Report as of FY2006 for 2006AR131B: "Occurrence and antibiotic resistance in fecal indicator bacteria upstream and downstream of wastewater treatment plants in northwest Arkansas"

Publications

- Dissertations:
  - In progress: Akiyama, Tatsuya, expected 2008, "Occurrence and antibiotic resistance in fecal indicator bacteria upstream and downstream of wastewater treatment plants in northwest Arkansas," MS Thesis, Department of Crop, Soil and Environmental Sciences, University of Arkansas, Fayetteville, Arkansas 72701.
- Other Publications:
  - Savin, M., 2007, Antibiotic resistance in aquatic bacteria downstream from effluent discharge, 2007 Arkansas Water Resources Center Conference, University of Arkansas, Fayetteville, Arkansas, April 24-25, 2007[CD].

Report Follows

Project title: Occurrence and antibiotic resistance in fecal indicator bacteria upstream and downstream of wastewater treatment plants in northwest Arkansas

Statement of critical regional or state water problem

Oklahoma has taken legal action against Arkansas for polluting its scenic waterways and drinking water sources. Some of the streams within the watersheds of concern, the Illinois, Euchaw, and Spavinow watersheds, receive runoff from agricultural lands. Others receive point sources of possible pollutants by receiving wastewater treatment effluent. The lawsuits have been concerned with excess nutrients, but nutrient loads are not the only concern associated with surface waters receiving runoff and wastewater effluent. Wastewater treatment plants are designed to remove BOD, nutrients, and pathogens, but do so with varying levels of success. In addition, wastewater treatment plants are not designed to remove many organic contaminants, and removal may be occurring with varying levels of success.

As far as screening for the presence of pathogens it is accepted protocol to use bacterial indicators. Ideally, indicators of fecal contamination should be organisms that are easily detected and useful in all water types. Indicators should not grow in water, but should be abundant in direct proportion to fecal contamination and should be present whenever a pathogen is present. Both fecal coliforms and fecal enterococci are members of intestinal microflora of warm-blooded animals. For freshwater systems, *Escherichia coli* is the accepted indicator species (U. S. EPA, 1986). The Arkansas Department of Environmental Quality requires that in primary contact waters fecal coliforms be no greater than 200 CFU/100mL, with a monthly maximum of 400CFU/100ml (ADEQ, 2004). Alternatively, *E. coli* numbers must be no greater than 126 CFU/100mL, with a monthly maximum of 410 CFU/100ml in rivers and streams (ADEQ, 2004).

The choice of indicator bacteria is not irrevocably established, however. Methods to measure bacterial indicators are not perfect and the appropriate indicator to use for different aquatic ecosystems may vary with local conditions. Fecal enterococci may be an appropriate choice for some locations (U. S. EPA, 1986). *Enterococcus faecalis* has a host range limited to humans and some wild birds (Wheeler et al., 2002). Unless the fecal loading rate from migratory or resident wild birds is high, water samples collected during baseflow conditions with high numbers of *Ent. faecalis* may indicate human fecal contamination (Kuntz et al., 2004). It is still not well known which test method best determines water safety or to what extent different indicators correlate with each other in different regions of the U.S.

The landscape, multiple sources of fecal contamination, and sediments may all contribute to different and varying numbers of indicator bacteria in surface water. While waste microflora may not be effective competitors in the environment, they may persist after entering the environment. For example, indicator organisms that enter surface waters can survive in sediments and elevated numbers are measured after sediments are disturbed (Burton et al., 1987; Hartel et al., 2004). Indicators may therefore represent present or past contamination. The ability to survive in sediments may pose an additional problem if the bacteria had developed antibiotic resistance in the intestine or later in the environment, and possession of antibiotic resistance genes continued to be of benefit under environmental conditions.

Antibiotic resistance of intestinal microflora is prevalent. For example, up to 5% of intestinal bacteria may carry the tetracycline resistance gene *tet*O (Aminov et al., 2001). Lateral transfer of genetic elements, once considered to be a rare event, is now known to occur frequently in bacteria. Genes conferring antibiotic resistance can be encoded on a variety of

mobile genetic elements and transferred to multiple and unrelated species through several mechanisms including conjugation, transformation, and transduction. All appear to be facilitating the rapid spread of antibiotic resistance in multiple bacterial lineages. For example, resistance to tetracycline is encoded on genetic elements in plasmids (Smalla et al., 2000; Aminov et al., 2001) and conjugative transposons (Salyers et al., 1995) that have facilitated the transfer of these genes across species (Nikolich et al., 1994).

Because antibiotics can pass through the human body and be excreted in wastes, certain amounts of antibiotics and intermediate break down products are transported to the wastewater treatment plant, and finally into the effluent due to the lack of removal. The addition of antibiotics and antibiotic residues to aquatic ecosystems can create both ecological and health concerns due to the potential development of antibiotic resistance in microbial populations. Recent studies have revealed the presence of antibiotics in streams receiving point sources of wastewater (e.g. Kolpin et al., 2002). In addition, a United States Geological Survey (USGS) study revealed the presence of low levels of antibiotics in northwest Arkansas streams, including Mud Creek in Fayetteville and Spring Creek in Springdale (Galloway et al., 2005; Table 1). Both Mud Creek and Spring Creek receive effluent from municipal wastewater treatment plants. Mud Creek flows through residential areas, whereas downstream from the wastewater treatment plant Spring Creek flows through a rural landscape where cattle, wildlife and other recreational users may be exposed to the surface waters. These two streams are tributaries to the Illinois River.

Table 1. Antibiotics previously found in Mud Creek in August 2004 (data from Galloway et al., 2005).

Antibiotic (ppm)	Mud Creek South of Hwy. 45[1]	Mud Creek at Township	Mud Creek at Old Wire Road
Erythromycin	ND[2]	0.175	0.154
Trimethoprim	ND	0.058	0.045
Tylosin	ND	0.012	0.008
Ciprofloxican	ND	0.039	0.027
Ofloxacin	ND	0.109	0.094
Sulfadimethoxine	ND	0.003	0.004
Sulfamethoxazole	ND	0.196	0.302

[1]Upstream of the wastewater treatment plant.
[2]Not detected, with a detection limit of 0.01 ppm for erythromycin and 0.005 ppm for the remaining antibiotics.

Natural resistance results from the production of antibiotics, such as streptomycin, trimethoprim, and erythromycin, by bacteria in the environment (Mazel et al., 1999). However, the presence of additional and "foreign" antibiotics may increase antibiotic resistance in pathogens, posing a threat to human health, and requires further investigation. Past studies have revealed the presence of antibiotic resistance in Swedish sewage (Iversen et al., 2002) and in the tributaries emptying into and inside Tillamook Bay, OR (Kelch and Lee, 1978). The addition of effluent from wastewater treatment plants has been shown in the Arga River in Spain to increase certain antibiotic resistance in strains of *Enterobacteriaceae* and *Aeromonas* downstream from the discharge (Goni-Urriza et al., 2000).

Compounding the problem of increased antibiotic resistance is the prevalence of genetic

elements encoding multiple resistance or associated with resistance to other stresses such as the presence of heavy metals. It remains unclear to what extent pressure to develop antibiotic resistance affects environmental communities, especially when antibiotic concentrations are found in very low levels. Higher levels of ampicillin resistant bacteria were isolated from stream waters adjacent to a farm receiving treated sludge than samples collected upstream or downstream from the farm (Selvaratnum and Kunberger, 2004). All ampicillin resistant isolates were also found to be resistant to at least one other antibiotic (Selvaratnum and Kunberger, 2004). Furthermore, antibiotic resistance analysis (ARA) to multiple antibiotics is a commonly used method to differentiate sources of fecal contamination within a watershed (Meays et al., 2004; Wiggins et al., 1999).

If possession of resistance mechanisms does not confer a competitive advantage under environmental conditions, genes encoding those mechanisms may not be retained or transferred among environmental community members. Rates of transfer of mobile genetic elements may decrease dramatically under (generally sub-optimal) environmental conditions, such as found among strains of *Enterococcus faecalis* in sewage treatment activated sludge basins (Marcinek et al., 1997). The presence of low levels of antibiotics in sewage did not increase transfer of gentamicin resistance plasmids in *Staphylococcus aureus* (Ohlsen et al., 2003). Plasmids have shown differing patterns of stability under non-selective conditions (Smalla et al., 2000). Such studies evoke questions of the stability of antibiotic resistance under various and dynamic environmental conditions. However, the influence of multiple stressors could mean that the presence of low levels of antibiotics could have additive or multiplicative effects in the environment. Even for discontinued antibiotics, resistance may be maintained if it is linked to genes encoding resistance to other currently used antibiotics (Bischoff et al., 2005). Clarification is still needed to understand the rate and extent of transfer and subsequent incorporation of resistance genes under *in-situ* environmental conditions.

A limited experiment investigating levels of antibiotics resistance in local streams was conducted during the summer of 2005 with high school students participating in the University of Arkansas Gifted and Talented Summer Course "The Good, the Bad, and the Genetically Engineered." Bacteria were enumerated using the Colilert defined substrate technology (IDEXX Laboratories, Westbrook, ME). Most probable numbers of *E. coli* in the presence and absence of tetracycline showed a decreased, but measurable number of *E. coli* growing in the presence of tetracycline in water collected from Mud Creek at Township (Fig. 1). The number of tetracycline resistant *E. coli* were about 40% of total *E. coli* counted in the absence of antibiotics. In contrast to these results, a stream running through an animal farm and a small area draining runoff from a residence showed that 15% and <1% of *E. coli*, respectively, were tetracycline resistant. If expression of antibiotic resistance among indicator bacteria is found to increase in streams receiving treated wastewater effluent, this suggests a potential for antibiotic resistance to increase in pathogens, which could have serious implications for treating diseases.

Statement of results and benefits

Government regulatory agencies need scientific data to make appropriate and sound decisions to protect water quality and human health. Understanding the occurrence and distribution of indicators of fecal contamination as recommended by U. S. EPA is essential to microbial source tracking and identification of public health risk. Additionally, to identify if streams receiving effluent from a point source are increasing in antibiotic resistance downstream

from that point source will enable regulators to develop preventive strategies to protect water quality in streams receiving wastewater discharge.

There is a wide variety of different phenotypic (based on characteristics expressed by the bacterium, such as ARA), genotypic (based on DNA), and chemical (based on chemicals associated with humans) methods to characterize bacteria and track their sources. If bacteria are acquiring resistance to antibiotics in the environment because of low levels of antibiotics being introduced with treated wastewater effluent, rather than through pressure in the intestine of various host animals, the basis of using ARA will be nullified. The information generated in this project, in conjunction with a parallel project conducted in the Stillwater creek watershed with our collaborator Shiping Deng at Oklahoma State University, will provide much needed information about potential microbial indicator organisms and levels of antibiotic resistance in streams receiving effluent that flow through both urban residential areas and agricultural production systems.

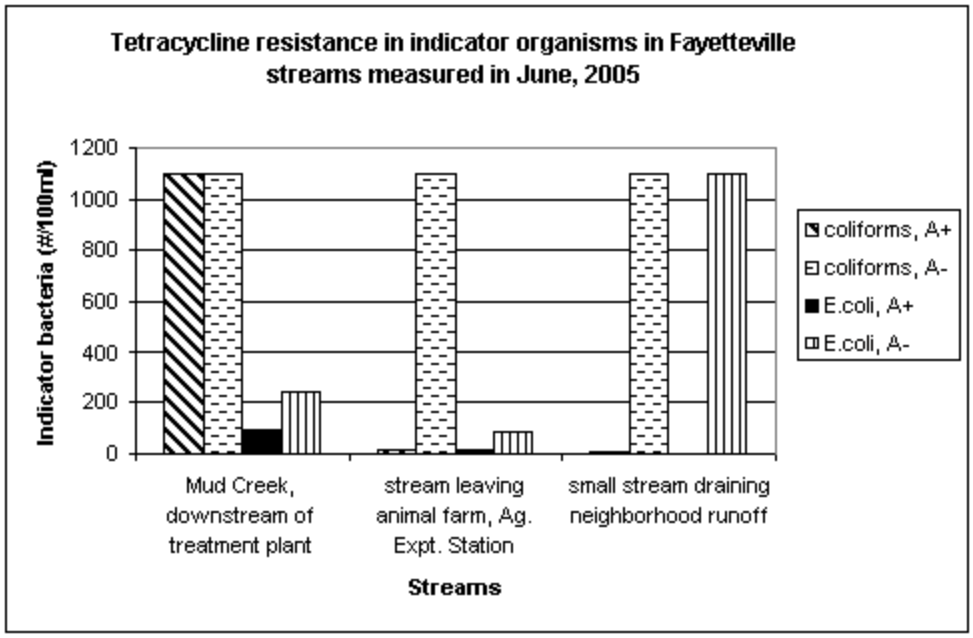


Fig. 1. Most probable numbers (MPN) of total coliforms and *Escherichia coli* were determined in surface waters in Fayetteville in the presence of tetracycline (A+ = antibiotic resistant MPN of bacteria) and without addition of tetracycline (A- = MPN with no antibiotics present, personal communication, M. Savin, 2005).

Nature, scope, and objectives

The goal of this project is to enumerate and compare distributions of *Enterococcus faecalis* and total coliform bacteria, *Escherichia coli* in particular, as indicators of fecal contamination. MPN will be determined for water and sediment bacteria. The exposure of bacteria in the environment to low levels of antibiotics from anthropogenic sources may lead to an increase in antibiotic resistance above natural levels. Therefore, *E. coli* MPN will be determined in the presence and absence of antibiotics. Mud Creek and Spring Creek were selected for testing because they receive effluent from wastewater treatment plants and were previously analyzed for the presence of antibiotics (Galloway et al., 2005). The presence of low

levels of seven antibiotics (Table 1) was found in August 2004 at sites downstream of the treatment plant in Mud Creek, after detection limits were improved ten-fold from previous detection limits utilized earlier in 2004. In order to test for the possibility of multiple antibiotic resistance, water and sediment samples will be exposed to five antibiotics, including antibiotics of two classes of antibiotics not previously detected in Mud Creek. We are including sediments as well as water samples in this experiment because sediments may serve as a reservoir for fecal indicator bacteria (Burton et al., 1987; Hartel et al., 2004). Samples will be taken during baseflow conditions because of the increased amount of bacteria introduced into the stream by runoff events and disturbance of sediments. Baseflow will be considered to be at least five days after a rain.

The methods proposed are based on cultivation. Cultivation techniques are known to be limited in the numbers and diversity of organisms grown. They provide a limited environment for growth that is not representative of water column conditions or the sediment. However, these techniques are commonly used and do provide a measure of indicator bacteria levels. Bacteria that grow in the presence of antibiotics must be resistant to the antibiotic present at that concentration. Thus, this method will give us a comparison of the level of resistance among different antibiotics for bacteria from the same source. We will isolate bacteria and preserve isolates in glycerol, so that population diversity and identity can be tested in future studies to differentiate fecal sources.

An inexpensive phenotypic method of bacterial source tracking is to determine the percentage of *Enterococcus faecalis* isolates. If Oklahoma changes regulations to enumerate *Ent. faecalis* and finds its streams out of compliance, there could be political and legal ramifications for Arkansas. Therefore, we will collaborate with Dr. Shiping Deng from Oklahoma State University to conduct parallel studies in each state to assess occurrence of both fecal coliforms and enterococci indicator bacteria.

We must also collect more data on the occurrence of indicators in streams. For standards to function as intended and represent current contamination, we need to know what background population levels are. Because there is more than one indicator species, the absolute and relative abundance of indicator organisms must be determined. Although the U.S. EPA has made recommendations of which bacteria to use as indicators, there is a lack of research of background levels of different bacteria in streams in northwest Arkansas. Additionally, since there apparently are resident populations in the environment, it is important to know if indicators are increasing in resistance.

Objective 1: To determine numbers of *E. coli* and *Ent. faecalis* in two Arkansas streams upstream and downstream of wastewater treatment plants.

> **Null Hypothesis 1:** There will be no difference in absolute numbers or relationships between numbers of *E. coli* and *Ent. faecalis*.

Objective 2: To determine antibiotic resistance of *E. coli* in the water and sediment for five different antibiotics.

> **Null Hypothesis 2:** The numbers of antibiotic resistant bacteria in the water and sediment will not be higher downstream from wastewater treatment plants in Mud Creek and Spring Creek, which receive effluent discharge and where low levels of antibiotics and antibiotic residues have been found.

Null Hypothesis 3: If resistant bacterial numbers are not higher downstream of the wastewater treatment plants, there will be no increase in antibiotic resistance to either antibiotics which have been found in the stream or antibiotics that were not detected in the stream. (Higher resistance to both antibiotics found in the streams and those not detected in the streams would suggest that bacteria that are antibiotic resistant carry genes encoding resistance to multiple antibiotics.)

Methods, procedures, and facilities

*Experimental approach***:** In order to determine the occurrence and distribution of indicator bacteria, each creek will be sampled upstream from the wastewater treatment plant and at two sites downstream from the wastewater treatment plant. Most probable numbers (MPN) will be calculated for total coliforms and *E. coli* using the Colilert® reagent and for fecal enterococci using Enterolert™ Systems (IDEXX Laboratories, Westbrook, ME). In addition, the MPN of *E. coli* will be determined both with and without the addition of an antibiotic (for a total of five antibiotics).

Sampling: At each sampling point, the location coordinates will be taken with a GPS device. Each location will be sampled aseptically in the spring, summer, and fall. Samples will be collected from the water column and sediments at the same time upstream and at two locations downstream from the waste treatment facility. Water samples will be collected aseptically in sterile, glass vials. Four grab samples will be collected from surface sediments, combined and stored in sterile WhirlPak bags. All samples will be kept on ice in the field and during transport to the laboratory and will be stored at 4ºC in the laboratory. During sample collection the water at each site will be measured for temperature, pH, dissolved oxygen, and conductivity. Turbidity will be determined immediately in the laboratory following sampling (absorbance at 595 nm).

Both water and sediment samples will be serially diluted with sterile distilled water to 10^{-1}, 10^{-2}, and 10^{-3} within six hours of collection and processed with the Colilert® and Enterolert™ Systems (IDEXX Laboratories, Westbrook, ME).

Enumeration of total coliforms and E. coli: Quantitative analysis of both total coliforms and *E. coli* will be determined using IDEXX Colilert® reagent immediately after sampling. Three repetitions of three dilutions will be incubated at 35˚C for 24 hours and MPN will be determined based on the dilution factor and manufacturer-supplied MPN tables. The Colilert® reagent contains a nutrient-indicator that can be metabolized by the coliform enzyme β-galactosidase that results in a change from colorless to yellow, and a product that fluoresces under long-wave ultraviolet light (366 nm) following metabolism of the *E. coli* enzyme β-glucuronidase.

Enumeration of fecal enterococci: A package of powdered Enterolert reagent will also be added to three replications of each dilution. After the reagent is dissolved in the sample, the contents of each bottle will be added to a Quanti-tray, a sterile disposable panel containing 97 wells. Each Quanti-tray will be mechanically sealed. The sealing distributes the sample uniformly into the wells. Each Quanti-tray will be incubated for 24 h at 41˚C. Fluorescing (positive) wells will be counted under UV light. The number of positive wells will be converted to a Most Probable Number (MPN) value based on the dilution factor and manufacturer-supplied MPN tables.

MPN will be "confirmed" by isolating the contents of positive (fluorescing) Quanti-tray wells and testing for the presence of fecal enterococci. To obtain these isolates, positive Quanti-tray wells will be labeled with an acetate marker. The back of the Quanti-tray will be surface-

disinfected with 70% ethanol, and wells will be punctured with a separate sterile pipette tip. A 10-µL aliquot will be removed from a well with the pipetter, and the aliquot will be spotted into one well of a 96-well microtiter plate containing Enterococcosel agar (Becton Dickinson, Sparks, MD). The plates will be incubated for 24 hours at 35˚C. Wells positive for esculin hydrolysis (black color) will be struck onto 5-cm plates containing brain heart infusion agar with 6.5% NaCl. Plates will then be incubated in Ziploc bags (DowBrands, Indianapolis, IN) at 35˚C. After 48 hours, colonies on the plates (positive growth) will be subjected to a catalase test with 8.82 M H_2O_2 to ensure that each isolate is catalase negative. Quanti-tray wells containing bacteria that conform to this definition of fecal enterococci (hydrolyze esculin, grow on brain heart infusion agar with 6.5 % NaCl, and are catalase negative) will be recorded as positive for fecal enterococci, and the wells will be counted to determine the MPN.

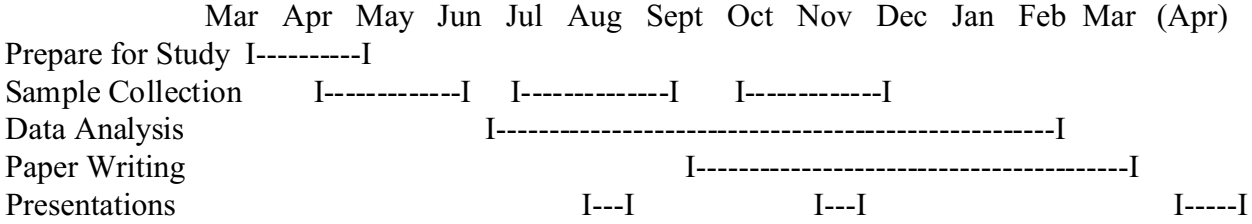
Determination of antibiotic resistance: Each sample will also be placed in five more sets of nine Colilert® tubes as described above; however, tubes will also contain one of five antibiotics. Selected antibiotics will include a representative of five classes of antibiotics: macrolides (erythromycin), quinolones (ofloxacin), sulfonamides (sulfamethoxazole), β-lactams (ampicillin), and tetracyclines (tetracycline). Three of the antibiotics, the macrolide, quinolone, and sulfonamide, have been detected previously in Mud Creek, one of the streams being tested in this study. National Committee for Clinical Laboratory Standards (NCCLS) breakpoints, where available, will be used to guide initial concentrations of antibiotics to be tested. Results of initial screenings will be used to adjust antibiotic concentrations as needed, with initial antibiotic concentrations ranging up to 8 µg/ml for ofloxacin, 80 µg/ml for sulfamethoxazole, and 50 µg/ml for erythromycin, ampicillin, and tetracycline.

Positive tubes will be plated on agar plates to isolate colonies for future identification of bacterial strains and diversity.

Facilities

The facilities and instrumentation needed for both objectives are available in Dr. Savin's laboratory located in Agriculture Building or Dr. Haggard's laboratory in the Engineering Building on the University of Arkansas campus in Fayetteville, with the exception of the Enterolert Quanti-tray sealer.

Timeline



Related Research

Previous research on isolates of the general bacterial community in soils and runoff from grass plots receiving poultry litter were inconclusive. The effect of repeated annual land applications of untreated and alum-treated poultry litter treatments varied with sampling time and antibiotic tested (Tomlinson and Savin, 2004). In addition, antibiotic resistance could be relatively high in control soil, which received no poultry litter. Plots were small, so litter may

have cross-contaminated plots, but we do not believe this to be the case because there were significant differences in biochemical and chemical properties among plots of different treatments. Because of the difficulty in obtaining information on what is fed to poultry, we may have chosen poorly in regard to the antibiotics tested. However, again, we do not believe this to be true because we had published and unpublished data on the antibiotics of choice in poultry rearing. Resistance in our previous work was most strongly related to antibiotic choice. One antibiotic that was tested did not target bacteria (monensin), another targeted gram-positive bacteria (bacitracin), and the third targeted all bacteria (tetracycline). As would be expected, resistance to antibiotics decreased in that order, respectively. Most likely the choice to investigate antibiotic resistance in isolates cultivated from the "entire" or general community may not have been selective enough to see differences among poultry litter treatments. Microbial communities change temporally, resulting in community shifts among cultivated bacteria as well. Bacteria that are active at one point in time may express differential levels of resistance than the community isolated at another sampling time. Hence, the seemingly different temporal response in expression of antibiotic resistance among treatments may be explained by changes in cultivated communities.

In this study, we will focus on bacterial indicators because they have been chosen as the organisms to indicate the presence of fecal contamination, and the possible presence of pathogens. We also have data for a number of antibiotics that have been detected and antibiotics that were not detected in the streams that we will sample. We have evidence of the absence of detectable levels of antibiotics upstream and the presence of detectable levels of antibiotics downstream of wastewater treatment plants (Galloway et al., 2005). Dr. Haggard participated in previous research where antibiotics and break down products were measured downstream of the treatment plants on Mud Creek and Spring Creek. The collaboration between Dr. Brian Haggard, a hydrologist with USDA-ARS in Fayetteville, and Dr. Savin should serve to ensure that high quality science of microbiology of surface waters is conducted.

Because of the profound implications for water quality, and because of the tenuous legal issues between Arkansas and Oklahoma, we are also collaborating with Dr. Shiping Deng of Oklahoma State University to compare results across state lines. Dr. Deng has collaborated with Dr. Peter Hartel at University of Georgia on a microbial source tracking project (unfunded) and practiced determining survival of enterococci in Boomer Lake. Both Dr. Savin and Dr. Deng are participants in the Cooperative Multistate Project S-1022 Basic and Applied Aspects of Bacterial Source Tracking.

Training potential

A portion of this project (antibiotic resistance in *E. coli* to three antibiotics in Mud Creek) is serving as the honors thesis research for an environmental, soil, and water science undergraduate student. The remainder of the research will constitute the focus of the research thesis of a graduate student (candidate for a M.S.) or, if a suitable M.S. student is not found, will be divided among undergraduate students pursuing independent research projects.

Literature cited

Aminov, R. I., N. Garrigues-Jeanjean and R. I. Mackie. 2001. Molecular ecology of tetracycline resistance: Development and validation of primers for detection of tetracycline resistance genes encoding ribosomal protection proteins. Appl. Environ. Microbiol. 67: 22-32.

Arkansas Department of Environmental Quality (ADEQ). 2004. Regulation No. 2. Regulation establishing water quality standards for surface waters of the state of Arkansas. Arkansas Pollution Control and Ecology Commission. [Online] www.adeq.state.ar.us/regs/files/reg02_final_051104.pdf. Accessed 11/1/05.

Bischoff, K. M., D. G. White, Michael E. Hume, T. L. Poole, and D. J. Nisbet. 2005. The chloramphenicol resistance gene *cmlA* is disseminated on transferable plasmids that confer multiple-drug resistance in swine *Escherichia coli*. FEMS Microbiol. Lett.

Burton, G. J., D. Gunnison, and G. R. Lanza. 1987. Survival of pathogenic bacteria in various freshwater sediments. Appl. Environ. Microbiol. 53:633-638.

Galloway, J. M., Haggard, B. E., Meyers, M. T., Green, and W. Reed. 2005. Occurrence of pharmaceuticals and other organic wastewater constituents in selected streams in northern Arkansas. U. S. Geological Survey, Reston, VI.

Goni-Urriza, M., M. Capdepuy, C. Arpin, N. Raymond, P. Caumette, and C. Quentin. 2000. Impact of an urban effluent on antibiotic resistance of riverine *Enterobacteriaceae* and *Aeromonas* spp. Appl. Environ. Microbiol. 66:125-132.

Hartel, P. G., K. Rodgers, S. N. J. Hemmings, J. L. McDonald, K. Gates, S. H. Jones, T. L. Bryant, B. O'Hara, E. Otero, and Y. Rivera–Torres. 2004. Desiccation as a mechanism of fecal enterococcal survival and regrowth for bacterial source tracking. Abstr. Annu. Meet. Am. Soc. Microbiol.

Iversen, A., I. Kühn, A. Franklin, and R. Möllby**.** 2002. High prevalence of vancomycin-resistant enterococci in Swedish sewage. Appl. Environ. Microbiol. 68**:**2838-2842.

Kelch, W. J., and J. S. Lee. 1978. Antibiotic resistance patterns of gram-negative bacteria isolated from environmental sources. Appl. Environ. Microbiol. 36:450–456.

Kolpin, D. W., E. T. Furlong, M. T. Meyer, E. M. Thurman, S. D. Zaugg, L. B. Barber and H. T. Buxton. 2002. Pharmaceuticals, hormones, and other organic wastewater contaminants in U.S. streams, 1999-2000: A national reconnaissance. Environmental Science Technology 36: 1202-1211.

Kuntz, R. L., P. G. Hartel, K. Rodgers, and W. I. Segars. 2004. Presence of *Enterococcus faecalis* in broiler litter and wild bird feces for bacterial source tracking. Water Res. 38: 3551-3557.

Marcinek, H., R. Wirth, A. Muscholl-Silberhorn and M. Gauer. 1997. *Enterococcus faecalis* gene transfer under natural conditions in municipal sewage water treatment plants. Appl. Environ. Microbiol. 64: 626-632.

Mazel, D., and J. Davies. 1999. Antibiotic resistance in microbes, Cell. Molec. Life Sci. 56:742-754.

Meays, C. L., K. Broersma, R. Nordin, A. Mazumder. 2004. Source tracking fecal bacteria in water: a critical review of current methods. J. Environ. Management 73:71-79.

Nikolich, M., G. Hong, N. Shoemaker and A. Salyers. 1994. Evidence for natural horizontal transfer of *tet*Q between bacteria that normally colonize humans and bacteria that normally colonize livestock. Appl. Environ. Microbiol. 60: 3255-3260.

Ohlsen, K., T. Ternes, G. Werner, U. Wallner, D. Loffler, W. Ziebuhr, W. Witte and J. Hacker. 2003. Impact of antibiotics on conjugational resistance gene transfer in *Staphylococcus aureus* in sewage. Environ. Microbiol. 5: 711-716.

Salyers, A. A., N. B. Shoemaker, A. M. Stevens and L.-Y. Li. 1995. Conjugative transposons: An unusual and diverse set of integrated gene transfer elements. Microbiol. Rev. 59: 579-590.

Selvaratnum, S. and J. D. Kunberger. 2004. Increased frequency of drug-resistant bacteria and fecal coliforms in an Indiana Creek adjacent to farmland amended with treated sludge. Can. J. Microbiol. 50:653-656.

Smalla, K., H. Heuer, A. Gotz, D. Niermeyer, E. Krogerrecklenfort and E. Tietze. 2000. Exogenous isolation of antibiotic resistance plasmids from piggery manure slurries reveals a high prevalence and diversity in IncQ-like plasmids. Appl. Environ. Microbiol. 66: 4854-4862.

Tomlinson P. J., and M. C. Savin. 2004. Antibiotic resistance in runoff and soil receiving poultry litter. *In* Annual Meetings Abstracts [CD-ROM]. ASA, CSSA, and SSSA, Madison, WI.

U.S. Environmental Protection Agency (U. S. EPA) 1986. Ambient water quality criteria for bacteria-1986. EPA-440/5-84/002. Office of Water Regulations and Standards, Criteria and Standards Division, Washington, D.C.

Wheeler A. L., P. G. Hartel, D. G. Godfrey, J. L. Hill, and W. I. Segars. 2002. Combining ribotyping and limited host range of *Enterococcus faecalis* for microbial source tracking. J. Environ. Qual. 31:1286–1293.

Wiggins, B. A., R. W. Andrews, R. A. Conway, C. L. Corr, E. J. Dobratz, D. P. Dougherty, J. R. Eppard, S. R. Knupp, M. C. Limjoco, J. M. Mettenburg, J. M. Rinehardt, J. Sonsino, R. L. Torrijos, and M. E. Zimmerman. 1999. Use of antibiotic resistance analysis to identify nonpoint sources of fecal pollution. Appl. Environ. Microbiol. 65:3483-3486.

MARY C. SAVIN
115 Plant Science Building, Dept. of Crop, Soil, and Environmental Sciences, University of Arkansas, Fayetteville, AR 72701, Phone: (501) 575-5740, E-mail: msavin@uark.edu

EDUCATION:

Ph.D.	University of Rhode Island, 1999 (Biological Sciences) Dissertation title: *Interaction of pore size distribution, nematodes, and carbon and nitrogen mineralization in soil*
M.S.	University of Rhode Island, 1995 (Natural Resources) Thesis title: *Role of edaphic factors and cranberry cultivation practices in the biodegradation of the herbicide norflurazon in a cranberry bog soil*
B.S.	University of Notre Dame, 1991 (Biology)

PROFESSIONAL EXPERIENCE:

2002-	Assistant Professor, Dept. of Crop, Soil and Environmental Sciences, University of Arkansas, Fayetteville, AR.
2000-2001	Post-Doctoral Research Associate, Dept. of Biological Sciences, University of Massachusetts Lowell, Lowell, MA.
1999-2000	Post-Doctoral Research Associate, Dept. of Natural Resources Science, University of Rhode Island, Kingston, RI.
1993-1999	Graduate Research Assistant, Dept. of Natural Resources Science, University of Rhode Island, Kingston, RI.
1991-1992	Research Assistant, Environmental Analysis Unit, Arthur D. Little, Cambridge, MA.

ADDITIONAL TRAINING:

2002	DNA Microarrays and T-RFLP Profiling for Environmental Microbiology, American Society for Microbiology, Salt Lake City, UT.
2002	Molecular Techniques for the Analysis of Microbial Community Structure in Soil and the Rhizosphere, Training Workshop in Support of the Multi-state Initiative in Soil Microbiology S-297, Cornell Univ., Ithaca, NY.
2002 - 2005	Teaching retreats, University of Arkansas Teaching and Faculty Support Center, Eureka Springs, AR.

HONORS AND AWARDS:

2005	Teaching Award of Merit, Gamma Sigma Delta The Honor Society of Agriculture, The Arkansas Chapter.

COURSES TAUGHT:

Agronomy Colloquium
Analysis of Environmental Contaminants (to be taught spring 2006)
Ecological Principles for ESWS Students, revised to Ecosystems Assessment with Lab
Intermediate Soils with drill, revised to Soil Resources and Nutrient Cycles with Lab
Introduction to Crop, Soil, and Environmental Sciences
Microbial Ecology
In-service Training for County Extension Agents, Introduction to Soil Biology
DBCAFLS Gifted & Talented Summer Institute Course, "The Good, the Bad, and the Genetically Engineered." 2004, 2005.

COMMITTEE MEMBERSHIPS: College: 4 Department: 7

CURRENT PROFESSIONAL ACTIVITIES/MEMBERSHIPS:

Cooperative Regional Project S-1022, Basic and Applied Aspects of Bacterial Source Tracking, 2005-2010.

Cooperative Regional Project S-297, Biodiversity and Microbial Community Structure in Soil and Rhizosphere, 2000-2005. Chair-elect and secretary, 2003; chair, 2004; annual meeting host, 2005.

International professional societies: 4; Honor societies: 3

GRANTS: Education: 3; Graduate student: 1; Undergraduate: 4
Research, External: 8; Internal: 2; Subcontracts: 2; Prior to appointment: 1

NON-PEER-REVIEWED PUBLICATIONS: 4

PUBLISHED ABSTRACTS: 39 (examples of 3 abstracts)

McClymont, A., B. E. Haggard, **M. C. Savin**. 2005. Surface runoff along the Illinois River and tributaries in northwest Arkansas and northwest Oklahoma. *In* Annual Meetings Abstracts [CD-ROM]. ASA, CSSA, and SSSA, Madison, WI.

Tomlinson P.J., and **M.C. Savin**. 2004.Antibiotic resistance in runoff and soil receiving poultry litter. *In* Annual Meetings Abstracts [CD-ROM]. ASA, CSSA, and SSSA, Madison, WI.

Tomlinson P.J., and **M.C. Savin**. 2004. Poultry litter, an influence on antibiotic resistance in soil? *In* Invited Papers and Abstracts of Contributed Papers [CD-ROM], Southern Branch of the American Society of Agronomy.

INVITED PRESENTATIONS: 15 (1 example presentation)

Antibiotic resistance of bacteria isolated from run-off and soils receiving poultry litter. **M. C. Savin** and P. J. Tomlinson. Arkansas Water Resources Center Conference, Fayetteville, AR, Apr, 20-21, 2004.

VOLUNTEERED PRESENTATION: 1

PEER-REVIEWED TEACHING PUBLICATIONS: 3 (+1 submitted)

PEER-REVIEWED RESEARCH PUBLICATIONS: 19 total (2004-2005 shown)

Amador, J.A. D. A. Potts, **M. C. Savin**, P. Tomlinson, J. H. Görres, and E. L. Nicosia. 2005. Mesocosm-scale evaluation of faunal and microbial communities of aerated and unaerated leachfield soil. *Journal of Environmental Quality.* In review.

Amador, J. A., J. H. Görres, and **M. C. Savin**. 2005. Effects of *Lumbricus terrestris* L. on carbon and nitrogen dynamics beyond the burrow. *Applied Soil Ecology*. In review.

Rooney-Varga, J. N., M. W. Giewat, S. Sood, **M. C. Savin**, M. LeGresley, J. L. Martin. 2005. Links between bacterial and phytoplankton diversity in a coastal marine environment. *Microbial Ecology*. 49:163-175.

Amador, J. A., J. H. Görres, and **M. C. Savin**. 2005. Role of soil water content in the carbon and nitrogen dynamics of *Lumbricus terrestris* L. burrow soil. *Applied Soil Ecology*. 28:15-22.

Savin, M. C., J. Martin, M. LeGresley, M. Giewat, and J. N. Rooney-Varga. 2004. Plankton diversity in the Bay of Fundy as measured by morphological and molecular methods. *Microbial Ecology.* 48:51-65.

Savin, M. C., J. H. Görres, and J. A. Amador. 2004. Microbial and microfaunal community dynamics in artificial and *Lumbricus terrestris* (L.) burrows. *Soil Science Society of America Journal*. 68:116-124.

BRIAN E. HAGGARD, Ph.D.
203 Engineering Hall, University of Arkansas, Fayetteville, AR 72701
Office: 479.575.2879, Fax: 479.575.2846,Cell: 479.790.7097, E-Mail: haggard@uark.edu

Educational Background:

Degree	Date	University	Major
B.S.	1994	University of Missouri - Rolla	Life Sciences
M.S.	1997	University of Arkansas	Environmental Soil and Water Science
Ph.D.	2000	Oklahoma State University	Biosystems Engineering

Professional Experience:

2000–2000	Hydrologist, GS-9, U.S. Geological Survey, Tulsa, Oklahoma
2000–2001	Hydrologist, GS-11, U.S. Geological Survey, Tulsa, Oklahoma
2001–2004	Research Hydrologist, GS-12, USDA–ARS Poultry Production and Product Safety Research Unit, Fayetteville, Arkansas
2005–Present	Research Hydrologist, GS-13, USDA–ARS Poultry Production and Product Safety Research Unit, Fayetteville, Arkansas
2001–Present	Adjunct Assistant Professor, Biological and Agricultural Engineering Department, University of Arkansas, Fayetteville, Arkansas
2003–2005	Associate Editor, Soil and Water Division, Applied Engineering in Agriculture & Transactions of the American Society of Agricultural and Biological Engineers

Honors and Awards:

Gentry Land and Water Resources Scholarship, University of Arkansas 1995
USDA National Needs Water Sciences Fellowship, Oklahoma State University 1997–1999
Williams Outstanding Thesis Award, College of Agriculture, Oklahoma State University 2000
USDA–ARS Outstanding Performance 2001, 2002, 2003, & 2004
American Society of Agricultural and Biological Engineers, Outstanding Reviewer 2003
American Society of Agricultural and Biological Engineers, Honorable Mention Paper Award 2005
USDA–ARS Southern Plains Area, Early Career Research Scientist 2005

Some Related Research Accomplishments and On-Going Projects:

Evaluated catchment–scale sources of phosphorus and mechanisms of phosphorus transport within the highly publicized Illinois River Drainage Area in Northwest Arkansas, tracing the elevated phosphorus concentrations at the Illinois River to one municipal wastewater treatment plant effluent discharge almost 47 km upstream.

Used a novel approach to evaluate nutrient retention efficiency in highly enriched streams, providing a means to quantify the wastewater treatment ecological service of streams.

Evaluated the occurrence of antibiotics, pharmaceuticals and other organic chemicals in water samples collected from 18 different sites across select streams in North Arkansas.

Maintained productive collaborations with hydrologists from the US Geological Survey and has used extensive regional databases of the US Geological Survey National Water Information Systems (NWIS) to evaluate water–quality trends and estimate nutrient load in select streams across Northwest Arkansas.

Related Publications: (2001-2005)

Haggard, B.E., Storm, D.E., Tejral, R.D., Popova, Y.A., Keyworth, V.G., and Stanley, E.H. 2001. Stream nutrient retention in three northeastern Oklahoma agricultural catchments. Transactions of the American Society of Agricultural Engineers 44(3):597-605.

Haggard, B.E., Storm, D.E., and Stanley, E.H. 2001. Effect of a point source input on stream nutrient retention. Journal of American Water Resources Association 37:1291-1299.

Petersen, J.C., Haggard, B.E., and Green, W.R. 2002. Hydrologic characteristics of Bear Creek near Silver Hill and the Buffalo River near St. Joe, 1999-2000. U.S. Geological Survey Water-Resource Investigations Report 02-4024. 36 pp.

Haggard, B.E., and Storm, D.E. 2003. Effect of leaf litter on phosphorus retention and hydrologic properties at a first order stream in northeast Oklahoma, USA. Journal of Freshwater Ecology 18(4):557-565.

Haggard, B.E., Moore, Jr. P.A., Chaubey, I., and Stanley, E.H. 2003. Nitrogen and phosphorus concentrations and export in an Ozark Plateaus catchment in the United States. Biosystems Engineering 86(1):75-85.

Haggard, B.E., Soerens, T.S., Green, W.R., and Richards, R.P. 2003. Using regression methods to estimating stream phosphorus loads at the Illinois River, Arkansas. Applied Engineering in Agriculture 19(2):187-194.

Pickup, B.E., Andrews, W.J., Haggard, B.E., and Green, W.R. 2003. Phosphorus concentrations, loads, and yields in the Illinois River Basin, Arkansas and Oklahoma, 1997-2001: US Geological Survey Water-Resources Investigations Report 03-4168. 39 pp.

Haggard, B.E., Ekka, S.A., Matlock, M.D., and Chaubey, I. 2004. Phosphate equilibrium between stream sediments and water: potential effects of chemical amendments. Transactions of the American Society of Agricultural Engineers 47(4):1113-1118.

White, K.L., Haggard, B.E., and Chaubey, I. 2004. Water quality at the Buffalo National River, 1991-2001. Transactions of the American Society of Agricultural Engineers 47(2):407-417.

Ekka, S.A., Haggard, B.E., Matlock, M.D., and Chaubey, I. 2005. Dissolved phosphorus concentrations and sediment-interactions in effluent dominated Ozark streams. Ecological Engineering *In Review*.

Galloway, J.M., Haggard, B.E., Meyers, M.T., and Green, W.R. 2005. Occurrence of pharmaceuticals and other organic wastewater constituents in selected streams in northern Arkansas, 2004. U.S. Geological Survey Scientific Investigations Report 2005-5140, 31 pp.

Haggard, B.E., Galloway, J.M., W.R. Green, and M.T. Meyers. 2005. Antibiotics, pharmaceuticals and other organic wastewater constituents in selected north-central and northwest Arkansas streams, 2004. Journal of Environmental Quality *In Review*.

Haggard, B.E., and Soerens, T.S. 2005. Potential influence of a small impoundment on phosphorus concentrations and transport at the Illinois River, Arkansas and Oklahoma, USA. Ecological Engineering *In Review*.

Haggard, B.E., Stanley, E.H., and Storm, D.E. 2005.Nutrient retention in a point source enriched stream. Journal of the North American Benthological Society 24:29-47.

White, K.L., Haggard, B.E., Matlock, M.D., and Kim, J-W. 2005. Periphytic chlorophyll a response to Triclosan: application of a passive diffusion periphytometer. Applied Engineering in Agriculture 21(2):307-311.