Amoxicillin in a Water Reclamation Plant--Lubbock, TX

Wastewater samples were collected from the influent, primary sludge, activated sludge basin, and the effluent of the Plant 4 of the LWRP. Samples were collected by LWRP personnel on the second Friday of each month from May to December. Samples were taken immediately to the ESL and filtered using a 0.45 μm filter. The primary sludge samples were centrifuged before filtering. The wastewater samples were filtered to prevent clogging of the C18 cartridges, which were required by the amoxicillin preparation procedure. All amoxicillin wastewater samples were analyzed in triplicate.

Antibiotic Resistance

The change in antibiotic resistance in the LWRP was investigated at four locations in the plant over eight months. To investigate the occurrence of antibiotic resistance in microorganisms in the systems examined in this research the disk diffusion susceptibility test was performed on samples obtained from the systems.

To investigate antibiotic resistance of the bacteria used in LWRP, samples were collected from the influent, activated sludge tank, primary sludge and effluent. Samples were collected, by LWRP personnel on the second Friday of each month from May to December in sterile, 1-L bottles. The samples were immediately taken to the ESL and analyzed.

The disk diffusion susceptibility test was performed to determine if heterotrophic organisms in the LWRP samples were resistant to multiple antibiotics. The LWRP samples were plated on nutrient agar and Hinton-Mueller plates. The antibiotics investigated in this study include amoxicillin with clavulanic acid and three other beta lactam antibiotics: penicillin, ampicillin, and cephalothin. Other antibiotics investigated in this study were bacitracin, ciprofloxacin, rifampin, streptomycin, tetracycline, and vancomycin. Table 3 lists the antibiotics and their concentrations used in this study. Table 4 presents a summary of the antibiotics investigated and their mechanisms.

Table 3. Antibiotics and concentrations of susceptibility disks.

Antibiotic	Concentration (μg)
Amoxicillin with clavulanic acid	30
Ampicillin	10
Bacitracin	10
Penicillin (units)	10
Cephalothin	30
Ciprofloxacin	30
Rifampin	5
Streptomycin	10
Tetracycline	30
Vancomycin	30

To create a lawn of bacteria, 0.1 mL of the wastewater was spread on the agar. After an incubation period, the zone of inhibition, which is the area around the disk without bacterial growth, was measured. The size of the zone of inhibition determined the susceptibility of the organisms to the antibiotic. The diameter of the paper disks were 0.7 cm. If the diameter of the zone of inhibition was between 0.7 and 1.0 cm, the organisms were considered resistant, because the organisms grew up to the disk. If the zone was between 1.0 and 1.2 cm, the bacteria were considered moderately susceptible and if the zone was greater than 1.2 cm, the microorganisms were considered susceptible. All samples were conducted in triplicate. Plates were incubated for 24 hours at 30^{o}C.

Media Preparation and Sterilization Procedures

Nutrient agar and Hinton-Mueller agar were used in this experiment. All of these products were prepared according to the manufacturer's directions.

Table 4. Summary of antibiotics and resistance mechanisms (Kimball, 2001).

Antibiotic	Class	Mechanism
Amoxicillin	Beta-lactam	Cell wall synthesis (PBPs)
Ampicillin	Beta-lactam	Cell wall synthesis (PBPs)
Bacitracin		Cell wall synthesis
Cephalothin	Beta-lactam	Cell wall synthesis (PBPs)
Ciprofloxacin	Quinolones	DNA gyrase inhibitor
Penicillin	Beta-lactam	Cell wall synthesis (PBPs)
Rifampin	Rifampin	Bacterial RNA Polymerase
Streptomycin	Aminoglycosides	70s ribosome subunit
Tetracycline	Tetracycline	30s ribosome subunit
Vancomycin	Glycopeptide	Cell wall synthesis (D-alanines)

Sterilization

Liquids, laboratory supplies, and media, when specified, were sterilized using an autoclave. The autoclave was operated for 15 minutes at 212^{o}F. The pressure was adjusted for Lubbock's elevation. Glassware was sterilized by dry sterilization, which requires glassware to be baked in an oven at 180^{o}C for four hours. Amoxicillin solution was sterilized by filtering the solution with a 0.22 μm filter.

Amoxicillin Quantification

A method developed by Sorenson and Snor (2001) was used to quantify the concentration of amoxicillin in the wastewater samples analyzed. Two milliliters of the sample was added to 20 mL of phosphate buffer 9.0 and mixed. Using a vacuum manifold, SPE cartridges (Waters) were washed with 2.0 mL of methanol and 5 mL of water. The samples were drawn through the cartridges at a flow rate of 2.0 mL/min. The column was washed three times with 2 mL of phosphate buffer 9.0. The samples were vacuumed dried for 1 minute. The cartridges were eluted with 2.5 mL of acetonitrile, which was collected in 15 mL polypropylene tubes and evaporated to dryness under nitrogen at a temperature of 55^{o}C. The residue was redissolved in 600 μL of phosphate buffer 9.0 and centrifuged filter through a cellulose membrane filter at 3000 x g for 15 minutes. Then, 500 μL of the filtrate was transferred to a 15 mL polypropylene tube. Next, 75 μL of derivitization reagent I was added and the sample was vortex-mixed for 30 seconds. After 10 minutes, 450 μL of derivitization reagent II was added and the sample was vortex-mixed for 60 seconds. The samples were placed in a water bath (55^{o}C) to react for 30 minutes. The samples were cooled in cool water and transferred to vials.   The samples were quantified using an HPLC. The injection volume was 500 μL and the mobile phase flow-rate was 1.0 mL/min.   The detection wavelength was 323 nm. The analytical instrument was calibrated on each day of analysis, and QC samples were run at a maximum of 20 samples, followed by blanks, to ensure that the instrument was still calibrated correctly.

RESULTS AND DISCUSSION

Fate of Amoxicillin in a Water Reclamation Plant--Lubbock, TX

To investigate the concentration of amoxicillin in a full-scale wastewater treatment plant, wastewater samples were collected from the Lubbock Wastewater Reclamation Plant (LWRP) in Lubbock, Texas. Samples were collected on the second Friday of every month between May and December 2002. The samples were immediately taken to the ESL and analyzed for amoxicillin and antibiotic resistance. All samples were analyzed in triplicate. The purpose of this experiment was to monitor the fate of amoxicillin in the LWRP to determine if amoxicillin was present in the plant's influent and effluent.

During the eight-month experiment, amoxicillin was detected in the influent of the plant only in the May samples; amoxicillin was not detected at any other sample locations during any of the months of this study. Table 5 presents a summary of the amoxicillin analysis. NA indicates not applicable. Due to equipment problems, samples could not be analyzed in December.

Table 5. Amoxicillin concentrations in the LWRP.

	Amoxicillin Concentration (mg/L)			
Month	Influent	Primary Sludge	Activated Sludge	Effluent
May	0.15	<0.10	<0.10	<0.10
June	<0.10	<0.10	<0.10	<0.10
July	<0.10	<0.10	<0.10	<0.10
August	<0.10	<0.10	<0.10	<0.10
September	<0.10	<0.10	<0.10	<0.10
October	<0.10	<0.10	<0.10	<0.10
November	<0.10	<0.10	<0.10	<0.10
December	NA	NA	NA	NA

During the entire course of the experiment, amoxicillin was not detected in the effluent of the LWRP. The results suggested that amoxicillin may not represent an environmental concern. This supported the hypothesis that amoxicillin would not be present at levels that may exert an impact on reclaimed wastewater end-users or aquatic life.

Antibiotic Resistance in the LWRP

A summary of the results of the antibiotic resistance tests performed using the LWRP wastewater are presented in Tables 6 through 9. Due to the large volume of data collected, a summary of the data is presented herein and the remaining data are presented in the appendices of this document. In the tables, S indicates the bacteria were susceptible to the antibiotic, MR indicates the bacteria were moderately resistant, and R indicates the bacteria were resistant to the antibiotic. In general, the bacteria in the plant were resistant to the beta-lactam antibiotics, including penicillin, ampicillin, and cephalothin. Bacteria in the influent were more resistant to the antibiotics examined than in the other flow streams. Bacteria in the plant were usually resistant to bacitracin and vancomycin. The resistance to amoxicillin with clavulanic acid, streptomycin, rifampin, and ciprofloxacin varied monthly.

The data showed that antibiotic resistance patterns changed in the plant, which may be a consequence of the organisms present and the fluctuations of organism population (Hassani et al., 1992; Imzilin et al., 1996). Of particular interest to this study was the resistance to the beta-lactam antibiotics. The organisms were resistant to the beta-lactam antibiotics. Generally, bacteria grew completely up to the disk. The resistance observed in the LWRP complemented the results obtained from the JSC-WRS and the TTU-WRS. Both systems illustrated resistance to the beta-lactam antibiotics. The addition of the beta-lactamase inhibitor, clavulanic acid, slightly reduced (moderately resistant versus resistant) the organisms' resistance to the amoxicillin, as compared to the resistance patterns of other beta-lactam antibiotics.

In addition, the bacteria exhibited different antibiotic resistant mechanisms. Table 4 contained the antibiotic and the mechanism of disinfection. The results of this experiment indicated the prevalence of antibiotic resistance in the LWRP and possible health effects and concerns to the ecosystems and end users of the water containing LWRP discharges. The concern of antibiotic resistance transfer from bacteria in the effluent of the LWRP to bacteria in the surrounding environment is a concern, especially if antibiotic resistance is spread to pathogenic bacteria.

Table 6. Antibiotic Resistance in the Influent of the LWRP.

Antibiotic	Jun	Jul	Aug	Sept	Oct	Nov	Dec
Amoxicillin with clavulanic acid	R	R	R	MR	S	S	R
Ampicillin	R	R	R	R	R	R	R
Bacitracin	R	R	R	NA	R	R	R
Cephalothin	R	R	R	R	R	R	R
Ciprofloxacin	S	R	R	S	R	S	MR
Penicillin	R	R	R	R	R	R	R
Rifampin	R	R	MR	MR	R	R	R
Streptomycin	MR	R	MR	R	S	R	R
Tetracycline	NA	R	R	R	NA	NA	NA
Vancomycin	R	R	R	R	R	R	R

Table 7. Antibiotic Resistance in the Primary Sludge of the LWRP.

Antibiotic	Jun	Jul	Aug	Sept	Oct	Nov	Dec
Amoxicillin with clavulanic acid	MR	S	MR	MR	MR	R	R
Ampicillin	R	R	R	R	R	R	R
Bacitracin	R	R	R	NA	R	R	R
Cephalothin	R	R	R	R	R	R	R
Ciprofloxacin	MR	S	MR	S	MR	MR	S
Penicillin	R	R	R	R	R	R	R
Rifampin	R	MR	MR	R	R	R	MR
Streptomycin	MR	MR	S	R	R	R	R
Tetracycline	NA	R	MR	R	NA	NA	NA
Vancomycin	R	R	R	R	R	R	R

Table 8. Antibiotic Resistance in the Activated Sludge of the LWRP.

Antibiotic	Jun	Jul	Aug	Sept	Oct	Nov	Dec
Amoxicillin with clavulanic acid	S	R	S	MR	S	S	S
Ampicillin	R	R	S	R	R	R	R
Bacitracin	R	R	R	NA	R	R	R
Cephalothin	R	R	R	R	R	R	R
Ciprofloxacin	S	R	S	S	R	S	MR
Penicillin	R	R	R	R	R	R	R
Rifampin	S	MR	MR	S	R	R	R
Streptomycin	MR	R	S	R	S	MR	S
Tetracycline	NA	R	S	MR	NA	NA	NA
Vancomycin	R	R	R	R	R	R	R

Table 9. Antibiotic Resistance in the Effluent of the LWRP.

Antibiotic	Jun	Jul	Aug	Sept	Oct	Nov	Dec
Amoxicillin with clavulanic acid	S	S	S	R	S	S	S
Ampicillin	R	R	MR	R	S	S	S
Bacitracin	R	R	R	NA	R	R	R
Cephalothin	R	R	MR	R	MR	MR	R
Ciprofloxacin	S	R	S	R	S	S	S
Penicillin	R	R	R	R	MR	R	R
Rifampin	MR	MR	S	R	R	S	MR
Streptomycin	S	S	R	R	S	S	S
Tetracycline	NA	S	S	R	NA	NA	NA
Vancomycin	R	R	S	R	R	R	R

In the LWRP, bacteria were resistant to multiple antibiotics. The greatest concern was that antibiotic resistant bacteria in the effluent of the LWRP may spread the genetic information encoding antibiotic resistance to organisms in the environment of the LWRP outfall. As a consequence, changing the antibiotic resistance properties of the bacteria in an ecosystem may disrupt the ecosystem, and the water may be a health hazard to end-users. For example, say a person uses the LWRP water to irrigate his/her farmland. During a visit at the farm, the farmer cuts their hand and the reclaimed wastewater comes into contact with the wound. Bacteria present in the water may infect the cut and the farmer may be hospitalized with a difficult infection to cure. This scenario is possible with the use of reclaimed wastewater. One solution to the aforementioned situation is to thoroughly disinfect the wastewater before releasing to the environment or the end users. This will minimize the bacterial population in the wastewater and minimize the exposure of the receiving ecosystem to antibiotic resistant bacteria.

Amoxicillin was detected in the influent of the LWRP on only one occasion during the eight-month study. Amoxicillin was not detected in the plant's flow streams (primary sludge, activated sludge or in the LWRP effluent). Due to the dilution of toilet flush water, 28 percent of interior residential water use (Metcalf and Eddy, 1991) with other domestic and municipal water flow streams, the concentration of amoxicillin is anticipated to be at or below the detection limit. In addition, it is unlikely that everyone served by the LWRP would be on antibiotics. Higher concentrations of amoxicillin may be observed in the effluent of a hospital or other medical facility; however, samples of this nature were not collected in this research. Based on the results of this study, amoxicillin, when present, is be believed to be degraded in the microbially-active sewer systems that transmit wastewater from the producers to the wastewater treatment plant. Thus, it is unlikely that amoxicillin would be present in the influent of the LWRP at concentrations greater than detected in this study.

CONCLUSIONS AND RECOMMENDATIONS

A recent concern of reusing wastewater for consumption is the presence of chemical contaminants, including a new category of compounds: personal care products and pharmaceuticals. Antibiotics are an especially troubling class of compounds due to their ability to produce antibiotic resistance in bacterial populations. Antibiotics enter the environment from a variety of sources including discharges from domestic wastewater treatment plants and pharmaceutical companies, runoff from animal feeding operations, infiltration from aquaculture activities, leachate from landfills, and leachate from compost made of animal manure containing antibiotics. However, antibiotics are not confined to the natural aquatic environment. Detectable concentrations of antibiotics have been observed in tap water (Herberer et al., 1998; Masters, 2001). The startling fact is that these compounds are passing through water treatment processes and contaminating drinking water supplies. The concentrations of these contaminants typically range from nanogram/liter (ng/L) to microgram/liter (µg/L); the consequence of their presence at these concentrations is unknown. The overall potential for antibiotic removal by biological and physiochemical treatment systems and simultaneous risk of antibiotic resistance development has been relatively unexplored. The objective of this research was to investigate the effect of a representative pharmaceutical in a biological water reclamation system. The antibiotic evaluated in this study was amoxicillin, which is a semi-synthetic, beta-lactam antibiotic used for a variety of infections. The objective of this particular project is to determine the fate of amoxicillin in the City of Lubbock's Wastewater Reclamation Plant and determine the antibiotic resistance patterns in the plant.

Amoxicillin was detected in the influent of the LWRP on only one occasion during the eight-month study. Amoxicillin was not detected in the plant's flow streams (primary sludge or activated sludge or in the LWRP effluent). Due to the small percentage of the cities population on amoxicillin at any given time and the ease at which amoxicillin is degraded, it is unlikely that amoxicillin would be present in the influent of the LWRP at concentrations greater than detected in this study. In the LWRP, bacteria were resistant to multiple antibiotics. Resistance to beta-lactam antibiotics was common, as indicated by the results of the and disk diffusion tests. The beta-lactam antibiotics investigated include penicillin, ampicillin, amoxicillin, and cephalothin. For the disk diffusion tests, amoxicillin was only available combined with the beta-lactamase inhibitor, clavulanic acid. In many cases, the beta-lactamase inhibitor was ineffective and organisms in the systems investigated were resistant to the beta-lactam, beta-lactamase inhibitor combination. Thus, the bacteria in the LWRP had the genetic mechanisms for beta-lactamase production, which provided resistance to beta-lactam antibiotics and beta-lactamase inhibiting compounds (i.e., clavulanic acid), which may be the consequence of overproduction of beta-lactamases or bacterial mutations in the clavulanic acid target.

The greatest concern is that antibiotic resistant bacteria in the effluent of the LWRP may spread the genetic information encoding antibiotic resistance to organisms in the environment of the LWRP outfall. As a consequence, changing the antibiotic resistance properties of the bacteria in the ecosystem may disrupt the ecosystem and the water may be a health hazard to end-users. For example, a person uses the LWRP water to irrigate their farmland. During an irrigation event, the farmer cuts his/her hand and the reclaimed wastewater comes into contact with the wound. Bacteria present in the water may infect the cut and the farmer may be hospitalized with a difficult infection to cure. This is a scenario is possible with the use of reclaimed wastewater.

One solution to the aforementioned situation is to thoroughly disinfect the wastewater before releasing to the environment or end-users. This will minimize the bacterial population in the wastewater and minimize the exposure of the receiving ecosystem to antibiotic resistant bacteria. However, disinfection requirements may need to become more stringent to protect the ecosystems downstream from the wastewater treatment plant’s outfall.

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APPENDIX

Table A.1. LWRP Susceptibility test results for June.

INFLUENT						
	Diameter of Disk (cm)			Average	Stnd Dev	Results
Amoxicillin w/ clav.acid	1.2	0.7	1.0	1.0	0.3	Resistant
Ampicillin	0.7	0.7	0.7	0.7	0.0	Resistant
Bacitracin	0.7	0.7	0.7	0.7	0.0	Resistant
Cephalothin	0.7	0.7	0.7	0.7	0.0	Resistant
Penicillin	0.7	0.7	0.7	0.7	0.0	Resistant
Ciprofloxacin	0.8	2.3	0.7	1.3	0.9	Susceptible
Rifampin	1.0	0.9	0.7	0.9	0.2	Resistant
Streptomycin	0.8	2.0	0.7	1.2	0.7	Mod. Resistant
Tetracycline	NA	NA	NA	NA	NA	NA
Vancomycin	0.8	0.7	0.7	0.7	0.1	Resistant
PRIMARY SLUDGE						
	Diameter of Disk (cm)			Average	Stnd Dev	Results
Amoxicillin w/ clav.acid	1.3	1.1	1.0	1.1	0.2	Mod. Resistant
Ampicillin	0.7	0.9	0.7	0.8	0.1	Resistant
Bacitracin	0.7	0.7	0.7	0.7	0.0	Resistant
Cephalothin	0.7	0.7	0.7	0.7	0.0	Resistant
Penicillin	0.7	0.7	0.7	0.7	0.0	Resistant
Ciprofloxacin	1.0	1.0	1.2	1.1	0.1	Mod. Resistant
Rifampin	0.9	1.2	0.9	1.0	0.2	Resistant
Streptomycin	1.0	1.2	1.0	1.1	0.1	Mod. Resistant
Tetracycline	NA	NA	NA	NA	NA	NA
Vancomycin	0.7	0.7	0.9	0.8	0.1	Resistant
ACTIVATED SLUDGE						
	Diameter of Disk (cm)			Average	Stnd Dev	Results
Amoxicillin w/ clav.acid	2.8	1.1	1.4	1.8	0.9	Susceptible
Ampicillin	0.7	0.7	1.0	0.8	0.2	Resistant
Bacitracin	0.8	1.0	0.8	0.9	0.1	Resistant
Cephalothin	0.7	0.8	0.7	0.7	0.1	Resistant
Penicillin	0.7	0.7	0.7	0.7	0.0	Resistant
Ciprofloxacin	1.6	1.0	1.7	1.4	0.4	Susceptible
Rifampin	1.2	2.1	1.4	1.6	0.5	Susceptible
Streptomycin	1.4	1.2	2.4	1.7	0.6	Susceptible
Tetracycline	NA	NA	NA	NA	NA	NA
Vancomycin	0.8	0.8	0.7	0.8	0.1	Resistant
EFFLUENT						
	Diameter of Disk (cm)			Average	Stnd Dev	Results
Amoxicillin w/ clav.acid	1.8	2.4	1.4	1.9	0.5	Susceptible
Ampicillin	0.7	0.7	0.7	0.7	0.0	Resistant
Bacitracin	0.7	0.7	1.1	0.8	0.2	Resistant
Cephalothin	0.7	0.7	0.7	0.7	0.0	Resistant
Penicillin	0.7	0.7	0.7	0.7	0.0	Resistant
Ciprofloxacin	2.1	1.9	1.9	2.0	0.1	Susceptible
Rifampin	1.2	1.2	1.1	1.2	0.1	Mod. Resistant
Streptomycin	1.9	2.2	2.2	2.1	0.2	Susceptible
Tetracycline	NA	NA	NA	NA	NA	NA
Vancomycin	0.8	0.8	0.9	0.8	0.1	Resistant

Table A.2. LWRP susceptibility test results for July.

	Diameter of Disk (cm)			Average	Stnd Dev	Results
INFLUENT						
Amoxicillin w/ clav.acid	1.2	0.9	1.0	1.0	0.2	Resistant
Ampicillin	0.7	0.7	0.7	0.7	0.0	Resistant
Bacitracin	0.7	0.7	0.7	0.7	0.0	Resistant
Cephalothin	0.7	0.7	0.7	0.7	0.0	Resistant
Penicillin	0.7	0.7	0.7	0.7	0.0	Resistant
Ciprofloxacin	0.7	0.7	0.7	0.7	0.0	Resistant
Rifampin	0.8	1.0	1.1	1.0	0.2	Resistant
Streptomycin	0.7	0.9	0.9	0.8	0.1	Resistant
Tetracycline	0.8	0.8	1.0	0.9	0.1	Resistant
Vancomycin	0.7	0.7	0.7	0.7	0.0	Resistant
PRIMARY SLUDGE						
	Diameter of Disk (cm)			Average	Stnd Dev	Results
Amoxicillin w/ clav.acid	1.1	1.7	1.4	1.4	0.3	Susceptible
Ampicillin	0.7	0.7	0.7	0.7	0.0	Resistant
Bacitracin	0.7	0.8	0.9	0.8	0.1	Resistant
Cephalothin	0.7	1.0	0.7	0.8	0.2	Resistant
Penicillin	0.7	0.7	0.7	0.7	0.0	Resistant
Ciprofloxacin	1.0	1.4	1.5	1.3	0.3	Susceptible
Rifampin	1.1	1.2	1.2	1.2	0.1	Mod. Resistant
Streptomycin	0.9	1.2	1.3	1.1	0.2	Mod. Resistant
Tetracycline	0.8	1.1	1.0	1.0	0.2	Resistant
Vancomycin	0.9	0.9	0.8	0.9	0.1	Resistant
ACTIVATED SLUDGE						
	Diameter of Disk (cm)			Average	Stnd Dev	Results
Amoxicillin w/ clav.acid	0.7	1.1		0.9	0.3	Resistant
Ampicillin	0.7	0.7		0.7	0.0	Resistant
Bacitracin	0.7	0.8		0.8	0.1	Resistant
Cephalothin	0.7	0.7		0.7	0.0	Resistant
Penicillin	0.7			0.7		Resistant
Ciprofloxacin	0.7	0.7	0.8	0.7	0.1	Resistant
Rifampin	1.0	1.5	0.8	1.1	0.4	Mod. Resistant
Streptomycin	0.7	0.7	0.7	0.7	0.0	Resistant
Tetracycline	1.0	0.7	0.7	0.8	0.2	Resistant
Vancomycin	0.7	0.7	0.7	0.7	0.0	Resistant
EFFLUENT						
	Diameter of Disk (cm)			Average	Stnd Dev	Results
Amoxicillin w/ clav.acid	1.8	1.8	1.6	1.7	0.1	Susceptible
Ampicillin	0.8	0.7	0.7	0.7	0.1	Resistant
Bacitracin	0.8	0.9	0.8	0.8	0.1	Resistant
Cephalothin	0.8	1.0	1.0	0.9	0.1	Resistant
Penicillin	0.7	0.7	0.7	0.7	0.0	Resistant
Ciprofloxacin	1.1	0.8	0.7	0.9	0.2	Resistant
Rifampin	1.2	1.4	1.0	1.2	0.2	Mod. Resistant
Streptomycin	1.6	1.4	8.0	3.7	3.8	Susceptible
Tetracycline	1.6	1.4	1.2	1.4	0.2	Susceptible
Vancomycin	0.8	1.0	0.8	0.9	0.1	Resistant

Table A.3. LWRP susceptibility test results for August.

	Diameter of Disk (cm)			Average	Stnd Dev	Results
INFLUENT						
Amoxicillin w/ clav.acid	0.7	0.8	1.0	0.8	0.2	Resistant
Ampicillin	0.7	0.7	0.7	0.7	0.0	Resistant
Bacitracin	0.7	0.7	0.7	0.7	0.0	Resistant
Cephalothin	0.7	0.7	0.7	0.7	0.0	Resistant
Penicillin	0.7	0.7	0.7	0.7	0.0	Resistant
Ciprofloxacin	0.9	0.7	1.0	0.9	0.2	Resistant
Rifampin	1.1	0.7	1.4	1.1	0.4	Mod. Resistant
Streptomycin	1.4	1.1	1.1	1.2	0.2	Mod. Resistant
Tetracycline	0.8	1.0	1.0	0.9	0.1	Resistant
Vancomycin	0.7	0.7	0.8	0.7	0.1	Resistant
PRIMARY SLUDGE						
	Diameter of Disk (cm)			Average	Stnd Dev	Results
Amoxicillin w/ clav.acid	1.1	1.0	1.3	1.1	0.2	Mod. Resistant
Ampicillin	0.8	0.7	0.7	0.7	0.1	Resistant
Bacitracin	0.7	0.7	0.7	0.7	0.0	Resistant
Cephalothin	0.7	0.7	0.7	0.7	0.0	Resistant
Penicillin	0.7	0.7	0.7	0.7	0.0	Resistant
Ciprofloxacin	1.2	1.1	1.1	1.1	0.1	Mod. Resistant
Rifampin	1.0	1.2	1.0	1.1	0.1	Mod. Resistant
Streptomycin	1.0	1.4	1.4	1.3	0.2	Susceptible
Tetracycline	1.2	1.0	1.2	1.1	0.1	Mod. Resistant
Vancomycin	0.7	0.7	0.7	0.7	0.0	Resistant
ACTIVATED SLUDGE						
	Diameter of Disk (cm)			Average	Stnd Dev	Results
Amoxicillin w/ clav.acid	1.8	1.7	1.2	1.6	0.3	Susceptible
Ampicillin	1.0	1.2	1.6	1.3	0.3	Susceptible
Bacitracin	0.7	0.7	0.7	0.7	0.0	Resistant
Cephalothin	0.7	0.7	0.7	0.7	0.0	Resistant
Penicillin	0.7	0.7	0.7	0.7	0.0	Resistant
Ciprofloxacin	1.4	2.8	1.8	2.0	0.7	Susceptible
Rifampin	1.0	0.8	1.1	1.0	0.2	Mod. Resistant
Streptomycin	1.4	1.9	1.2	1.5	0.4	Susceptible
Tetracycline	1.0	1.6	1.8	1.5	0.4	Susceptible
Vancomycin	0.7	0.7	0.7	0.7	0.0	Resistant
EFFLUENT						
	Diameter of Disk (cm)			Average	Stnd Dev	Results
Amoxicillin w/ clav.acid	1.2	2.2	2.4	1.9	0.6	Susceptible
Ampicillin	1.0	1.2	1.0	1.1	0.1	Mod. Resistant
Bacitracin	0.8	1.0	0.7	0.8	0.2	Resistant
Cephalothin	0.7	0.7	2.2	1.2	0.9	Mod. Resistant
Penicillin	0.7	0.7	1.2	0.9	0.3	Resistant
Ciprofloxacin	1.0	2.0	2.4	1.8	0.7	Susceptible
Rifampin	1.8	1.6	2.2	1.9	0.3	Susceptible
Streptomycin	1.1	0.8	0.7	0.9	0.2	Resistant
Tetracycline	3.8	4.0	3.8	3.9	0.1	Susceptible
Vancomycin	1.2	1.0	1.8	1.3	0.4	Susceptible

Table A.4. LWRP susceptibility test results for September.

	Diameter of Disk (cm)			Average	Stnd Dev	Results
INFLUENT						
Amoxicillin w/ clav.acid	1.0	1.1	1.4	1.2	0.2	Mod. Resistant
Ampicillin	0.7	0.7	0.7	0.7	0.0	Resistant
Bacitracin	-	-	-	-	-	-
Cephalothin	0.7	0.7	0.7	0.7	0.0	Resistant
Penicillin	0.7	0.7	0.7	0.7	0.0	Resistant
Ciprofloxacin	1.3	1.4	1.2	1.3	0.1	Susceptible
Rifampin	-	-	1.1	1.1	-	Mod. Resistant
Streptomycin	-	0.8	-	0.8	-	Resistant
Tetracycline	0.8	1.1	0.9	0.9	0.2	Resistant
Vancomycin	0.7	-	-	0.7	-	Resistant
PRIMARY SLUDGE						
	Diameter of Disk (cm)			Average	Stnd Dev	Results
Amoxicillin w/ clav.acid	1.2	1.2	1.2	1.2	0.0	Mod. Resistant
Ampicillin	0.7	0.7	0.7	0.7	0.0	Resistant
Bacitracin	-	-	-	-	-	-
Cephalothin	0.7	0.7	0.7	0.7	0.0	Resistant
Penicillin	0.7	0.7	0.7	0.7	0.0	Resistant
Ciprofloxacin	1.3	1.4	1.2	1.3	0.1	Susceptible
Rifampin	1.0	-	-	1.0	-	Resistant
Streptomycin	-	1.0	-	1.0	-	Resistant
Tetracycline	1.0	1.0	1.0	1.0	0.0	Resistant
Vancomycin	-	-	0.7	0.7	-	Resistant
ACTIVATED SLUDGE						
	Diameter of Disk (cm)			Average	Stnd Dev	Results
Amoxicillin w/ clav.acid	0.9	1.3	1.2	1.1	0.2	Moderately Resistant
Ampicillin	0.7	0.7	0.7	0.7	0.0	Resistant
Bacitracin	-	-	-	-	-	-
Cephalothin	0.7	0.7	0.7	0.7	0.0	Resistant
Penicillin	0.7	0.7	0.7	0.7	0.0	Resistant
Ciprofloxacin	1.1	1.6	1.6	1.4	0.3	Susceptible
Rifampin	1.5	-	-	1.5	-	Susceptible
Streptomycin	-	0.8	-	0.8	-	Resistant
Tetracycline	1.1	1.1	1.0	1.1	0.1	Mod. Resistant
Vancomycin	-	-	0.7	0.7	-	Resistant
EFFLUENT						
	Diameter of Disk (cm)			Average	Stnd Dev	Results
Amoxicillin w/ clav.acid	1.3	0.8	1.0	1.0	0.3	Resistant
Ampicillin	0.7	0.7	0.7	0.7	0.0	Resistant
Bacitracin	-	-	-	-	-	-
Cephalothin	0.7	0.7	0.7	0.7	0.0	Resistant
Penicillin	0.7	0.7	0.7	0.7	0.0	Resistant
Ciprofloxacin	0.7	0.8	0.8	0.8	0.1	Resistant
Rifampin	1.0	-	-	1.0	-	Resistant
Streptomycin	1.1	0.7	-	0.9	0.3	Resistant
Tetracycline	0.7	0.7	0.8	0.7	0.1	Resistant
Vancomycin	0.7	-	0.7	0.7	0.0	Resistant

Table A.5. LWRP susceptibility test results for October.

	Diameter of Disk (cm)			Average	Stnd Dev	Results
INFLUENT						
Amoxicillin w/ clav.acid	1.2	1.5	1.4	1.4	-	Susceptible
Ampicillin	0.7	0.7	-	0.7	-	Resistant
Bacitracin	0.7	0.7	-	0.7	-	Resistant
Cephalothin	0.9	0.7	-	0.8	-	Resistant
Penicillin	0.7	0.7	-	0.7	-	Resistant
Ciprofloxacin	0.7	0.7	-	0.7	-	Resistant
Rifampin	0.8	0.8	-	0.8	-	Resistant
Streptomycin	1.9	1.0	-	1.5	-	Susceptible
Tetracycline	-	-	-	-	-	-
Vancomycin	0.7	0.7	-	0.7	-	Resistant
PRIMARY SLUDGE						
	Diameter of Disk (cm)			Average	Stnd Dev	Results
Amoxicillin w/ clav.acid	0.9	1.2	-	1.1	-	Mod. Resistant
Ampicillin	0.7	0.7	-	0.7	-	Resistant
Bacitracin	0.7	0.7	-	0.7	-	Resistant
Cephalothin	0.7	0.7	-	0.7	-	Resistant
Penicillin	0.7	0.7	-	0.7	-	Resistant
Ciprofloxacin	1.4	0.9	-	1.2	-	Mod. Resistant
Rifampin	0.7	0.7	-	0.7	-	Resistant
Streptomycin	0.7	0.7	-	0.7	-	Resistant
Tetracycline	-	-	-	-	-	-
Vancomycin	0.7	0.7	-	0.7	-	Resistant
ACTIVATED SLUDGE						
	Diameter of Disk (cm)			Average	Stnd Dev	Results
Amoxicillin w/ clav.acid	1.6	1.6	-	1.6	-	Susceptible
Ampicillin	0.7	0.7	-	0.7	-	Resistant
Bacitracin	0.7	0.7	-	-	-	Resistant
Cephalothin	0.7	0.7	-	0.7	-	Resistant
Penicillin	0.7	0.7	-	0.7	-	Resistant
Ciprofloxacin	0.9	0.7	-	0.8	-	Resistant
Rifampin	0.7	0.7	-	0.7	-	Resistant
Streptomycin	0.8	1.7	-	1.3	-	Susceptible
Tetracycline	-	-	-	-	-	-
Vancomycin	0.7	0.7	-	0.7	-	Resistant
EFFLUENT						
	Diameter of Disk (cm)			Average	Stnd Dev	Results
Amoxicillin w/ clav.acid	1.8	2.2	-	2.0	-	Susceptible
Ampicillin	1.2	1.4	-	1.3	-	Susceptible
Bacitracin	0.7	0.8	-	0.8	-	Resistant
Cephalothin	1.2	1.2	-	1.2	-	Mod. Resistant
Penicillin	1.2	0.9	-	1.1	-	Mod. Resistant
Ciprofloxacin	2.0	1.2	-	1.6	-	Susceptible
Rifampin	1.0	-	-	1.0	-	Resistant
Streptomycin	2.0	1.6	-	1.8	-	Susceptible
Tetracycline	-	-	-	-	-	-
Vancomycin	0.7	0.7	-	0.7	-	Resistant

Table A.6. LWRP susceptibility test results for November.

	Diameter of Disk (cm)			Average	Stnd Dev	Results
INFLUENT						
Amoxicillin w/ clav.acid	0.7	1.9	2.0	1.5	0.7	Susceptible
Ampicillin	1.1	1.2	0.7	1.0	0.3	Resistant
Bacitracin	0.7	0.7	-	0.7	-	Resistant
Cephalothin	0.7	0.7	0.7	0.7	0.0	Resistant
Penicillin	0.8	0.8	0.7	0.8	0.1	Resistant
Ciprofloxacin	2.0	1.5	1.6	1.7	0.3	Susceptible
Rifampin	0.7	0.7	-	0.7	-	Resistant
Streptomycin	0.7	0.8	-	0.8	-	Resistant
Tetracycline	-	-	-	-	-	-
Vancomycin	0.7	-	-	0.7	-	Resistant
PRIMARY SLUDGE						
	Diameter of Disk (cm)			Average	Stnd Dev	Results
Amoxicillin w/ clav.acid	1.1	0.8	0.8	0.9	0.2	Resistant
Ampicillin	0.7	0.7	0.8	0.7	0.1	Resistant
Bacitracin	0.7	0.7	-	0.7	-	Resistant
Cephalothin	0.7	0.7	0.7	0.7	0.0	Resistant
Penicillin	0.7	0.7	0.7	0.7	0.0	Resistant
Ciprofloxacin	1.0	1.0	1.5	1.2	0.3	Mod. Resistant
Rifampin	0.8	0.8	-	0.8	-	Resistant
Streptomycin	1.0	0.7	-	0.9	-	Resistant
Tetracycline	-	-	-	-	-	-
Vancomycin	0.7	0.7	-	0.7	-	Resistant
ACTIVATED SLUDGE						
	Diameter of Disk (cm)			Average	Stnd Dev	Results
Amoxicillin w/ clav.acid	1.6	1.6	2.0	1.7	0.2	Susceptible
Ampicillin	1.2	0.7	0.8	0.9	0.3	Resistant
Bacitracin	0.7	0.7	-	0.7	-	Resistant
Cephalothin	0.7	0.7	0.7	0.7	0.0	Resistant
Penicillin	0.7	0.7	0.7	0.7	0.0	Resistant
Ciprofloxacin	1.4	2.0	1.2	1.5	0.4	Susceptible
Rifampin	0.7	1.0	-	0.9	-	Resistant
Streptomycin	0.7	1.4	-	1.1	-	Mod. Resistant
Tetracycline	-	-	-	-	-	-
Vancomycin	0.7	0.7	-	0.7	-	Resistant
EFFLUENT						
	Diameter of Disk (cm)			Average	Stnd Dev	Results
Amoxicillin w/ clav.acid	2.0	2.2	1.1	1.8	0.6	Susceptible
Ampicillin	1.5	1.3	2.4	1.7	0.6	Susceptible
Bacitracin	0.7	0.7	-	0.7	-	Resistant
Cephalothin	0.7	1.0	1.6	1.1	0.5	Mod. Resistant
Penicillin	0.7	1.2	0.7	0.9	0.3	Resistant
Ciprofloxacin	2.4	1.4	2.2	2.0	0.5	Susceptible
Rifampin	1.7	0.9	-	1.3	-	Susceptible
Streptomycin	1.6	0.9	-	1.3	-	Susceptible
Tetracycline	-	-	-	-	-	-
Vancomycin	0.7	0.7	-	0.7	-	Resistant

Table A.7. LWRP susceptibility test results for December.

INFLUENT						
	Diameter of Disk (cm)			Average	Stnd Dev	Results
Amoxicillin w/ clav.acid	0.9	0.9	0.9	0.9	0.0	Resistant
Ampicillin	0.7	0.7	0.8	0.7	0.1	Resistant
Bacitracin	0.7	0.7	0.7	0.7	0.0	Resistant
Cephalothin	0.8	0.7	0.7	0.7	0.1	Resistant
Penicillin	0.7	0.7	0.7	0.7	0.0	Resistant
Ciprofloxacin	1.2	1.1	1.0	1.1	0.1	Mod. Resistant
Rifampin	0.7	0.7	0.8	0.7	0.1	Resistant
Streptomycin	0.7	0.7	0.8	0.7	0.1	Resistant
Tetracycline	-	-	-	-	-	-
Vancomycin	0.7	0.7	0.7	0.7	0.0	Resistant
PRIMARY SLUDGE						
	Diameter of Disk (cm)			Average	Stnd Dev	Results
Amoxicillin w/ clav.acid	0.9	1.0	1.0	1.0	0.1	Resistant
Ampicillin	0.7	0.8	0.8	0.8	0.1	Resistant
Bacitracin	0.7	0.7	-	0.7	-	Resistant
Cephalothin	0.7	0.7	0.7	0.7	0.0	Resistant
Penicillin	0.7	0.7	0.7	0.7	0.0	Resistant
Ciprofloxacin	1.5	1.5	1.1	1.4	0.2	Susceptible
Rifampin	1.1	1.1	-	1.1	-	Mod. Resistant
Streptomycin	0.8	0.8	-	0.8	-	Resistant
Tetracycline	-	-	-	-	-	-
Vancomycin	0.7	0.7	-	0.7	-	Resistant
ACTIVATED SLUDGE						
	Diameter of Disk (cm)			Average	Stnd Dev	Results
Amoxicillin w/ clav.acid	1.0	1.6	1.4	1.3	0.3	Susceptible
Ampicillin	0.8	0.7	0.7	0.7	0.1	Resistant
Bacitracin	0.8	0.8	-	0.8	-	Resistant
Cephalothin	0.7	0.7	0.7	0.7	0.0	Resistant
Penicillin	0.7	0.7	0.8	0.7	0.1	Resistant
Ciprofloxacin	1.2	1.2	1.2	1.2	0.0	Mod. Resistant
Rifampin	0.9	0.8	-	0.9	-	Resistant
Streptomycin	1.6	1.1	-	1.4	-	Susceptible
Tetracycline	-	-	-	-	-	-
Vancomycin	0.7	0.7	-	0.7	-	Resistant
EFFLUENT						
	Diameter of Disk (cm)			Average	Stnd Dev	Results
Amoxicillin w/ clav.acid	2.8	2.0	1.0	1.9	0.9	Susceptible
Ampicillin	1.0	1.4	2.0	1.5	0.5	Susceptible
Bacitracin	0.7	1.0	1.6	1.1	0.5	Resistant
Cephalothin	1.4	0.9	0.7	1.0	0.4	Resistant
Penicillin	2.0	1.0	0.7	1.2	0.7	Resistant
Ciprofloxacin	2.0	1.6	2.2	1.9	0.3	Susceptible
Rifampin	1.5	1.2	0.7	1.1	-	Mod. Resistant
Streptomycin	3.1	1.8	2.0	2.3	-	Susceptible
Tetracycline	-	-	-	-	-	-
Vancomycin	0.7	1.0	0.9	0.9	-	Resistant

Urban Forested Wetland Restoration

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URBANIZING WATERSHEDS AND CHANGING RIVER FLOOD DYNAMICS: IMPLICATIONS FOR URBAN WETLAND RESTORATION

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Abstract. Urbanization alters river hydrology, morphology, water quality, and habitat and ecology. Most of these associated changes are due to an increase in impervious surface cover (ISC) throughout the watershed. But the spatial location of urban areas within the watershed also greatly influences river hydrology. As river hydrology changes, functions and structure of riverine wetlands associated with the hydrology may be impacted. In order to increase the long-term success of wetland restoration attempts within an urbanizing environment, it is necessary to quantify potential impacts to the wetlands as hydrology continues to change. This study investigated the effects of increased ISC on stream hydrology between 1972 and 1995 for a sub-watershed located north of Dallas, Texas. Moving window and FRAGSTATS analyses calculated the degree and location of ISC throughout the basin, and U.S. Geologic Survey stream-gauge data were analyzed to determine changes in steam hydrology between the 2 time periods. Average ISC for the watershed increased from 2 to 11% between 1972 and 1995, but highest cover (50–80%) occurred along the southern and eastern borders. Annual river flooding frequency and duration doubled between 1972 and 1995, and flooding velocity increased from 31.4 to 35.4 m3/sec. Wetland restoration attempts within the watershed should address the potential for future hydrologic changes as ISC continues to increase.

Key words: urbanization; impervious surface cover; wetlands; wetland restoration; hydrologic regime

INTRODUCTION

The process of urbanization impacts streams in many ways that include alterations to hydrology, morphology, water quality, and habitat and ecology (Schueler 1992). Changes to these stream attributes are caused by an increase of impervious surface cover (ISC) associated

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with the process of urbanization. With increasing ISC, soil water infiltration decreases, which increases surface runoff. The degree of urban surface runoff is typically proportional to the amount of ISC. For example, as ISC reaches 10–20% within a catchment basin, runoff increases twofold; 35–50% ISC causes a threefold increase in runoff; and 75–100% ISC increases surface runoff more than fivefold over forested regions (Arnold and Gibbons 1996).

The increased runoff associated with urbanization affects stream hydrology in many ways. The time lag between the center of the precipitation event to the center of the peak flow discharge decreases as urbanization increases (Espey et al. 1965). Stream velocity and the associated erosive forces increase, as do the magnitude and frequency of flooding events (Schueler 1992).

Although the degree of urbanization, as measured by the percent cover of impervious surface cover, is one of the most important factors determining stream hydrography, the location of urbanization within the watershed also plays an important role. Sheeder et al. (2002) found dual urban and rural hydrograph peaks based on the degree and spatial location of urbanization within the watershed. The first peak is a result of drainage from the urban area followed closely by drainage from the rural area. Although not addressed in the article, flood duration may increase as a result of the two flooding peaks occurring consecutively. Hirsch et al. (1990) theorize that if lower reaches of a basin become urbanized with little development in the upper basin, water from the lower urban portion could drain before the arrival of water from the upper portion, thus decreasing flooding magnitude but increasing duration.

The effects of urbanization on stream hydrology can have serious implications for the long-term sustainability and health of riverine wetlands. Riverine wetlands are those located within floodplains of rivers and streams. As hydrology is the most important determinant of wetland structure and function (Mitsch and Gosselink 2000), and because the hydroperiod of riverine wetlands are inextricably connected with the flooding regime of the associated river, an altered hydroperiod caused by urbanization may significantly alter wetland structure and function that had developed under pre-urban conditions.

Not only is the changing hydrology an important consideration for potential impacts on urban wetlands, but this has important implications for urban wetland restoration attempts as well. Most wetland restoration failures have been attributed to a failure to restore a proper hydrologic regime (Kunz et al. 1988, Kusler 1990, Mitsch and Gosselink 2000, Stolt et al. 2000).

For example, restoration projects resulting in wetlands becoming too wet for bottomland species may limit above ground production, while those resulting in dry conditions may not support obligate wetland species (Niswander and Mitsch 1995). Wetland restoration attempts within urbanizing watersheds need to consider the changing hydrology in order to ensure hydrologic levels suitable for optimal wetland production. Hydrologic conditions at the time of restoration may be very different from those some years down the road as urbanization within the watershed continues to increase. It is, therefore, imperative to quantify the degree and location of urbanization within the watershed with the associated hydrologic alterations over time, and be able to predict future hydrologic impacts based on past and present land-use changes.

A bottomland hardwood forest restoration project is currently being undertaken in Dallas County, Texas. As urbanization within the watershed has increased, river hydrologic regimes have been altered. Because the potential for continued urbanization exists, it is necessary to quantify past land use change to predict impacts of future land use changes on the restored wetland. The objectives of this study were to (1) determine the location and degree of urbanization within the East Fork Trinity watershed before and during urbanization and relate it to hydrologic changes of Rowlett Creek, which is located within the watershed, and (2) given the degree of urbanization and hydrologic alteration, determine necessary steps to take to ensure the long-term success of restored wetlands within the watershed.

METHODS

Study area

The East Fork Trinity watershed is located north of Dallas, Texas, in Dallas and Collin counties and was delineated by the US Environmental Protection Agency (2002). A sub-watershed within the East Fork Trinity watershed was derived from a digital elevation model with 30-m resolution to limit the watershed to include only the hydrologic area of interest directly influenced by urbanization. The extent of this watershed is 374 km^2.

The climate of the region includes mild winters and hot summers averaging 9º and 29ºC, respectively. Average annual precipitation is 94 cm, which occurs throughout the year but peaks slightly during the spring (NOAA 2003). The Dallas metropolitan area has experienced rapid urban growth, as much as 400% since 1970 (Knauss 2001).

Image preparation and analysis

Aerial photographs from 1972 were used for pre-urban analyses. DOQQs from 1995 with 1-m resolution were downloaded from the Texas Natural Resources Information System website (TNRIS 2003) for use of comparison to the 1972 photos. It became apparent that time would limit the amount of the watershed that could be analyzed for this study. Consequently, a sub-watershed was then chosen based on its small size (31 km^2) and close proximity to the U.S. Geologic Survey (USGS) stream-gauge station used for the hydrologic analyses.

Land cover types for the grids created from the photos and DOQQs were classified using unsupervised classification in ERDAS IMAGINE 8.6 (ERDAS IMAGINE 2002). The resulting 50 classes were then manually combined into 5 classes based on visual estimates from the photos and DOQQs. These cover classes included woody, herbaceous, bare ground, impervious surface, and water. The classification was still not entirely accurate as much of the actual water and herbaceous cover were classified as woody. These areas were subsequently manually delineated and appropriately reclassified.

The amount of cover of each class was determined for the entire sub-watershed in ArcView (ESRI 1998), but because spatial location of urbanization within a watershed is also important in determining hydrologic changes over time, a moving window approach was used in combination with FRAGSTATS v. 3 (McGarigal and Marks 1995) to determine patch attributes at a finer scale. Patch attributes calculated included cover, number of patches, mean patch size, and edge density.

Moving windows with a 356-m radius were systematically shifted in 178-m steps across the classified grids. At least 50% of each window needed to include data from within the sub-watershed in order to be included in the analyses. An ArcView script was then used to perform the FRAGSTATS metrics in all moving windows.

Hydrologic analyses

To compare hydrologic changes over time, stream-gauge data for Rowlett Creek were downloaded from the USGS website (NWISWeb Data for USA 2003). Analysis of the data included 5-year intervals centered on the 2 dates for which aerial photos and DOQQs were available, 1972 and 1995, respectively. Annual flooding frequency and duration were calculated for 2 flooding heights, 28 and 89 cm above base flow elevations. The average duration of a single flooding event was calculated by dividing the total annual flood duration by the number of annual floods. Average flooding magnitude (m^3/sec) of floods greater than 28 cm was also calculated for each of the time periods.

RESULTS

Image analyses

Percent cover values of each cover class were calculated for the entire sub-watershed for 1972 and 1995 (Table 1). Percent woody cover almost doubled between years from 9 to 17%. Because it was difficult to distinguish between bare ground and ISC during the classification procedure, these 2 cover classes are listed both separately and combined. ISC alone increased over fivefold between years from 2 to 11%, while combining bare ground and ISC resulted in a little under double the cover in 1995 compared to 1972 (8 to 15%).

Table 1. Percent cover of different land-cover classes summarized for the entire sub-watershed in 1972 and 1995.

	1972	1995
Woody	9%	17%
Herbaceous	81%	67%
Bare ground + ISC	8%	15%
Bare ground	6%	4%
ISC	2%	11%
Water	0.8%	1.2%

Results of FRAGSTATS analyses showed differing changes in cover for different areas of the sub-watershed between years. Percent woody cover as well as the size and numbers of woody patches have increased over much of the sub-watershed (Fig. 1). Edge density also increased between years (Fig. 1d). The most pronounced increases in numbers and cover of woody patches in 1995 were centered around areas where patch number and cover were highest in 1972.

An increase in ISC between years was mainly restricted to a few scattered areas throughout the sub-watershed (Fig. 2). ISC cover was highest in 1995 along the southern and eastern edges of the basin (Fig. 2a). These areas are located close to the mouth of the basin. The number of ISC patches increased between years, but the mean patch size decreased (fig. 2b, c). These results, in combination with high edge densities of 3000/ha (Fig. 2d), indicate that ISC increased but became more fragmented between years.

Hydrologic analyses

Because urbanization has a greater effect on smaller floods (Hirsch et al. 1990), analyses of flooding frequency and duration were separated into small floods (28 cm) and larger floods (89 cm). Despite this separation, both annual flooding frequency and duration of Rowlett Creek doubled between years for both heights (Table 2).

Table 2. Average annual flooding frequency and duration for small and larger floods for 2 time periods. The data represent 5-yr averages centered on 1972 and 1995.

	Frequency (#/yr)		Duration (days/yr)	
	1972	1995	1972	1995
Small floods (28 cm)	9.6	18.4	15.8	31.6
Larger floods (89 cm)	4.6	8.8	6	11.4

Disregarding flooding height, the average duration for a single flooding event increased from 40 hrs to 42hrs between 1972 and 1995. The average flood velocity remained the same between the 2 time periods (Fig. 3), but when extreme low and high floods were discounted flood velocities increased from 31.4 to 35.4 m^3/sec.

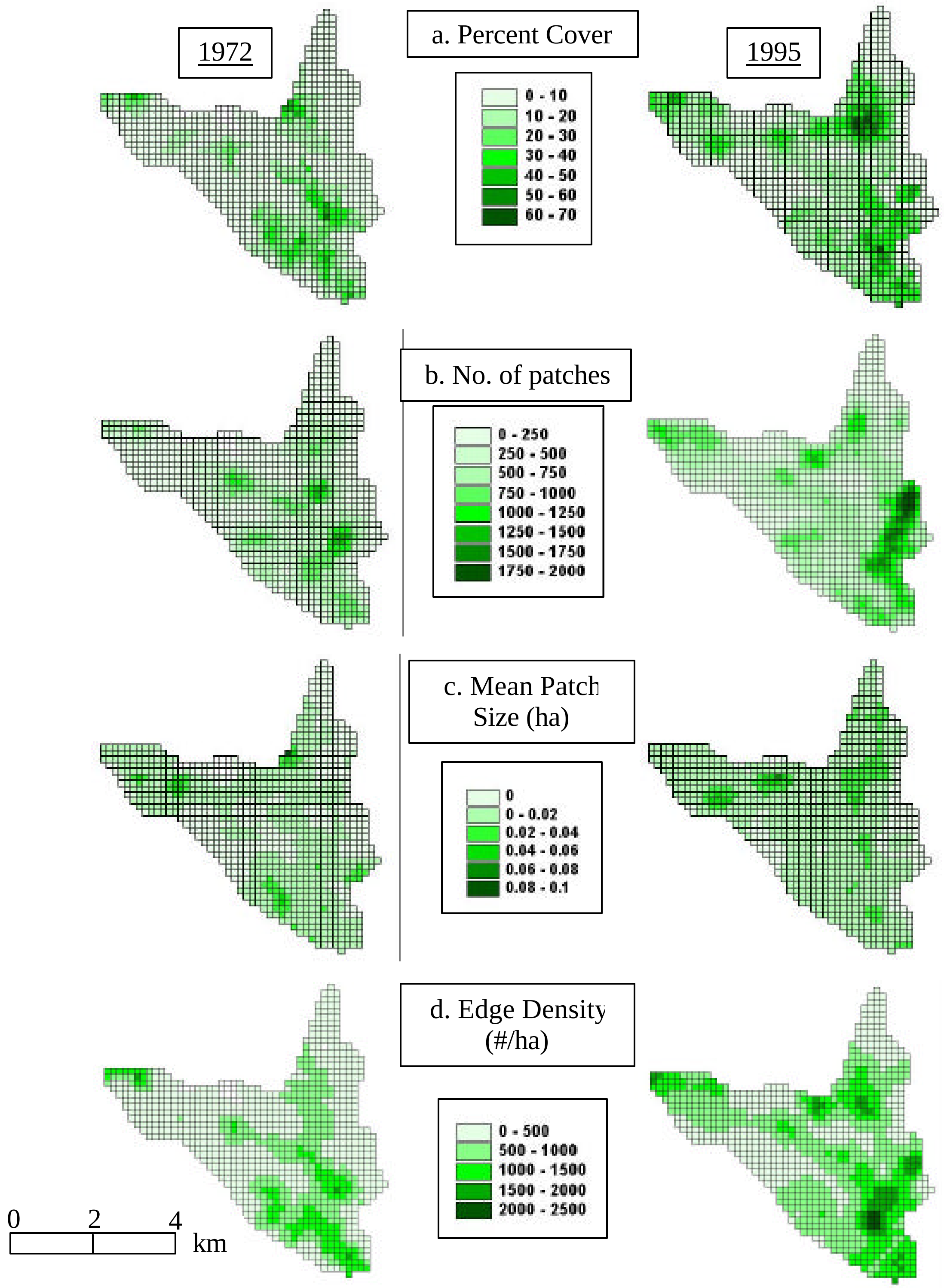

Figure 1. Results of FRAGSTATS analyses for woody cover within the sub-watershed performed on grids generated from moving window analyses. Figures in the left column refer to 1972 and those in the right to 1995. (a) Percent cover, (b) number of patches, (c) mean patch size, and (d) edge density.

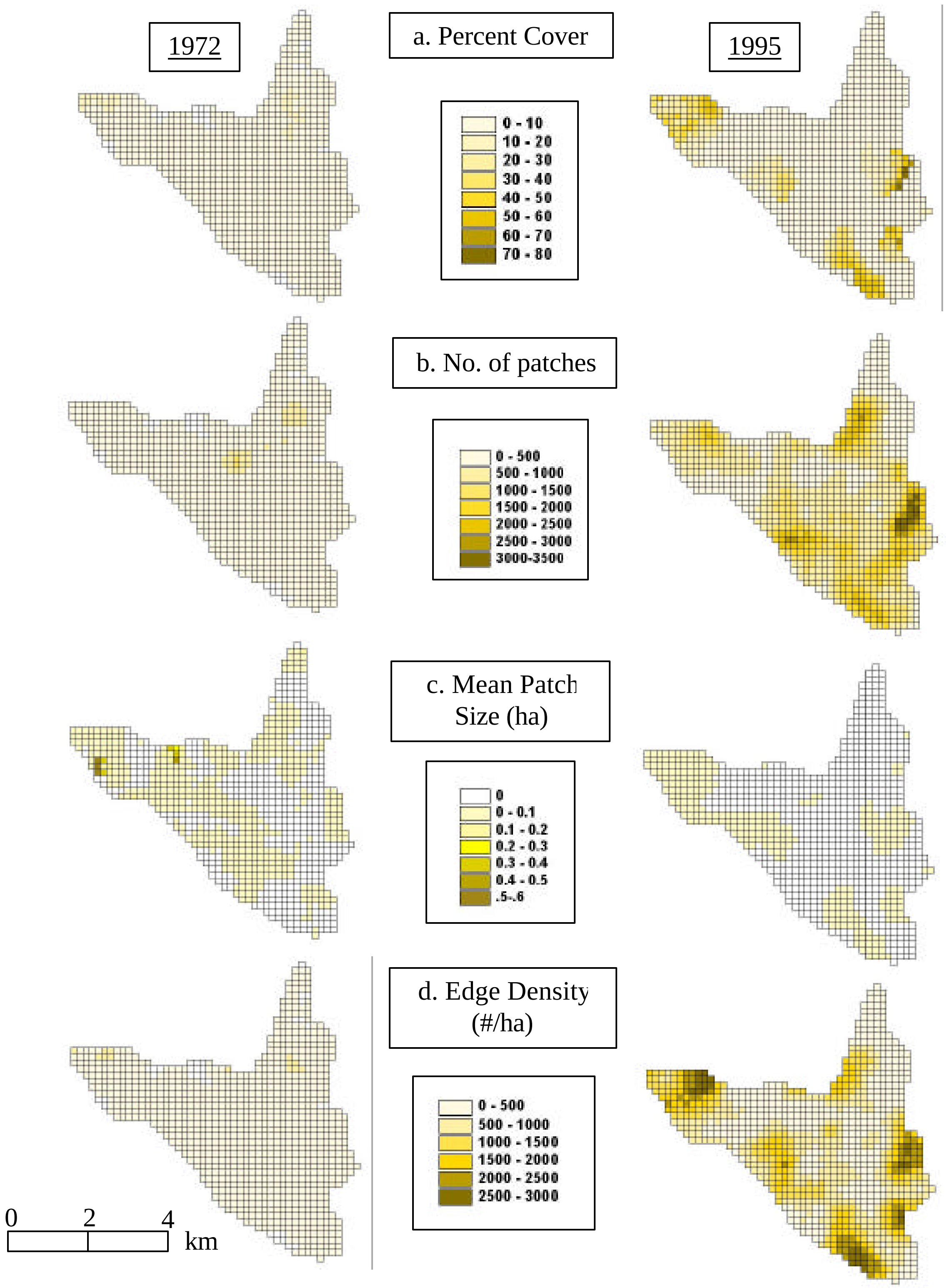

Figure 2. Results of FRAGSTATS analyses for ISC within the sub-watershed performed on grids generated from moving window analyses. Figures in the left column refer to 1972 and those in the right to 1995. (a) Percent cover, (b) number of patches, (c) mean patch size, and (d) edge density.

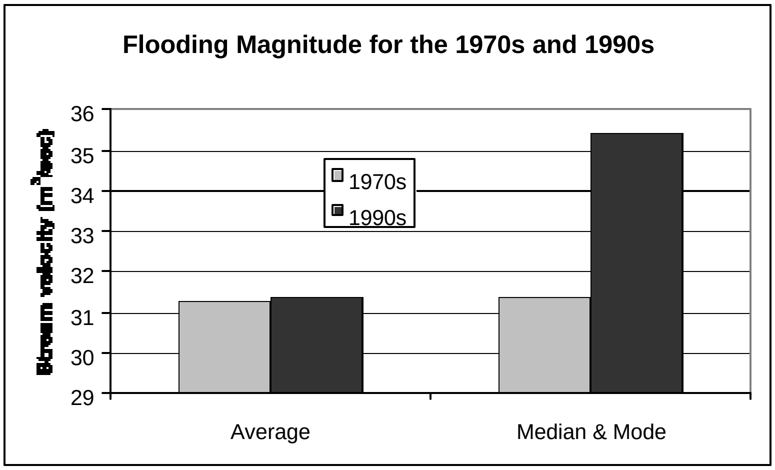

Figure 3. Average, median, and mode flooding magnitude for the 5-yr periods from 1970 to 1974 and 1993 to 1997.

DISCUSSION

The results of the FRAGSTATS analyses seem initially counterintuitive. Woody cover increased with an increase in number of patches and patch size, while ISC increased with increasing number of patches but decreasing patch size. One would expect that as urbanization increases, ISC patch size would also increase and woody cover would decrease. These results can be explained by 2 phenomena: (1) land that was used for agriculture had been cleared of woody cover, but the conversion to urban land allowed woody cover to increase, and (2) this doubling of woody cover would appear to fragment the ISC from an aerial photo or DOQ, thus creating smaller ISC patch sizes. Viewing the original 1972 aerial photos revealed very little woody cover in presumably newly developed urban areas. By 1995, the trees had grown to an extent that they covered housetops and streets, thereby fragmenting ISC. Analyses of patch attributes may have yielded more accurate results had the woody patches within ISC patches been discounted.

Spatial location of urban patches varied throughout the sub-watershed. This spatial complexity makes it difficult to directly relate the influence of spatial location of ISC to specific hydrologic variables. However, a clear relationship does exist between changes in the amount of ISC and changes to the hydrologic regime. In 1972, only 2% of the sub-watershed was covered by ISC. In 1995, ISC increased to 11%. By that time annual flooding frequency and duration had doubled, and average flooding magnitude and single flood duration also increased.

Although 11% ISC is not much of the total cover, Arnold and Gibbons (1996) state that ISC over 10% can cause volume of runoff to double over similar forested watersheds. With 84% of the sub-watershed still covered by woody and herbaceous vegetation, the potential for future urbanization is high. Although difficult to accurately predict, it is safe to say that if urbanization continues, future flooding frequency, duration, and magnitude will increase as ISC increases.

Increasing ISC tends to affect smaller floods to a greater extent than larger floods (Hirsch et al. 1990, Paul and Meyer 2001). Increased ISC doubled the frequency and duration of floods in Rowlett Creek without respect to flood size. However, large floods in this study were considered all those greater than 89 cm. Flooding heights of 89 cm may still have been too small to see differences in flood responses to increased ISC. Analyzing larger floods may reveal results consistent with other published studies.

The increased flooding frequency, duration, and magnitude associated with an increase in ISC has important implications for wetland restoration endeavors. Wetlands that develop under pre-urban conditions may become degraded with rapidly changing hydrologic conditions brought about by urbanization. When attempting to restore such wetlands, it is necessary to quantify the effect that past land-use changes have had to bring about current hydrologic conditions. It is then necessary to predict influences of future land-use changes on the future hydrologic regime. Steps must be taken during initial restoration efforts to establish sufficient plasticity within the system to allow for adaptation to future hydrologic variations, thus increasing the self-sustainability of the wetland. For example, sufficient microtopography could be created to produce a hydrologic gradient that would allow species colonization to fluctuate with fluctuating water tables (Barry et al. 1996). Also, species introduced to the site should include those that are tolerant of a wide range of hydrologic conditions, thus increasing the likelihood that species will be in place that can tolerate future conditions. And, although outside the realm of self-sustaining, variable water control structures could be incorporated to allow for manual manipulation of the hydrology.

CONCLUSIONS

Hydrologic restoration in an urbanizing environment presents a unique question: how can wetlands be restored given the fact that hydrology will continue to change? When attempting to restore wetlands within an urbanizing environment, it is necessary to determine changes in the degree and spatial location of urbanization over time. This study has shown that flooding frequency, duration, and magnitude increased as urbanization increased. It is important to note that these conclusions were based on analyses completed on a portion of the watershed. However, it is safe to say that as urbanization continues flooding dynamics will continue to change. Instead of fighting against them, allowing the system to respond to these changes, by creating hydrologic gradients, introducing species with wide environmental tolerances, and installing variable water control structures, will increase the probability of continued restoration success.

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Adsorption and Desorption of Atrazine on Selected Lake Sediments in Texas

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Adsorption and Desorption of Atrazine from Various Lake Sediments in Texas

Report

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June 1, 2003

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LITERATURE REVIEW[1]

Atrazine (2-chloro-4-ethylamino-6-isopropylamino-s-triazine) has been used extensively throughout the United States for broadleaf weed control in corn, sorghum, and sugarcane production, as well as in several minor crops. In 2002, 32.0 million ha of corn and 3.9 million ha of sorghum were planted in the U.S (NASS 2003). In 2002, 0.8 million ha of corn and 1.3 million ha of sorghum were planted in Texas (TASS 2002). Approximately 34.7 million-kg of atrazine are applied yearly in the U.S., making it one of the most commonly used pesticides in this country. Eighty-six percent of this quantity is used in corn production, 10% is used in sorghum production, and 4% on remaining crops (EPA 2002).

Due to this extensive use, atrazine has been one of the most detected herbicides in U.S. surface water (Ma et al. 1997; Seybold et al. 1999). It has been estimated that 0.5% to 6.0% of the atrazine applied in agricultural systems is removed by surface runoff (Hall et al. 1972; Hall 1974; Triplett et al. 1978; Wauchope 1978). This leads to the main source of pesticide contamination in lakes (Spalding et al. 1994). Approximately 20% of the 50,000 community drinking water systems in the U.S. are supplied by surface water. Approximately 3,600 drinking water systems of the 20% supplied by surface water have had atrazine detections. The maximum contaminant level (MCL) of 3 ug L^{-1} for atrazine has been met or surpassed in 200 of these 3,600 water systems. These 200 water systems are located in Alabama, Ohio, Illinois, Indiana, Iowa, Kansas, Kentucky, Louisiana, Michigan, Missouri, and Texas (EPA 2003).

[1] This proposal is in the format of Weed Science.

Due to the detections in Texas, some monitoring programs have been initiated. Eight lakes around the Dallas area of North Texas, were analyzed by the Texas A&M Pesticide Fate Laboratory in College Station, TX for the presence of atrazine in the lake water. These eight lakes consisted of Big Creek Lake, Joe Pool Lake, Lavon Lake, Bardwell Lake, Lake Waxahachie, Lake Aquilla, Lake Tawakoni, and Richland-Chambers Reservoir. Concentrations have primarily ranged from 0.3 ug L^{-1} to 0.5 ug L^{-1} through out the year in the source water of these lakes. Occasionally, concentrations have ranged from 30 to 50 ug L^{-1} atrazine during the spring months following atrazine applications. Higher concentrations of pesticides typically have been found in both ground and surface water following initial herbicide application (Pionke et al.1990; Spalding et al. 1989; Triplett et al.1978; Wauchope 1978; Wu et al. 1983). All eight lakes have been placed on the Texas 2000 Clean Water Act Section 303(d) list because the atrazine concentrations in finished drinking water indicated contamination of source water. Seven of these lakes were classified as "threatened" lakes meaning that the finished drinking water from these lakes had a concentration of 1.5 ug L^{-1} atrazine or 50% of the MCL. Aquilla Lake was classified as an impaired body of water due to the fact that its finished drinking water exceeded the MCL of 3 ug L^{-1} (TNRCC 2002).

Although not deemed a human carcinogen, atrazine has become a growing concern regarding human and wildlife health due to its presence in surface water. Sexual abnormalities in frogs and a decrease in length and weight have been linked to atrazine contamination (Carr et al. 2003; Diana et al. 2000; Morgan 1996; Reeder et al. 1998; Tavera-Mendoza 2002). Lab studies on frogs have shown observed sexual abnormalities at atrazine concentrations of >20 ug L^{-1}. Above 200 ug L^{-1} of atrazine, both frog length and weight decreased (Diana et al. 2000). High atrazine concentrations had no effect on survival (Diana et al. 2000). It was concluded in these studies that atrazine concentrations in natural water were too low to cause a threat to wildlife (Carr et al. 2003; Diana et al. 2000; Morgan 1996; Reeder et al. 1998; Tavera-Mendoza 2002). Recently it was reported that concentrations as low as 0.1 ug L^{-1} were shown to cause sexual abnormalities in frogs in the laboratory (Hayes et al. 2002). This concentration is below the EPA's MCL. The same study reported seeing frog abnormalities in natural water at concentrations ranging from 0.2 ug L^{-1} and greater although no other contaminants were studied (Hayes et al. 2002). A study on cricket frogs (*Acris crepitans*) concluded that 2.7% of frogs collected from different sites in Illinois during a 3-year period were intersexed. Along with atrazine, polychlorinated biphenyl (PCB) and polychlorinated dibenzofuron (PCDF) were found in the samples collected (Reeder et al. 1998). More research is needed to determine if atrazine is the sole cause of such abnormalities.

Atrazine, a symmetrical triazine, has several different fates in lake water including hydrolysis, photolysis, adsorption, and desorption (Gao et al. 1998; Goswami et al. 1971; Konstantinou et al. 2001; Mersie et al. 1998a; Moreau et al. 1997; Wauchope et al. 1985). Atrazine has a relatively low water solubility (33 mg L^{-1}) and moderate adsorption (average K_{oc} = 100 ml g^{-1}), but little is known about the interaction of atrazine and sediment (Mersie et al. 1998b). Along with atrazine, three of its metabolites have been found in lake water (EPA 2003). These metabolites are desethyl atrazine (DEA), desisopropyl atrazine (DIA), and diaminochlorotriazine (DACT).

Often sediment has been questioned as a potential storage reservoir, transport vehicle, and ultimately a long-term contamination source of organic compounds like atrazine. Adsorption and desorption of organic compounds to soil depends on several different factors, including sand, silt, and clay contents, organic matter, pH, and aerobic and anaerobic environment. Soil and water pH plays a primary role in the adsorption behavior of atrazine. As pH decreases, the cationic fraction is increased, increasing atrazine adsorption to soil (Clay et al. 1990a; Gao et al. 1998; Rae et al. 1998; Weber 1970). Likewise, atrazine tends to desorb as pH increases (Jenks et al. 1998).

Atrazine adsorbs to both organic matter and clays. Organic matter has a greater affinity to atrazine compared to clay (Gao et al. 1998; Laird et al. 1994; Ying et al. 2000). Atrazine affinity to soil increases as organic matter increases (Patakioutas et al. 2002). This increase in organic matter decreases the amount desorbed (Jenks et al. 1998).

A decrease in soil particle size increases the adsorption of atrazine to soil and decreases the amount susceptible to desorption. Its high affinity to clay and silt particles is greater due to greater surface area compared with coarser textures (Sonon et al. 1995). However, if pH decreases and organic matter increases, or a combination of the two, the affinity of a sandy soil to atrazine increases (Gao et al. 1998).

In several studies, it has been shown that the total amount of atrazine adsorbed to a soil or sediment is not completely desorbed. Normally small fractions (5.5 to 40 %) of the amount adsorbed were desorbed demonstrating that relatively large amounts of atrazine potentially are adsorbed or degraded (Clay 1990b; Gao 1998; Jenks et al. 1998; Pignatello et al. 1991; Seybold 1996).

As in soils, sediments may have different adsorptive characteristics and, therefore, may have different affinities for organic compounds like atrazine. Unlike soil, sediment is found in an anaerobic environment and often contains a greater amount of organic matter, which may affect adsorption capacities of sediments (Seybold 1999).

A classification system for adsorption isotherms was designed by Giles et al. (1960). This design classified adsorption patterns of soils in four different categories. The different isotherms included the S, C, L, and H-type isotherms. The S-type occurs where lower concentrations of the pesticide remain in water, mid-level concentrations adsorb, and high concentrations reach a maximum adsorption. The C-type isotherm, demonstrates constant partitioning of a compound across a range of concentrations and a maximum adsorption is never reached. The L-type isotherm (Langmuir) demonstrates a relatively high affinity at lower concentrations, but then reaches maximum adsorption at higher concentrations. The H-type isotherm (high affinity) is similar to the L-type but has a higher affinity at low concentrations and reaches a maximum (Weber 1970).

The difference between the amount of a herbicide adsorbed to a soil or sediment and the amount of the herbicide desorbed from the same soil or sediment is known as hysteresis. No one mechanism influences the adsorption and desorption process of a compound in the same way. Several types of bonds, varying in strength between the sediment and herbicide, can cause adsorption. The same bonds influences the herbicides desorption. Bonds range from weak bonds such as London-van der Waals forces to strong bonds such as covalent bonds. In desorption, the weaker bonds are broken, but the stronger ones may not be over come. Without the release of these stronger bonds, equilibrium during the desorption process is never met. This in part explains the amount of certain herbicides, which remain adsorbed even after several desorption cycles (Koskinen et al. 1990).

Another concern in the possible sediment storage and recontamination factor is the degradation and persistence of atrazine once adsorbed to the sediment. Past studies, have shown that the half-life of atrazine is variable among soils and sediments. The average field half-life is 60 d, but the half-life has ranged from 39 d to 261 d (Vencill 2002). In sediments collected from estuarine water, atrazine's half-life of 15 to 20 d was reported (Jones et al. 1982), and a half-life of 145 d was reported from Wisconsin lake sediments (Armstrong et al. 1967; Jones et al. 1982).

Many different factors contribute to atrazine degradation once adsorbed to soil. Atrazine is susceptible to breakdown by UV light (photolysis) after application into hydroxyatrazine and *N*-desethylated atrazine (Vencill 2002; Jordan et al. 1970). Photolysis is faster in soil systems than in water because photolysis is hindered by the presence of organic matter in surface water (Konstantinou et al. 2001). In soils at a moderately acidic pH ranging between 5.5 to 6.5, atrazine degradation to hydroxyatrazine is due to chemical hydrolysis, and at moderately neutral pH in soils, hydrolysis is partially driven by an increase in microbial activity (Vencill 2002; Blumhorst et al. 1994). Hydrolysis is slower at more basic values ranging from 7.5 to 8 in soils (Vencill 2002; Blumhorst et al. 1994). In anaerobic environments, pH is typically higher than aerobic situations and has a low rate of atrazine hydrolysis and also atrazine dealkylation by microbes (Goswami et al. 1971; Jones et al. 1982; Klint et al. 1993).

Soil bacteria can drive microbial hydroxylation and mineralization. Some *Pseudomonas* species have been observed to mineralize atrazine in both aerobic and anaerobic environments (Shapir et al. 1998). In one study, over 30 atrazine-degrading bacteria cultures were found in soil samples. In this study, it was concluded that hydrolysis of atrazine mediated by bacteria is wide spread in soils (Mandelbaum et al. 1993).

Soil characteristics also play a role in degradation of atrazine. Soils with higher organic matter typically degrade atrazine more efficiently through microbial degradation (McCormick et al. 1965). Soil characteristics can influence the sequestration of atrazine. Organic carbon, soil texture, and cation exchange capacity play a large role in holding atrazine unexcessable for microbes. Radosevich et al. 1997). Estimated rates of atrazine degradation in soil and sediment have differed substantially depending on the study. Atrazine degradation was slower in submerged soils than in aerobic soil (Goswami et al. 1971). In this study, actual soil samples treated with atrazine were submerged. Jones et al. (1982) found that atrazine degradation was faster in sediment/water systems than in soil systems. In this study both sediment and soil were studied. Although some work has been documented regarding atrazine behavior, it is not known what effect differing sediment characteristics have on atrazine contamination in surface water.

HYPOTHESIS

Lake sediments differ in characteristics and, therefore, differ in their ability to adsorb and desorb atrazine.

OBJECTIVE

To determine relative adsorption and desorption of various Texas lake sediments to atrazine.

MATERIALS AND METHODS

Eight lake sediments were collected from sites where atrazine was detected during an earlier study. All eight lakes were located in northeast Texas and were used as community water use systems.

Sediment collection and preparation.

Sediment samples were collected using an Eckman dredge from a bridge or boat depending on site availability at each individual lake. Samples were placed in 3.75-L Ziploc bags and placed in a cooler for transport back to the laboratory. Once back at the laboratory, samples were dried on paper in the greenhouse. The sediment samples were then ground with a soil grinder and then a mortar and pestle as needed to get uniform particle size. After grinding, sediments were passed through a 2-mm sieve. Subsamples were characterized by the Texas A&M Soil Characterization Laboratory for texture, percent organic carbon, pH, CEC, extractable bases, and % base saturation.

Batch equilibrium adsorption and desorption.

Four 2-g samples were weighed out for each collection and placed into separate 35-ml centrifuge tubes. Initial standard solutions were made using a ring-labeled atrazine standard combined with an analytical grade atrazine standard in methanol. One hundred uL of the initial standard solutions were added to each centrifuge tube along with 5-ml of 0.01M $CaCl_2$ solution to provide a constant ionic strength during the adsorption-desorption experiment. This provided a concentration of approximately 8.41 Bq mL^{-1} ^{14}C material in all samples, and 0.5, 1.0, 2.0, and 5.0 ug mL^{-1} of analytical grade, non-radio labeled atrazine.

The samples were placed on a table shaker and shaken for 24 h. Tubes were then removed and centrifuged for 10 min. A 2-mL aliquot were then removed from the supernant of each sample and placed in glass scintillation vials along with 10 ml of scintillation fluid for the adsorption study. The remaining solution (1ml) was removed from the samples and replaced with fresh 0.01M $CaCl_2$ solution. Samples were then shaken again for 24 h. Another 2-ml aliquot were removed and placed in glass scintillation vials along with 10 ml of scintillation fluid for analysis of atrazine desorption. The procedure was repeated two more times in a 48-h period. The aliquots in scintillation fluid were then placed on a Beckman LS6500 Multipurpose scintillation counter. Each sample was counted for 20 min.

The relative affinity of atrazine to a sediment was determined by the Freundlich equation.

$$X/M = KC^{1/n} \qquad (1)$$

$$\log X/M = \log K + 1/n \log C \qquad (2)$$

Where, X/M equals the amount adsorbed per amount adsorbent, C represent the solution concentration at equilibrium, and K and n are constants. K represents the affinity of the herbicide to the sediment, and 1/n is the slope. If n is approximately 1, K represents the distribution coefficient, K_d.

Isotherms were characterized, by graphing X/M on the y-axis and C on the x-axis. Hysteresis was determined, by graphing the adsorption amounts using log X/M on the y-axis and log C on the x-axis. Desorption of two initial concentrations of 1.0 ug mL^{-1} and 5.0 ug mL^{-1} were chosen to determine desorption behavior of atrazine from the selected sediments. These desorption amounts were subtracted from the total amount adsorbed at the specified concentration. The result was the amount of atrazine remaining adsorbed after the consequent desorption steps. The difference between adsorption and desorption showed the amount of atrazine that was desorbed. This also provided a way of determining the degree of hysteresis.

The experiment was executed in a completely randomized block design and replicated four times. Linear regression was used to determine K_d. Percent desorption was determined using total amount atrazine adsorbed and total amount of atrazine remaining adsorbed after desorption process. The general linear model (GLM) procedure was used to determine differences between K_d values and percent desorption between the different lake sediments (SAS 1985). Tukey's studentized range was used for mean separation of K_d values and percent desorption to determine differences between adsorption and desorption of the various sediments.

RESULTS AND DISCUSSION

The texture of the sediments varied from a loamy fine sand at Richland-Chambers Reservoir to a clay at both Big Creek Lake and Aquilla Lake (Table 1). The organic carbon content ranged from a 0.45% at Richland-Chambers Reservoir to a 2.98% at Big Creek Lake (Table 2). This is approximately a difference of 2.5%. The pH of the sediments ranged from a 6.4 at Big Creek Lake to a 7.8 at Lake Tawakoni (Table 3). Both Big Creek Lake and Aquilla Lake are both clays and high in % organic carbon, but the sediment at Aquilla Lake has a pH of 7.4, a one-point increase in pH from the sediment of Big Creek Lake. It has been stated, as pH decreases, it increases atrazine adsorption (Clay et al. 1990a; Gao et al. 1998; Rae et al. 1998; Weber 1970).

On the graphical representation of the Freundlich Equation for Big Creek Lake, Lake Waxahachie, Richland Chambers Reservoir, and Aquilla Lake, log of X/M is found on the y-axis and log of C is found on the x-axis (graph 1, 2, 3, &4). The solid black line represents the adsorption curve with points located for initial concentration 0.5 ug ml^{-1}, 1.0 ug ml^{-1}, 2.0 ug ml^{-1}, and 5.0 ug ml^{-1}. The y-intercepts for Big Creek Lake, Lake Waxahachie, Richland Chambers Reservoir, and Aquilla Lake were 0.65, 0.48, 0.26, and 0.40 respectively. The y-intercept is used to determine K adsorption values for each lake. All adsorption curves were linear and had n values ranging from 0.98 to 1.02. Since n was approximately 1.00, $K = K_d$ can be assumed. Initial concentrations of 1.0 ug ml^{-1} and 5.0 ug ml^{-1} were chosen to draw desorption curves. Desorption curves (dashed lines) for Big Creek Lake, Lake Waxahachie, Richland Chambers Reservoir, and Aquilla Lake show that 78%, 84%, 92%, and 88% of the atrazine initially adsorbed remains adsorbed to the sediment respectively (Graphs 1, 2, 3, &4). Hysteresis is present due to the difference in adsorption and desorption (Graph 1, 2, 3, &4). If hysteresis did not occur, the amount initially adsorbed would then have been desorbed, causing the desorption curves to fall on top of the adsorption curve.

K_d values ranged from 1.8 at Richland Chambers Reservoir to a 4.5 at Big Creek Lake (Table 4). Sediment from Richland Chambers Reservoir was a loamy fine sand, low in organic carbon, and had a pH of 7.7. Sediment from Big Creek Lake consisted of clay, high organic carbon and a pH of 6.4. Sediment from Aquilla Lake had a K_d value of 2.5, which differed by 2 points from Big Creek Lake. Aquilla Lake was also a clay, high in organic carbon, but had a pH of 7.4.

The K_d value of Big Creek Lake was significantly different than all other sediments. The K_d values of Richland Chambers Reservoir and Lake Tawakoni were not significantly different from each other but were significantly different than all other sediments. There was a +.84 correlation between percent organic carbon and K_d, there was a -.75 correlation between pH and K_d, and a +.74 correlation between percent clay and K_d.

Percent desorption ranged from 7.6% at Richland Chambers Reservoir to 21.6% at Big Creek Lake, a difference of 14% (Table 4). Lake Tawakoni and Richland Chambers Reservoir consisted of fine, loamy, sandy type materials low in organic carbon. The percent desorption of these two lakes were significantly different than the percent desorption of Joe Pool Lake, Lavon Lake, and Big Creek lake, which consisted of higher clay content and higher organic carbon content.

A correlation of +.81 is seen between K_d values and percent desorption (Graph 5). The more atrazine a sediment adsorbs, the more atrazine a sediment desorbs. The less atrazine a sediment adsorbs, the less atrazine a sediment desorbs.

Conclusion

There were differences in atrazine adsorption and desorption to the various sediments. This suggests that availability in water once sediment has been contaminated depends on characteristics of individual sediments.

Sediment Data

Texture

Big Creek Lake	Clay
Joe Pool Lake	Silty clay
LavonLake	Silty clay
Bardwell Lake	Silty clay loam
LakeWaxahachie	Silty clay loam
Aquilla Lake	Clay
Lake Tawakoni	Very fine sandy loam
Richland-Chamber Reservoir	Loamy fine sand

Table 1. Texture characteristics of 8 various lake sediments. Samples were characterized by the Texas A&M Soil Characterization Laboratory, College Station, TX

Sediment Data

% Organic Carbon

Big Creek Lake	2.98
Joe Pool Lake	1.87
Lavon Lake	2.07
Bardwell Lake	1.37
Lake Waxahachie	1.45
Aquilla Lake	2.08
Lake Tawakoni	0.58
Richland-Chamber Reservoir	0.45

Table 2. Organic Carbon content of 8 various lake sediments. Samples were characterized by the Texas A&M Soil Characterization Laboratory, College Station, TX

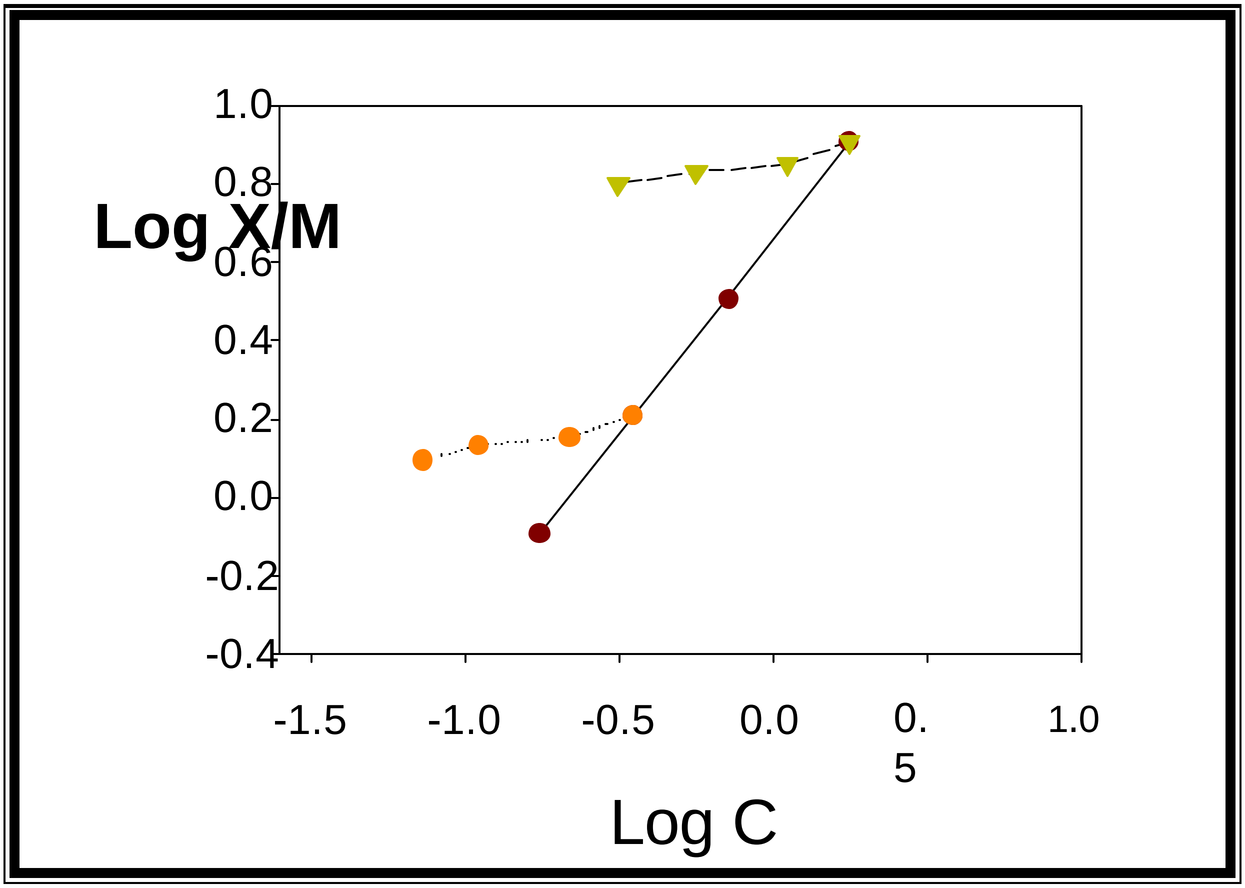
Sediment Data

pH

Big Creek Lake	6.4
Joe Pool Lake	7.5
Lavon Lake	7.4
Bardwell Lake	7.7
Lake Waxahachie	7.5
Aquilla Lake	7.4
Lake Tawakoni	7.8
Richland-Chamber Reservoir	7.7

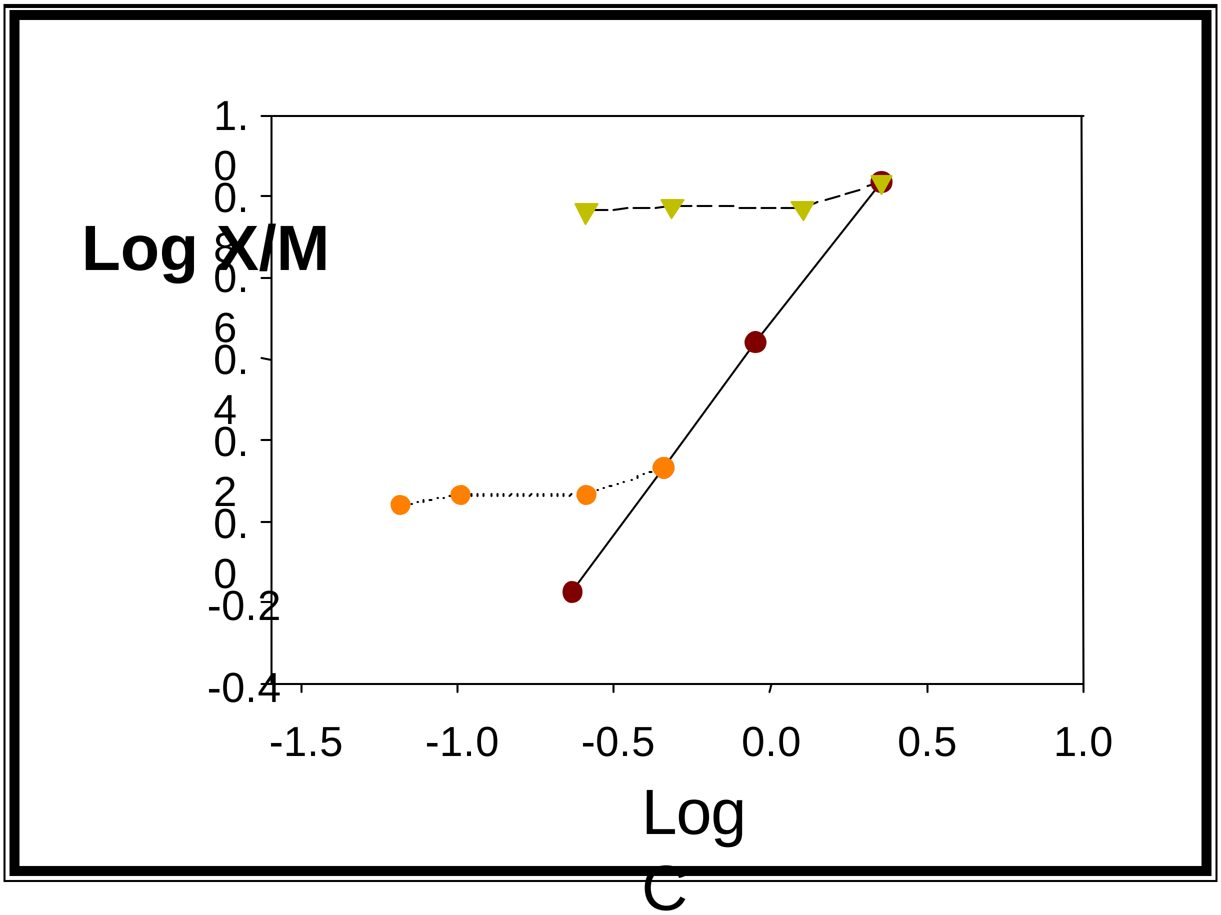
Table 3. pH of 8 various lake sediments. Samples were characterized by the Texas A&M Soil Characterization Laboratory, College Station, TX

Big Creek



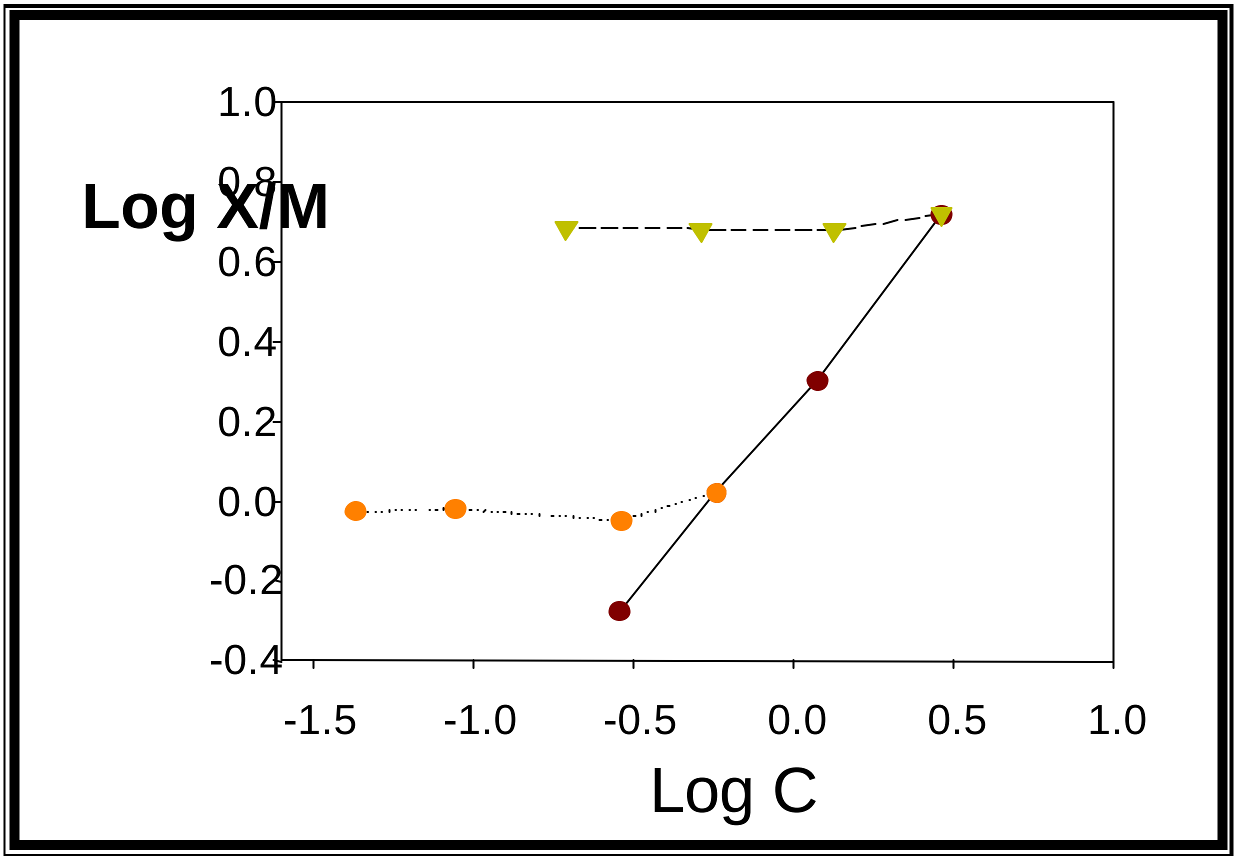
Graph 1. Graphical representation of the Freundlich equation.

Waxahachie



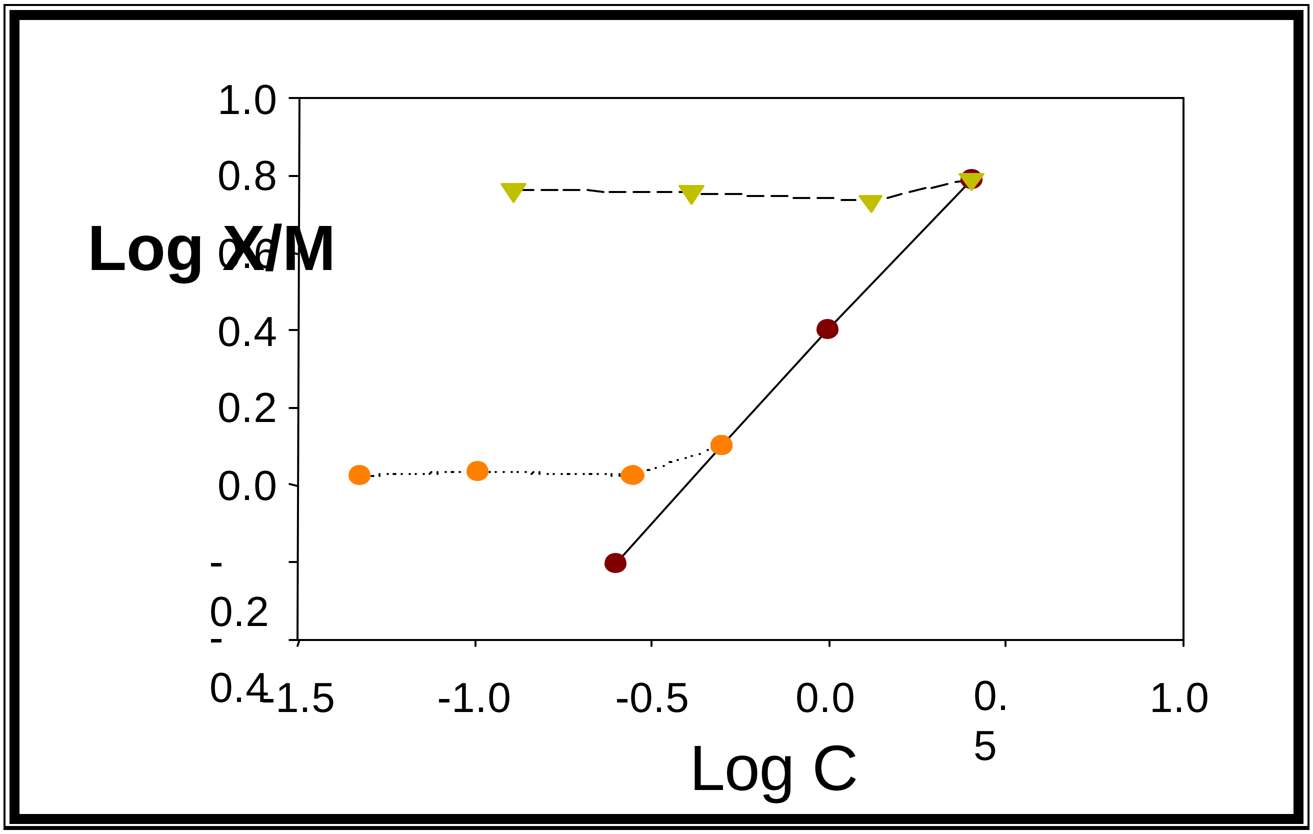
Graph 2. Graphical representation of the Freundlich equation.

Richland Chambers



Graph 3. Graphical representation of the Freundlich equation.

Aquilla

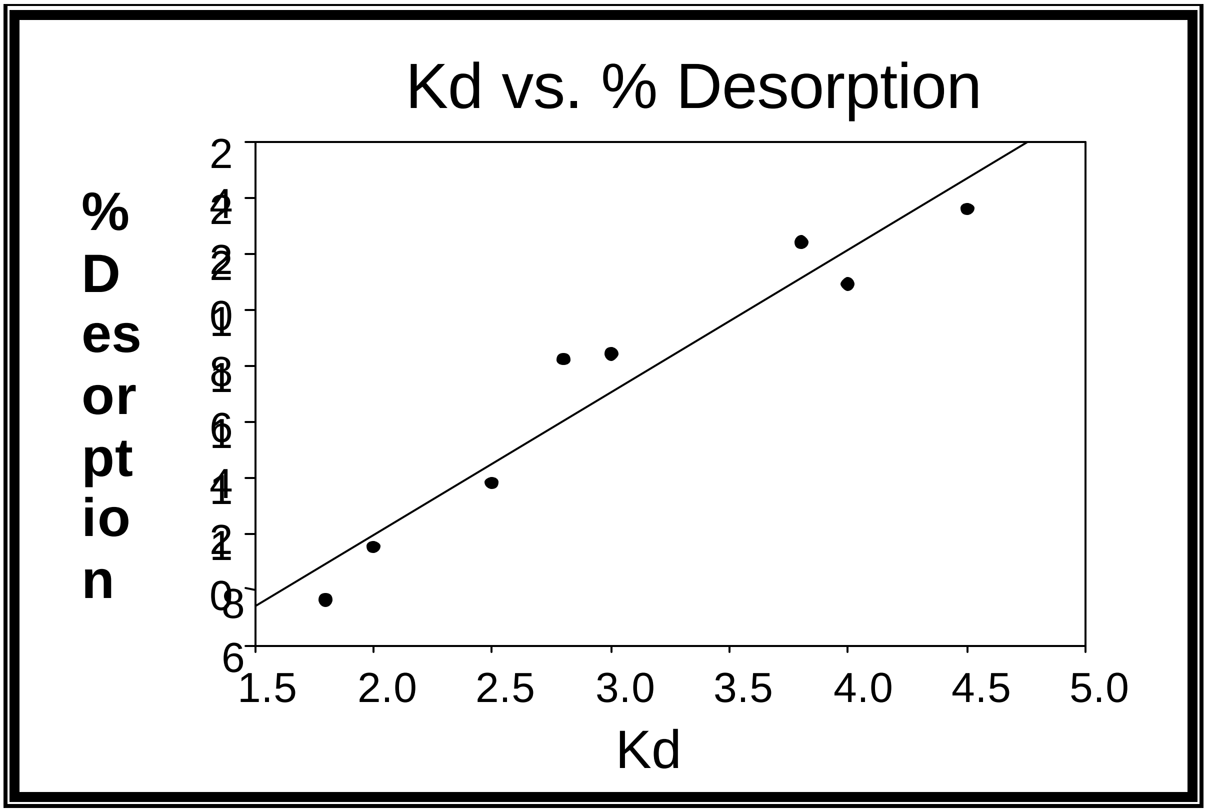


Graph 4. Graphical representation of the Freundlich equation.

Adsorption/Desorption

Collection Site	Kd	n	% Desorption
Richland Chambers Reservoir	1.8	0.98	7.6
Tawakoni Lake	2.0	0.98	9.5
Aquilla Lake	2.5	0.99	11.7
Lake Waxahachie	2.8	1.00	16.2
Bardwell Lake	3.0	1.02	16.4
Lavon Lake	3.8	0.97	20.4
Joe Pool Lake	4.0	0.99	18.9
Big Creek Lake	4.5	0.99	21.6

Table 4. pH of 8 various lake sediments. Samples were characterized by the Texas A&M Soil Characterization Laboratory, College Station, TX



Graph 5. Relationship between Kd and % Desorption.

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PUBLICATIONS:

Vader, J.A., S.A. Senseman, and M.C. Dozier. 2003. Adsorption and desorption of atrazine in various Texas lake sediments. Proc. South. Weed Sci. Soc. 56:000. In press. Presentation

Vader, J.A., S.A. Senseman, and M.C. Dozier. 2002. Adsorption and desorption of atrazine from various lake sediments in Texas. Texas Plant Protection Assoc. Conf. Proc. Second place poster

Vader, J.A., S.A. Senseman, and M.C. Dozier. 2002. Adsorption and desorption of atrazine from various lake sediments in Texas. Proc. South. Weed Sci. Soc. 55:184. Poster

The Role of Suspended Clays in Phosphrus Processing by Lotic Periphyton

Basic Information

Title:	The Role of Suspended Clays in Phosphrus Processing by Lotic Periphyton
Project Number:	2002TX63B
Start Date:	3/1/2002
End Date:	2/1/2003
Funding Source:	104B
Congressional District:	11th
Research Category:	None
Focus Category:	Surface Water, Water Quality, Nutrients
Descriptors:	None
Principal Investigators:	june.e..wolfe.iii.1, Owen T. Lind

Publication

WOLFE - TWRI Graduate Research Enhancement Grant Progress Report
March 2002 - February 2003

Project Title: Nutrient processing by in-stream periphyton in a reservoir-watershed landscape

Principle Investigator: Mr. June E. Wolfe, III
Co-Principle Investigator: Dr. Owen T. Lind

First Quarter Activity - (March-May 2002):

- Received grant funds and assigned an account number through the Baylor University budget office.
- Ordered supplies – Dionex Ion Chromatograph (IC) Atlas Suppressor.
- Became familiar with ion chromatography techniques for orthophosphate determination in laboratory stream samples.
- Became familiar with spectrophotometric techniques for total phosphorus determination in laboratory stream samples.

Second Quarter Activity - (June-August 2002):

- Ordered supplies – Micro-filtration glassware, filters, and long path equipment for Genesys 5 spectrophotometer.
- Paid student fees for summer seminar course – Basin Watershed Modeling (1 hr).
- Conducted preliminary experiments to develop operating procedures for laboratory streams and determine phosphorus sorption potential of laboratory stream construction materials.
- Developed handling and storage procedures for laboratory stream water samples.
- Developed spreadsheets for data handling.

Third Quarter Activity - (September-November 2002):

- Paid student fees for two courses (Biology Seminar – 1 hr, Interdisciplinary Teaching – 1 hr).
- Logged 1349 travel miles (Temple to Waco and return).
- Completed the required Dissertation Research Proposal and filed with Baylor Biology Department.
- Further development of laboratory techniques for phosphorus analysis.
- Developed methodologies for determining periphyton biomass.

Fourth Quarter Activity – (December 2002– February 2003)

- Paid student fees for one course (Wetland Ecology – 4 hrs)
- Ordered supplies - Polyethylene sample bottles, plastic centrifuge tubes, high temperature crucibles, chemical reagent.
- Requested reimbursement for travel expensed incurred during fall semester commute.
- Became familiar with techniques for measuring clay turbidity in laboratory streams.
- Developed turbidity curves for clays under investigation.
- Became familiar with techniques for separating suspended clays from water samples using a centrifuge.
- Evaluated three laboratory stream substrates as phosphorus sources/sinks.

Information Transfer

Basic Information

Title:	Information Transfer
Project Number:	2002TX65B
Start Date:	3/1/2002
End Date:	2/1/2003
Funding Source:	104B
Congressional District:	8th
Research Category:	None
Focus Category:	Law, Institutions, and Policy, Management and Planning, Waste Water
Descriptors:	None
Principal Investigators:	Ric W. Jensen, C. Allan Allan Jones

Publication

1. Gerston, Jan. December 2002. Water and Wastewater Utilities Enhance System Security. Texas Water Resources newsletter.
2. Jensen, Ric. September 2002. TWRI Mills Scholars Program Aids TAMU Graduate Students; Soil and Water Grants Support Research, Extension Efforts, Texas Water Resources newsletter.
3. Jensen, Ric. 2002. New Waves newsletter (3 issues).
4. Alexander, Rachel. 2002. Rio Grande Basin Initiative Outcomes newsletter (2 issues).

Information Transfer Program
TWRI
2002

PI: Ricard W. “Ric” Jensen, Ph.D.

Articles describing research projects of the Texas Water Resources Institute (TWRI) were publicized in the “Texas Water Resources” and “New Waves” newsletters. These articles presented information about TWRI research to the public in easily understood terms.

At the same time, significant efforts were made to describe TWRI research projects on the World Wide Web. This includes a full description of each US Geological Survey (USGS) Project awarded by TWRI and links to popular articles.

Special efforts were made to obtain products of funded studies (for example, journal articles, papers, posters, and presentations) and to then make these available to the public. For example, more than 70 special reports and technical reports were web-posted during 2002 that summarize the results of Institute research.

TWRI continues efforts to assure the timely communication and dissemination of water resources news via email through the “TWRI WaterTalk” list server. More than 400 people now subscribe to the list server. They are sent 5 messages per day about water resources news, new books and other resources, and meetings and conferences.

Significantly, the list server is used to alert researchers and policy makers about opportunities to take part in funding opportunities and to form collaborations for building team partnerships.

As a result of publicizing these programs through the list server and Web site, TWRI has significantly increased the number of individuals that apply for competitive grants programs, including those funded by USGS and NIWR. This creates, in turn, more opportunities for collaborative research and extension efforts.

More than ever, TWRI is expanding its role to include several research and extension programs supported by external grants. For example, the Institute is working with Texas Cooperative Extension to manage the Rio Grande Basin Initiative, which is funded by the U.S. Department of Agriculture Cooperative State Research Education and Extension Service (USDA/ CSREES). Other significant grants now being administered and publicized by the Institute include projects to market composted dairy manure, efforts to advance hydrologic modeling, and work to investigate the ecological effects of brush control.

In addition to publication and Web site, TWRI has been involved in several meetings, workshops, and conferences. Some of these efforts involve hosting or co-hosting events dealing with bacterial source tracking, the desalination of coastal and inland waters, advances in water related computer modeling, and other topics. Training supported by TWRI included the education of groundwater district managers, and training of small water systems personnel about wellhead protection.

Information Transfer Program

USGS Summer Intern Program

Student Support

Student Support					
Category	**Section 104 Base Grant**	**Section 104 RCGP Award**	**NIWR-USGS Internship**	**Supplemental Awards**	**Total**
Undergraduate	3	6	0	0	9
Masters	5	3	0	0	8
Ph.D.	5	0	0	0	5
Post-Doc.	0	0	0	0	0
Total	13	9	0	0	22

Notable Awards and Achievements

Jordan Furnans, Ph.D. student at the University of Texas Austin, received a prestigious Fullbright Scholarship to help him continue research funded under the USGS 104 program.

Jennifer Hadley, Masters student in Forest Science, Texas A&M University, won the TAMU Vice Chancellors award for Teaching in 2003.

Matt Simmons, a Ph.D. graduate student in the department of Rangeland Ecology and Management at Texas A&M University, was asked to present research findings at the Ecological Society of America annual conference.

Judy Vader, Masters student in Soil and Crop Sciences, Texas A&M University, won 2nd place at the 2003 Texas Plant Protection Society Conference with a poster depicting her USGS based research.

Publications from Prior Projects