Attachment 10:

Alternatives to minimize the environmental impact of large swine production units
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J R Miner

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Alternatives to Minimize the Environmental Impact of Large Swine Production Units[1,2]

J. Ronald Miner

Bioresource Engineering Department, Oregon State University, Corvallis 97331

ABSTRACT: Large swine production facilities have become controversial additions to the agricultural landscape as their numbers and sizes have increased. In addition to being larger enterprises, these units have involved greater specialization, the influx of outside capital, and the employment of labor without extensive investment in the enterprise. Major complaints have included water pollution and odors. Water pollution complaints have been related to surface and groundwater resources. Accidental spills, structural failure, and purposeful discharges have been noted. Odor problems are most often related to manure management techniques. Large anaerobic lagoons and irrigation of lagoon effluent have the potential to emit odors that travel long distances. Fortunately, technology and management alternatives exist to achieve higher levels of environmental acceptability. More effective water pollution and odor control alternatives generally increase construction and operating costs. Producers, regulatory officials, and the local public have an opportunity to interact to achieve progress in establishing acceptable compromises. This article identifies the range of existing and evolving alternative strategies and provides some assistance to producers and neighbors in achieving the necessary equilibrium.

Key Words: Odors, Water Pollution, Animal Manures, Lagoons

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Introduction

The recent concern over the swine industry in several U.S. states indicates that livestock production is in a time of significant transition. The traditional small farm practice of having a few pigs to supplement the other agricultural operations is a declining part of the overall industry. Tables 1, 2, and 3 show this decline on a national basis and the major increases in the number of larger facilities in certain states (National Agricultural Statistics Service, 1996). This rapid shift to larger, highly specialized swine ownership has been accompanied by a change in the nature of swine enterprise ownership. Owners may be a corporate structure with management hundreds of kilometers away and with only operating personnel on-site. The operating laborers, rather than being the owner and his or her family, are more likely to be hired employees. These personnel may be local residents or may be primarily recent immigrants starting their employment. Thus, the overall enterprise may convey a sense of being run by outsiders rather than by a neighbor who is raising pigs.

In addition to this change in management and in community ties, there is frequently a change in the nature of land ownership. Most large-scale swine enterprises are not involved in crop production. As a result, they may not own the land necessary for manure application. Where that is the case, they will depend on nearby land owners for suitable land application sites.

The Issues

Two frequent concerns expressed whenever large-scale swine enterprises are discussed are water pollution and odors. Both of these are valid, and they have been frequent issues following the construction of large facilities. Both are, however, issues that have solutions. Other issues have included increased vehicle traffic, noise, flies, and lighting.

Water Pollution

Water pollution can occur whenever large quantities of organic waste materials are concentrated in a single area. Simultaneously, the concentration of

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Table 1. Number of farms selling hogs and pigs, 1969–1992[a]

Year	1 to 1,000 animals marketed	More than 1,000 animals marketed
1969	597,000	6,600
1978	454,700	15,800
1982	293,400	21,700
1987	215,000	24,000
1992	160,400	27,750
1969–1992 Percentage change	−73	+320

[a]Source: U.S. Census of Agriculture (USDA, 1995).

Table 2. Hogs and pigs: number of operations by size group in the United States, 1994–1996[a]

Size, number of animals	1994	1995	1996
1 to 999	195,280	170,310	145,520
1,000 to 1,999	8,070	7,720	7,050
More than 2,000	4,630	4,750	4,880

[a]Source: Hogs and Pigs Report (National Agricultural Statistics Service, 1996).

waste material on a single site also offers the opportunity for more effective management and a greater degree of control.

Most of the large-scale swine operations constructed to date have incorporated anaerobic lagoons into the overall manure treatment and storage strategies. Well-designed lagoons provide three specific functions:

1. They provide a location for anaerobic bacteria to decompose the residual organic compounds into more easily handled end products. This means a conversion of much of the solid material into liquids. This is not unlike the breakdown of solids that takes place in household septic tank systems.
2. They provide a storage volume to accumulate treated and partially treated manure until the time for convenient or economically productive application of the material to cropland.
3. They provide a facility to capture and store any contaminated runoff that might be generated by rainfall on manure-contaminated surfaces that may be outside the roofed areas.

There are two potential pollution pathways. If a lagoon overflows or if lagoon contents are purposefully drained into a surface waterway, this material will contaminate the receiving water. Because lagoons for large livestock operations require a permit from the appropriate state water pollution control agency, plans are reviewed to ensure there is no direct discharge from the lagoon to the receiving water course. Discharge is prohibited at all times except in connection with a 25-yr, 24-h storm event. Under conditions of such a storm, the assumption is made that there will be sufficient water in the water course to minimize the effects of the escaping lagoon contents. Lagoon dike specifications are written to ensure the dikes are stable and will not fail under even the most extreme weather. Unfortunately, experience has been that a few producers have discharged lagoon contents into receiving waterways, and some lagoon dikes have failed. Neither of these two situations is acceptable or inevitable. Engineers know how to design and build dikes that are secure, and managers know not to discharge lagoon contents into public waters. Regulatory enforcement of proper lagoon maintenance is an essential ingredient of water pollution control. Most state water pollution control agencies have a commitment to water quality but over the past decade have had their budgets reduced to the point they are unable to provide the field-based inspections necessary to ensure proper operation of the waste storage and treatment facilities. It is interesting to note that in response to lagoon failures in North Carolina, the state s most recent regulations require an annual inspection by the water pollution control agency personnel and one by the appropriate federal technical service agency (personal communication, F. Humenik, NCSU, June 1998).

There is also a concern over the possibility of groundwater pollution from large-scale livestock enterprises. Again, there are two very likely possibilities. One is the possibility of leakage from the lagoon or manure storage reservoir moving downward into groundwater. The second is the possibility of applying excessive effluent to cropland and creating a situation in which more nitrogen is being applied than is being used by the crop. If that is the case, excess nitrogen will be transported beyond the root zone and will eventually appear in the groundwater as an increased nitrate concentration. Excessive application of rainfall or irrigation water when not needed can also contribute to nitrate escape.

Nutrient management strategies are designed to provide the crops with the full amount of plant nutrients needed while minimizing the amount of soluble nutrients that escape from the root zone. Coupled with soil and plant testing, it is possible to manage nitrogen in a very precise manner. To be effective, nutrient management requires that the livestock producer have an adequate amount of crop or

Table 3. Hogs and pigs: number of operations by size group in North Carolina, 1994–1996[a]

Size, number of animals	1994	1995	1996
1 to 999	5,830	5,120	4,500
1,000 to 1,999	350	380	300
More than 2,000	820	1,100	1,200

[a]Source: Hogs and Pigs Report (National Agricultural Statistics Service, 1996).

pasture land for the nitrogen available, that a sufficient number of manure or lagoon effluent samples be analyzed to provide the necessary inventory information, and that detailed records be maintained so the fate of nutrients on the farm can be evaluated on an ongoing basis.

Odor Release

Swine confinement buildings are a source of objectionable odors if manure is allowed to accumulate on the floors, manure is stored within the buildings, the animals are allowed to become covered with manure, or the flush water has a volatile odor due to anaerobic storage. Odors are primarily the products of anaerobic decomposition of manure. One of the more frequent sources of odor complaints is the manure storage/ treatment system. Another major odor emission source is the sprinkler or nozzle that is used to distribute liquid manure as part of the land spreading. Liquid manure on the surface of the ground continues to emit odor until it dries or is absorbed by the soil.

Anaerobic lagoons have a particularly bad reputation. Heavily loaded lagoons are a notorious odor source. However, manure management options to reduce odor do exist. For example, enclosed anaerobic digesters can reduce organic content by 90% while producing a biogas that can be converted to electricity. Lagoons can be covered and the collected gas diverted to a soil absorption system. Permeable covers for lagoons are also available which greatly decrease odors (Miner and Pan, 1995). Aerobic alternatives include aerated lagoons and modified activated sludge processes (MWPS, 1985).

Disposal of dead animals, if it is not accorded a high level of management attention, has the potential to produce an odor that is highly offensive and suggestive of unhealthy conditions. Options include prompt removal to a rendering plant, burial, or composting.

Manure following treatment or storage is most often applied to crop or pasture land as a source of plant nutrients. Dilute liquid manure is least expensively applied using irrigation equipment. When pumping liquid manure through a high-pressure sprinkler an extensive surface area is generated, causing significant escape of manure odorants to the air. Widespread complaints can ensue.

Technical Solutions

Technological solutions exist that can prevent the escape of manure from treatment/storage systems. Similarly, odors can be significantly reduced or eliminated. Some of the alternatives add cost either to facilities or to management; however, the current transition within the industry may be the appropriate time to boldly adopt a higher level of environmental stewardship.

Lagoon Construction and Management

Other industries and municipalities are able to construct and manage lagoons that have a relatively low rate of failure. Site selection is a key. Construction away from streams and rivers will avoid the problem of immediate stream discharge should a relatively minor problem arise. In addition, by having lagoons out of the flood plane, erosion damage to the outside of the dike will be reduced. Width of top berms can be selected to avoid erosive failure. Frequent inspection associated with an effective maintenance program will help avoid unanticipated failure. Record keeping and the use of a depth gauge in the lagoon will make it possible to determine when a lagoon is reaching the level when application to cropland is essential.

Construction and testing techniques are sufficiently developed to achieve the required rates of infiltration or seepage. Soil scientists and design engineers are able to design and construct lagoons that do not leak. Typically, regulatory agencies specify that infiltration rates are to be less than 10^{-7} cm (Missouri Department of Natural Resources, 1989). In many soils, this degree of water tightness can be achieved with native material. Where the soils are high in sand and gravel or where layered rock is encountered, it is often necessary to import a clay material to produce a water-tight seal. An alternative is to install a synthetic water-tight liner. If a liner is used, it is typical to install observation wells beneath the lagoon to check for potential leakage.

Nutrient Management

Crop nutrient requirements are sufficiently well established, analytical procedures available, and application equipment sufficiently refined that the manager has precise control over the application rates of nitrogen, phosphorus, and potassium. Evapotranspiration rates are also sufficiently well established to prevent transporting nitrate nitrogen to the groundwater with responsible management.

Application of phosphorus in excess of the crop utilization rate is common in much of the United States because relatively less phosphorus than nitrogen is lost during storage. There are locations in which this practice is of increasing concern. Livestock and poultry producers should be particularly alert to this matter if phosphorus becomes the basis for land application regulations because it can have a significant effect on the amount of land required for manure utilization or for the disposal of treated effluent.

The amount of land required for large-scale confinement livestock operations can be difficult to assess. If the operation has adequate land for the utilization of the available nutrients it generates, this additional land, if properly located, can provide a buffer in case of odor release. However, increasing the amount of land requires livestock and cropping expertise. Whether the operation includes cropland or not, adequate land must be available based on a reasonable/conservative nutrient management plan.

When sufficient land is not available for the utilization of all the nutrients through agronomic practices, nutrient reduction technologies exist. It is possible to produce an effluent that meets very strict water quality standards; however, the economic and energy costs increase as the removal efficiencies increase. Nitrogen can be removed from lagoon effluent by raising the pH to promote volatilization, for example. Alternate aerobic and anaerobic treatment processes will remove nitrogen by nitrification then denitrification. Phosphorus can be precipitated by chemical addition. If potassium reduction is required only a modification of the diet will be effective.

Building Odor Control

Great progress has been made in designing livestock confinement buildings that minimize animal contact with the manure and that can be maintained at optimal humidity and temperature. Slotted floors, flushed gutters, and fill and dump tanks are among the possibilities. The choice is largely a local decision to best utilize the particular geographic advantages that may be available.

Flushed buildings and the fill and dump under-floor storage tanks (pit recharge system) are a special concern if recirculated water is being used for flushing. When water is recycled from a lagoon, the water may have a sufficient concentration of odorous gases that it contributes to, rather than reduces, building odors. Under those conditions, it may be necessary to aerate or otherwise deodorize the recycled water as part of the wastewater treatment process.

Heating and ventilation is another variable that can be manipulated to achieve in-building odor control. One of the constraints of a few existing buildings is that they have no or minimal supplemental heating capabilities. Under those conditions, the only means of maintaining appropriate temperatures under cold weather conditions is to reduce ventilation rates. This results in increased humidity, the buildings become more odorous, and the air that escapes becomes more objectionable.

Lagoon Odor Control

A great variety of manure management technologies are available to the confinement swine producer. Most often, the designer will first think of using an anaerobic lagoon because of its low construction and operating costs relative to the typical alternatives. There are a number of possible hazards associated with anaerobic lagoons:

1. Lagoon loading rates are generally out of date with regard to the size of swine confinement facilities being constructed. The engineers that assembled the data that form the basis for lagoon loading rates were dealing with facilities envisioned to house 400 to 1,000 animals. Loading rates were identified that would provide sufficient anaerobic digestion capacity that the unmixed lagoon would emit odors that would be objectionable at an appropriately located neighboring residence on a sufficiently infrequent basis so as to be acceptable. Designers and regulatory officials have tended to accept these loading rates as being independent of herd size and create very large lagoons that emit odors of the same intensity and quantity per unit area as the “maximum acceptable.” Thus, as a result of the larger surface area, the residents located in accord with the established policy find themselves facing an odor that is more intense and of a greater frequency than the guidelines were meant to allow.
2. Most of the regulatory officials being asked to review plans for confinement livestock operations are employed by water pollution control agencies with primary goals of avoiding surface and groundwater pollution. They, therefore, support the use of the “standard loading rates” because there is no justification from a water pollution control perspective to build a lagoon that will operate at a lower loading. This interchange provides additional security to the designer but does little to address the concerns of the surrounding residents.
3. Designers have erroneously believed that state or professional organization design standards were selected to serve in all situations. Anaerobic lagoon loading rates are an appropriate example. Lagoon sizing criteria are established by state water pollution control agencies to protect surface water quality, and in some states to provide limited guidance regarding odor intensity. These values should not be confused with design recommendations for a particular location. Wherever there are particular concerns relative to odors, where a site evaluation suggests there are likely to be odor conflicts, or where the facility developer wants to increase the likelihood of success, alternate waste treatment and storage facilities can be considered and may represent a better choice.

For these reasons, this is an appropriate time to abandon the “standard lagoons only” policy that has been adopted by the large-scale swine farm designers and consider alternatives that better serve the public and contribute sustainability to the industry. This will increase public acceptance of these enterprises and help avoid legislated constraints.

Water Quality Protection

Anaerobic lagoons are not unlike a large number of other large relatively shallow basins designed to be constructed in the ground. Clearly, these units require careful consideration of soil type, construction techniques, local rainfall amounts, and rainfall intensity. The bottom line is that engineers and construction professionals have the ability to construct basins that do not leak or that have dikes that will not erode and dump large quantities of manure into receiving streams. In addition, groundwater sampling and monitoring techniques have improved sufficiently to identify when lagoons are leaking.

Similarly, agronomists have identified the nutrient requirements of those crops to which treated manure is applied. Manure application rates in excess of the uptake rates are destined to cause leaching or runoff. Responsible crop producers can monitor manure or lagoon effluent application rates to avoid the escape of nitrates to the groundwater. It has also become clear that irrigation management is another essential ingredient in the overall water quality protection effort. In areas with high winter rainfall rates and relatively low evapotranspiration, fall applications of manure or lagoon effluent create an opportunity for large and uncontrolled loss. These observations suggest that the earlier practice of lowering lagoon levels in late fall is no longer the universally appropriate mode of operation.

Swine producers also bear a degree of responsibility to institute a regular and effective monitoring program. For many operations, this translates into regular inspection by the state and local permitting agency to ensure proper operation and maintenance. This oversight has tended to decrease in recent years as state and local government agencies with these responsibilities have learned to operate with shrinking budgets and depleted rosters. The solution options seem clear. Either we as resident taxpayers must provide the resources necessary to have these facilities inspected or the permit fees must be increased sufficiently to allow that degree of service. Conventional wisdom would suggest that neither of these options is fully acceptable based on the behavior of our individual state governments.

Odor Control

There is a perception that odor is somehow inevitable around confined livestock facilities and there is nothing that can be done to avoid its escape into the surrounding community. Clearly, that is not the case. Technology and management techniques exist to produce livestock in confinement at any desired level of odor production. There are problems associated with odor control such as variability, difficulty of measurement, and subjective responses; however, those difficulties do not preclude odor control at a level that protects the rights of neighboring residents to the full enjoyment of their property.

The use of anaerobic lagoons for the collection, treatment, and storage of swine manure presents challenges for odor control. Many swine producers in the late 1960s felt that a lagoon had failed if it had become full of liquid manure. Somehow their expectations were that an anaerobic lagoon was going to accomplish some kind of volume reduction and that the manure would “go away.” There are other unrealistic expectations of lagoons today. One of these is that they can be built as large as needed and they will cause the odor to “go away.” That expectation is no more realistic today than the one that was held 30 yr ago.

Anaerobic lagoons can be made less odorous by reducing the load on the lagoon. The organic load can be reduced by pretreatment with an anaerobic digester, separating solids from the waste before it enters the lagoon (Vetter et al., 1990), aerating the lagoon, or adding either a permeable cover to oxidize the escaping gases (Miner and Pan, 1995) or an impermeable cover to capture the gases. Anaerobic lagoons are not the only option available for the storage and treatment of manure from large-scale livestock and poultry operations. Enclosed anaerobic digesters followed by aeration produce an effluent suitable for reuse as a flush water (Schulte et al., 1985).

Implications

Livestock production in the United States is at a significant transition point. Opportunities exist to create environmentally sustainable systems. Water pollution and odor are controllable. The challenge to livestock producers, the design community, and the regulatory community is to envision and implement these sustainable systems.

Literature Cited

Miner, J. R., and H. Pan. 1995. A floating permeable blanket to prevent odor escape. In: Proc. Intl. Livestock Odor Conference, Iowa State Univ., Ames. pp 28–34.

Missouri Department of Natural Resources. 1989. Design guidelines for animal waste management for concentrated animal feeding operations. Manual 121, Water Pollution Control Program, Missouri DNR, Jefferson City.

MWPS. 1985. Livestock Waste Facilities Handbook. Midwest Plan Service. MWPS-18 (2nd Ed.). Ames, IA.

National Agricultural Statistics Service. 1996. Hogs and Pigs Report, December 31.

Schulte, D. D., D. A. Kottwitz, and T. J. Siebenmorgen. 1985. Design and operation of a flexible cover, pre-cast concrete digester for swine manure. In: Agricultural Waste Utilization and Management. pp 509–515. American Society of Agricultural Engineers, St. Joseph, MI.

USDA. 1995. U. S. Census of Agriculture. United States Government Printing Office, Washington, DC.

Vetter R. L., D. J. Frederick, and P. H. Huntington. 1990. Full scale anaerobic digester and waste management system for a 300 cow dairy. In: Agriculture and Food Processing Waste. pp 236–249. American Society of Agricultural Engineers, St. Joseph, MI.

Attachment 11:

Antimicrobial residues in animal waste and water resources
proximal to large-scale swine and poultry feeding operations
(Campagnolo et al. 2002)

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Antimicrobial residues in animal waste and water resources proximal to large-scale swine and poultry feeding operations

Enzo R. Campagnolo[a,b], Kammy R. Johnson[b,*], Adam Karpati[a,b], Carol S. Rubin[b], Dana W. Kolpin[c], Michael T. Meyer[d], J. Emilio Esteban[b], Russell W. Currier[e], Kathleen Smith[f], Kendall M. Thu[g], Michael McGeehin[b]

[a]*Epidemic Intelligence Service, Epidemiology Program Office, Centers for Disease Control and Prevention, 1600 Clifton Rd. NE, Atlanta, GA 30333, USA*
[b]*Health Studies Branch, Division of Environmental Hazards and Health Effects, National Center for Environmental Health, Centers for Disease Control and Prevention, 1600 Clifton Rd. NE, Atlanta, GA 30333, USA*
[c]*Water Resources Division, United States Geological Survey, 400 S. Clinton St., Iowa City, IA 52244, USA*
[d]*Water Resources Division, United States Geological Survey, 3916 Sunset Ridge Rd., Raleigh, NC 27607, USA*
[e]*Iowa Department of Public Health, 321 East 12th St., Lucas State Office Building, Des Moines, IA 50319, USA*
[f]*Ohio Department of Health, Bureau of Infectious Disease Control, 35 E. Chestnut St., Columbus, OH 43215, USA*
[g]*Institute for Rural and Environmental Health, Oakdale Campus, The University of Iowa, Iowa City, IA 52242, USA*

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Abstract

Expansion and intensification of large-scale animal feeding operations (AFOs) in the United States has resulted in concern about environmental contamination and its potential public health impacts. The objective of this investigation was to obtain background data on a broad profile of antimicrobial residues in animal wastes and surface water and groundwater proximal to large-scale swine and poultry operations. The samples were measured for antimicrobial compounds using both radioimmunoassay and liquid chromatography/electrospray ionization-mass spectrometry (LC/ESI-MS) techniques. Multiple classes of antimicrobial compounds (commonly at concentrations of >100 μg/l) were detected in swine waste storage lagoons. In addition, multiple classes of antimicrobial compounds were detected in surface and groundwater samples collected proximal to the swine and poultry farms. This information indicates that animal waste used as fertilizer for crops may serve as a source of antimicrobial residues for the environment. Further research is required to determine if the levels of antimicrobials detected in this study are of consequence to human and/or environmental ecosystems. A comparison of the radioimmunoassay and LC/ESI-MS analytical methods documented that radioimmunoassay techniques were only appropriate for measuring residues in animal waste samples likely to contain high levels of antimicrobials. More sensitive LC/ESI-MS techniques are required in environmental samples, where low levels of antimicrobial residues are more likely.

Keywords: Animal feeding operation (AFO); Animal manure; Manure storage lagoon; Swine; Poultry; Chemical pollutants; Antimicrobial agents; Surface water; Groundwater

*Corresponding author. Tel.: +1-404-498-1354; fax: +1-404-498-1355.
E-mail address: kdj8@cdc.gov (K.R. Johnson).

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1. Introduction

Since the 1970s, animal production has tended toward consolidation and intensification into larger, confinement-type operations (US GAO, 1995). The expansion and intensification of large-scale animal feeding operations (AFOs) in the United States has raised important questions about environmental pollution and the potential public health impact of contaminants in animal manure entering surface and groundwater (Schiffman, 1988; Thu and Durrenberger, 1994, 1998; Thu, 1995; Thu et al., 1997; DeLind, 1995; Herrick, 1995; Jackson et al., 1995; Schiffman et al., 1995; US GAO, 1995; Reynolds et al., 1997; US Senate, 1997; Jongbloed and Lenis, 1998)

Animal manure is commonly applied to agricultural crop fields as a source of organic fertilizer to increase crop yields. However, when applied to agricultural land in amounts greater than can be utilized by crops and retained by the soil, manure constituents may be transported to surface water and groundwater through runoff and infiltration (Stewart and Mathers, 1971; Jongbloed and Lenis, 1998; Mackie et al., 1998; US GAO, 1999). Animal waste components also potentially affect water quality and public health by polluting drinking water supplies and recreational waterways (US GAO, 1999).

The composition of animal manure depends on several factors, such as the species from which it originates, diet, production style, and the waste management plan of the AFO. Animal manure is a mixture of organic and inorganic material and represents a potential source of agricultural nutrients, such as nitrogen and phosphorus (Voorburg, 1991; Jongbloed and Lenis, 1998; Mackie et al., 1998). However, animal manure may also contain other chemical constituents, such as heavy metals, hormones and antimicrobial compounds (Mackie et al., 1998; US GAO, 1999), which result from on-farm livestock management (enriched feedstuffs) and veterinary practices (pharmaceutical usage).

Antimicrobial agents are administered to livestock at therapeutic doses or to prevent illness (prophylaxis). At much lower doses (subtherapeutic), antimicrobial agents are used as feed additives to increase the rate of growth and to improve feed efficiency (Addison, 1984). Irrespective of dosage, an estimated 75% of antimicrobial agents administered to confined livestock and poultry may be excreted back into the environment (Addison, 1984). The effect of these agents upon environmental biota, particularly aquatic biota, is of concern, but is not fully understood (Daughton and Ternes, 1999). Recent evidence suggests that the interaction between bacterial organisms and antimicrobial agents in the environment may contribute to the development of antimicrobial-resistant bacterial strains, with groundwater serving as a potential source of antimicrobial-resistant pathogens in the human food chain (Chee-Sanford et al., 2001).

Research is limited about the direct and indirect effects on the environment and human health from environmental contamination resulting from AFOs (CDC, 1998a). Gathering baseline information about the existence and extent of these environmental pollutants is an essential first step toward understanding the environmental impact or human health effects potentially associated with large-scale AFOs and their activities. To address the need for baseline information, we conducted two investigations, one involving large-scale swine operations in Iowa and the other involving large-scale poultry operations in Ohio. These investigations assessed whether chemical constituents (including antimicrobial compounds) present in animal wastes were also present in water resources on or immediately proximal to large-scale swine and poultry operations. The purpose of this paper is to describe the antimicrobial residue results of this study. A detailed discussion of the results for the other chemical constituents measured has been published elsewhere (CDC, 1998b,c).

2. Materials and methods

Environmental samples (surface water, groundwater and liquefied waste from swine manure storage lagoons) were collected proximal to swine and poultry AFOs where the farm operators were willing to participate in the investigation. Because convenience sampling was employed for this study, the samples were not considered to be representative of any particular livestock-raising practice or manure management strategy.

2.1. Farm site selection

A swine farm site was defined as an operation consisting of several buildings (farrowing, nurseries or finishing), one or more manure lagoons and agricultural crop fields upon which manure from the operation was applied. Nine swine farms were included in the study. Of the nine, two were farrowing operations (birthing facilities), three were nurseries (facilities where pigs are weaned) and four were finishing operations (facilities where pigs are raised until reaching market weight). A total of 48 swine barns and 96 300 swine were represented in the study (CDC, 1998b).

A poultry (chicken or turkey) farm site was defined as an operation with poultry houses and an agricultural field upon which manure from the poultry houses was applied. Five farms were included in the study: two broiler operations, three egg-layer operations, and one turkey operation. Eight poultry houses and at least 239 000 birds were represented in the study (CDC, 1998c).

The interval between manure application and sample collection was not determined, nor was the amount of manure applied to the crop fields monitored. The animal feeding operations and agricultural fields upon which manure from the operations was applied could be adjacent or separate.

2.2. Sample collection

Samples were collected from swine farms from October through December 1998. A total of 23 samples were collected from or near participating swine farms, including manure waste lagoon samples (seven) and water samples (16) from or immediately proximal to the farm (CDC, 1998b). Water samples from the surface water (three), field tile lines (six) and drainage wells (two) were collected 24–48 h after substantial (1 in.) rainfall. Groundwater samples (four) were from selected pre-existing monitoring wells (approx. 18–20 ft. deep) located down-gradient of the manure storage lagoons and a private well (one).

Water samples were collected from or proximal to poultry farms over 3 days during a 1-week period in October 1998. A total of 18 samples were collected, including samples from field streams (eight), field springs (two), field wells (two), field tiles (five) and a river (one) (CDC, 1998c). Groundwater sampling methods included geoprobe drilling, direct spring sampling and direct well sampling; methods were chosen on site and varied among farm sites, depending upon well type and availability.

Samples for antimicrobial analysis were collected unfiltered and immediately chilled to near freezing; they were shipped to the laboratory for analysis within 24 h of collection.

2.3. Laboratory analysis

Samples from swine and poultry farm sites were analyzed for antimicrobial residues using radioimmunoassay and liquid chromatography/electrospray ionization-mass spectrometry (LC/ESI-MS) techniques. The water and liquid manure samples were filtered through a 0.45-μm glass fiber filter and refrigerated until they were screened for fluoroquinolones using a commercially available strip immunoassay and for the β-lactam, tetracycline, sulfonamide and macrolide classes of antimicrobials using commercially available radioimmunoassays, with procedures adapted to analyze water samples (Hirsch et al., 1998; Meyer et al., 2000).

The solid-phase extraction LC/ESI-MS method was modeled after the method of Hirsch et al. (1998). The LC/ESI-MS was operated in a positive-ion mode using selected-ion monitoring. Lincomycin, trimethoprim, sulfamethazine, sulfadimethoxine and erythromycin–H_2O (erythromycin metabolite) were tested using gradient separation (Hirsch et al., 1998) on a 4.6×150 mm, 5-μm phenylhexyl column. Chlortetracycline, tetracycline, oxytetracycline, norfloxacin, ciprofloxacin, enrofloxacin and sarafloxacin were tested using gradient separation (Hirsch et al., 1998) on a 4.6×150 mm, 5-μm end-capped C_8 LC column. The limit of quantitation was 0.05 μg/l for all of the above compounds, except the tetracyclines, which was 0.5 μg/l.

Table 1
Environmental antimicrobial residue levels, measured by radioimmunoassay, proximal to large-scale animal feeding operations, Iowa and Ohio, 1998

Farm	Farm type	Collection site	Antimicrobial (μg/l)				
			Tetracycline[a]	Sulfonamide[b]	β-Lactam[c]	Macrolide[d]	Fluoroquinolone[e]
1	Swine	Lagoon	250	>20	BDL	227	NA
2	Swine	Lagoon	11	>20	BDL	BDL	NA
3	Swine	Lagoon	150	>20	BDL	60	NA
4	Swine	Lagoon	68	>20	3.5	BDL	NA
5	Swine	Lagoon	66	>20	2.1	81	NA
7	Swine	Lagoon	540	>20	2.1	275	NA
8	Swine	Lagoon	110	>20	2.9	15	NA
8	Swine	Monitoring well	BDL	7.6	BDL	BDL	NA
5	Poultry	River	TR	BDL	BDL	BDL	5.0

BDL, the concentration was below the limit of detection of the assay. TR, detected below the limit of detection but above the 95% confidence interval of the negative control (Meyer et al., 2000). NA, not applicable; samples were not tested for this agent.
[a] Tetracycline concentrations as chlortetracycline, 1 μg/l (ppb) limit of detection.
[b] Sulfonamide concentrations as sulfamethiazine, 5 μg/l (ppb) limit of detection.
[c] β-Lactam concentrations as penicillin G, 2 μg/l (ppb) limit of detection.
[d] Macrolide concentrations as erythromycin, 10 μg/l (ppb) limit of detection.
[e] Fluoroquinolone concentration as enrofloxacin, 5 μg/l (ppb) limit of detection.

3. Results and discussion

Multiple classes of antimicrobial compounds were detected in all seven swine-waste storage lagoon samples (Table 1). Concentrations of individual antimicrobials commonly exceeded 100 μg/l and the total antimicrobial residues (summation of all antimicrobials detected in a given sample) approached 1 mg/l (Tables 1 and 2). Thus, as previously noted, at least a portion of the antimicrobial compounds administered to swine is excreted by the animals (Addison, 1984). Antimicrobial use information for individual farms was not collected as part of this study; however, with the exceptions of sulfadimethoxine and trimethoprim, all of the antimicrobials we evaluated in the swine manure samples are approved for use in swine for therapeutic purposes and/or growth promotion (USDA, 1999; US FDA, 2002a). The use of antimicrobials on swine farms is not uncommon, with 70–80% of farms administering antimicrobials to piglets in feed (USDA, 1995, 2001). Furthermore, the percentage of swine farms that use antimicrobials in feed for growing swine continues to increase over time (USDA, 1995, 2001).

Antimicrobial compounds were found in five of the water samples (31%) collected proximal to swine farms and in 12 of the water samples (67%) collected proximal to poultry farms (Tables 1 and 2). Multiple classes of antimicrobial residues were detected in two of the water samples (13%) collected proximal to swine farms and in four of the water samples (22%) collected proximal to poultry farms (Tables 1 and 2).

With the exceptions of sulfadimethoxine and trimethoprim, all of the antimicrobial residues we tested for and detected in the water samples proximal to swine farms are approved for use in swine for therapeutic purposes and/or growth promotion (USDA, 1999; US FDA, 2002a). However, sulfadimethoxine and trimethoprim are approved for use in cattle and sulfadimethoxine is also approved for use in poultry (US FDA, 2002a). The presence of other meat-producing species or the use of their waste as a potential source of antimicrobial-containing waste was not evaluated on the swine farms or near the water collection sites, nor was antimicrobial usage data reviewed for the farms. The samples collected from or proximal to swine farms were not tested for fluoroquinolones, since this class of antimicrobials is not approved for use in swine (US FDA, 2002a).

Water samples collected proximal to poultry farms were screened for antimicrobials that are

Table 2
Environmental antimicrobial residue levels, measured by LC/ESI-MS, proximal to large-scale animal feeding operations, Iowa and Ohio, 1998

Farm ID	Farm type	Collection site	Antimicrobial (μg/l) CTC	TET+OXT	LIN	SMZ	SDX	TMP	ERY	SFX	EFX	NFX	CPX
1	Swine	Lagoon	870.0	130.0	240.0	400.0	2.5	2.5	2.5	NA	NA	NA	NA
2	Swine	Lagoon	68.0	27.0	2.5	2.5	2.5	2.5	2.5	NA	NA	NA	NA
3	Swine	Lagoon	95.0	35.0	80.0	160.0	2.5	2.5	2.5	NA	NA	NA	NA
4	Swine	Lagoon	190.0	25.0	2.5	2.5	2.5	2.5	2.5	NA	NA	NA	NA
5	Swine	Lagoon	250.0	100.0	68.0	100.0	2.5	2.5	2.5	NA	NA	NA	NA
7	Swine	Lagoon	1000.0	410.0	210.0	380.0	2.5	2.5	2.5	NA	NA	NA	NA
8	Swine	Lagoon	70.0	25.0	2.5	2.5	2.5	2.5	2.5	NA	NA	NA	NA
3	Swine	Monitoring well	BDL	BDL	BDL	BDL	TR	BDL	BDL	NA	NA	NA	NA
4	Swine	Monitoring well	BDL	BDL	BDL	BDL	TR	BDL	TR	NA	NA	NA	NA
8	Swine	Monitoring well	BDL	BDL	1.4	BDL	BDL	BDL	BDL	NA	NA	NA	NA
2	Swine	Field tile	BDL	BDL	0.4	0.3	BDL	BDL	BDL	NA	NA	NA	NA
3	Swine	Field tile	2.0	BDL	BDL	BDL	BDL	BDL	BDL	NA	NA	NA	NA
2	Poultry	Field stream	BDL	BDL	BDL	BDL	BDL	0.06	BDL	BDL	BDL	BDL	BDL
2	Poultry	Field stream	1.5	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL
1	Poultry	Field stream	BDL	1.0	BDL	BDL	BDL	0.27	BDL	4.0	BDL	BDL	BDL
3	Poultry	Field stream	BDL	BDL	BDL	BDL	BDL	0.15	BDL	BDL	BDL	BDL	BDL
3	Poultry	Field stream	BDL	BDL	BDL	BDL	0.35	BDL	BDL	3.0	BDL	BDL	BDL
4	Poultry	Field stream	BDL	BDL	BDL	BDL	BDL	0.15	BDL	BDL	BDL	BDL	BDL
5	Poultry	Field stream	BDL	BDL	BDL	BDL	BDL	BDL	BDL	3.0	BDL	BDL	BDL
5	Poultry	River	BDL	BDL	0.5	BDL	0.05	BDL	BDL	3.0	BDL	BDL	BDL
3	Poultry	Field tile	BDL	BDL	BDL	BDL	BDL	TR	BDL	BDL	BDL	BDL	BDL
2	Poultry	Farm spring	BDL	2.0	BDL	BDL	BDL	TR	BDL	BDL	BDL	BDL	BDL
1	Poultry	Farm spring	BDL	1.0	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL
2	Poultry	Field well	BDL	1.0	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL

BDL, the concentration was below the limit of detection of the assay. TR, detected below the limit of detection, but above the 95% confidence interval of the negative control (Meyer et al., 2000). NA, not applicable; samples were not tested for this agent. CTC, chlortetracycline; 0.5 μg/l (ppb) limit of detection (LOD); TET+OXT, tetracycline and oxytetracycline, 0.5 μg/l (ppb) LOD; LIN, lincomycin, 0.05 μg/l (ppb) LOD; SMZ, sulfamethazine, 0.05 μg/l (ppb) LOD; SDX, sulfadimethoxine, 0.05 μg/l (ppb) LOD; TMP, trimethoprim, 0.05 μg/l (ppb) LOD; ERY, erythromycin–H_2O, 0.05 μg/l (ppb) LOD; SFX, sarafloxacin, 0.05 μg/l (ppb) LOD; EFX, enrofloxacin, 0.05 μg/l (ppb) LOD; NFX, norfloxacin, 0.05 μg/l (ppb) LOD; and CPX, ciprofloxacin, 0.05 μg/l (ppb) LOD.

approved for use in poultry; in addition, the water was screened for trimethoprim, norfloxacin and ciprofloxacin, which are not approved for use in poultry. All of the antimicrobial residues, except trimethoprim, that were detected in those water samples were antimicrobials that have been approved for use in poultry. Trimethoprim is approved for use in cattle (US FDA, 2002a) and the presence of nearby cattle farms or the use of cattle waste as a potential source of antimicrobial-containing waste was not evaluated for the poultry farms. Although other potential sources of antimicrobials, such as private septic systems, were not evaluated as part of this study, they are unlikely sources of significant amounts of the antimicrobial agents identified. The absence of norfloxacin and ciprofloxacin is not surprising, since these agents are only approved for use in humans (US FDA, 2002b). Furthermore, the presence of sarafloxacin in water proximal to poultry farms implicates poultry waste used as fertilizer as a source of environmental antimicrobials, since its sole approved use is for poultry (US FDA, 2002a,b).

The prevalence of antimicrobial compounds in water samples proximal to swine and poultry farms, coupled with the results from the animal waste samples, provides evidence that animal waste stored in lagoons or applied to agricultural fields as fertilizer to increase crop yields appears to act as a non-point source of antimicrobial residues in water resources. This non-point source of antimicrobial residues in water resources likely contributed to the frequent detection of antimicrobial compounds found in streams of the United States (Kolpin et al., 2002).

A comparison of the radioimmunoassay and LC/ESI-MS results documents that radioimmunoassay techniques were only effective at measuring antimicrobial residues in samples likely to contain high levels of these compounds (such as animal waste) due to the higher reporting limits that are currently available through these analytical methods. Only one of the water samples (6%) proximal to swine farms and one sample (6%) proximal to poultry farms were found to contain antimicrobial compounds via radioimmunoassay methods (Table 1). In comparison, 31% of the water samples proximal to swine farms and 67% proximal to poultry farms were found to contain antimicrobial compounds using LC/ESI-MS methods (Table 2). Thus, the more sensitive LC/ESI-MS methods are required to adequately determine the presence of antimicrobial residues in samples likely to contain low levels of these compounds.

4. Conclusions

This study evaluated the presence of antimicrobial compounds in animal waste and surface and groundwater resources proximal to selected large-scale swine and poultry AFOs. High concentrations of multiple classes of antimicrobial compounds were found in all samples from swine-manure storage lagoons, with total antimicrobial concentrations approaching 1 mg/l. Antimicrobial residues were prevalent in environmental water samples proximal to swine (31%) and poultry farms (67%). Documenting the presence of high levels of antimicrobial compounds in animal-waste storage lagoons, coupled with their prevalence in water samples proximal to swine and poultry farms, suggests that animal waste applied to agricultural fields as fertilizer may act as a non-point source of antimicrobial residues in water resources. However, this study represents a one-time sample; there may be cyclical environmental contamination corresponding to when manure is applied and subsequent degradation of the antimicrobials. More research is needed to assess the fate of antimicrobial agents in the environment.

Further investigation is needed to understand the transport mechanisms for antimicrobial agents in the environment, to determine whether these agents are biologically active, and to determine the potential impact of these agents on the environment, environmental biota and public health. This study serves as a first step toward assessing the presence of these substances and differing methodologies for measuring them, an essential starting point in evaluating the environmental contamination and potential public health risks associated with waste from AFOs. Further studies are needed to better define the relation between AFOs, the environment and public health.

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References

Addison JB. Antibiotics in sediments and run-off waters from feedlots. Residue Rev 1984;92:1–28.

CDC. The Confinement Animal Feeding Operation Workshop, Washington DC, 23–24 June 1998. Atlanta, GA: Centers for Disease Control and Prevention, National Center for Environmental Health, 1998.

CDC. Report to the State of Iowa Department of Public Health on the Investigation of the Chemical and Microbial Constituents of Ground and Surface Water Proximal to Large-scale Swine Operations, October–December, 1998. Atlanta, GA: Centers for Disease Control and Prevention, National Center for Environmental Health, 1998.

CDC. Report of a Pilot Environmental Investigation around Large Poultry Operations in Ohio, November 1998. Atlanta, GA: Centers for Disease Control and Prevention, National Center for Environmental Health, 1998.

Chee-Sanford JC, Aminov RI, Krapac IJ, Garrigues-Jeanjean N, Mackie RI. Occurrence and diversity of tetracycline resistance genes in lagoon and groundwater underlying two swine production facilities. Appl Environ Microbiol 2001;67:1494–1502.

Daughton CG, Ternes TA. Pharmaceuticals and personal care products in the environment: agents of subtle change? Environ Health Perspect 1999;107(Suppl 6):907–938.

DeLind L. The state, hog hotels and the 'right to farm': a curious relationship. Agric Hum Values 1995;12(3):23–44.

Herrick JB. Animal waste—the smell of success or the stench of trouble? J Am Vet Med Assoc 1995;206(2):162–163.

Hirsch R, Ternes TA, Haberer K, Mehlich A, Ballwanz F, Kratz KL. Determination of antibiotics in different water compartments via liquid chromatography-electrospray tandem mass spectrometry. J Chromatogr A 1998;815:213–223.

Jackson L, Melvin S, Thu K, Lasley P, Thorne P. Understanding the impacts of large-scale swine production. Proceedings from an Interdisciplinary Scientific Workshop, Des Moines, IA, 29–30 June. 1995.

Jongbloed AW, Lenis NP. Environmental concerns about animal manure. J Anim Sci 1998;76:2641–2648.

Kolpin DW, Furlong ET, Meyer MT, Thurman EM, Zaugg SD, Barber LB, Buxton HT. Pharmaceuticals, hormones, and other organic wastewater contaminants in US streams, 1999–2000: a national reconnaissance. Environ Sci Technol 2002;36:1202–1211.

Mackie RI, Stroot PG, Varel VH. Biochemical identification and biological origin of key odor components in livestock waste. J Anim Sci 1998;76:1331–1342.

Meyer MT, Bumgarner JE, Varns JL, Thurman EM, Hostettler KA, Daughtridge JV. Use of radioimmunoassay as a screen for antibiotics in confined animal feeding operations and confirmation by liquid chromatography/mass spectrometry. Sci Total Environ 2000;248:181–187.

Reynolds SJ, Donham KJ, Stookesberry J, Subramanian P, Thu K, Whitten P. Air quality assessments in the vicinity of swine production facilities. J Agromed 1997;4(1–2):37–46.

Schiffman SS. Livestock odors: implications for human health and well-being. J Anim Sci 1988;76:1343–1355.

Schiffman SS, Sattely-Miller EA, Suggs MS, Graham BG. The effect of environmental odors emanating from commercial swine operations on the mood of nearby residents. Brain Res Bull 1995;37(4):369–375.

Stewart BA, Mathers AC. Soil conditions under feedlots and land treated with large amounts of animal manure. International Symposium on Identification and Measurement of Environmental Pollutants. Ottawa, Canada: National Council of Canada, Campbell Publishing Co, 1971. p. 81–83.

Thu K. Piggeries and politics: rural development and Iowa's multibillion dollar swine industry. Culture Agric 1995;53:19–23.

Thu K, Durrenberger EP. North Carolina's hog industry. The rest of the story. Culture Agric 1994;49:202–203.

Thu K, Durrenberger EP. Pigs, Profits, and Rural Communities. Albany, NY: State University of New York Press, 1998.

Thu K, Donham K, Ziegenhorn R, Reynolds S, Thorne PS, Subramanian P. A control study of the physical and mental health of residents living near a large-scale swine operation. J Agric Saf Health 1997;3(1):13–26.

USDA. Part I: Reference of 1995 Swine Management Practices. Fort Collins, CO: US Department of Agriculture, National Animal Health Monitoring System, 1995.

USDA. Antimicrobial Resistance Issues in Animal Agriculture, December 1999. US Department of Agriculture, Animal Plant Health Inspection Service, Veterinary Services, Centers for Epidemiology and Animal Health, Center for Emerging Issues, 1999. Available at www.aphis.usda.gov/vs/ceah/cei.

USDA. Part I: Reference of Swine Health and Management in the United States, 2000. Fort Collins, CO: US Department of Agriculture, National Animal Health Monitoring System, 2001. #N338.0801.

US FDA. The FDA Approved Animal Drug List (The Green Book). Blacksburg, VA: US Food and Drug Administration, Drug Information Laboratory, College of Veterinary Medicine, 2002. http://www.fda.gov/cvm/greenbook/greenbook.html. Accessed February 2002.

US FDA. Approved Drug Products with Therapeutic Equivalence Evaluations (Electronic Orange Book)US Food and Drug Administration, Center for Drug Evaluation and Research, Office of Information Technology, Division of Data Management and Services, 2002. . http://www.fda.gov/cder/ob/default.htm. Accessed February 2002.

US GAO. Animal Agriculture: Information on Waste Management and Water Quality Issues. Briefing Report to the Committee on Agriculture, Nutrition, and Forestry, US SenateUS General Accounting Office, 1995. p. 1995.

US GAO. Animal Agriculture: Waste Management Practices. Report to the Honorable Tom Harkin, Ranking Minority Member, Committee on Agriculture, Nutrition, and Forestry, US SenateUS General Accounting Office, 1999. .

US Senate. Animal Waste Pollution in America: An Emerging National Problem. Environmental Risks of Livestock and Poultry Production. Report Compiled by the Minority Staff of the United States Senate Committee on Agriculture, Nutrition, and Forestry for Senator Tom Harkin 1997. .

Voorburg JH. Pollution by animal production in the Netherlands: solutions. Review of science and technology. Off Int Epizootics 1991;10(3):655–668.

Attachment 12:

Nutrient transport through a Vegetative Filter Strip with subsurface drainage
(Bhattarai et al. 2009)

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Nutrient transport through a Vegetative Filter Strip with subsurface drainage

Rabin Bhattarai, Prasanta Kumar Kalita*, Mita Kanu Patel

Department of Agricultural and Biological Engineering, University of Illinois at Urbana-Champaign, Urbana, 1304 W. Pennsylvania Avenue, Urbana, IL 61801, USA

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ABSTRACT

The transport of nutrients and soil sediments in runoff has been recognized as a noteworthy environmental issue. Vegetative Filter Strips (VFS) have been used as one of the best management practices (BMPs) for retaining nutrients and sediments from surface runoff, thus preventing the pollutants from reaching receiving waters. However, the effectiveness of a VFS when combined with a subsurface drainage system has not been investigated previously. This study was undertaken to monitor the retention and transport of nutrients within a VFS that had a subsurface drainage system installed at a depth of 1.2 m below the soil surface. Nutrient concentrations of NO_3-N (Nitrate Nitrogen), PO_4^- (Orthophosphorus), and TP (Total Phosphorus) were measured in surface water samples (entering and leaving the VFS), and subsurface outflow. Soil samples were collected and analyzed for plant available Phosphorus (Bray P1) and NO_3-N concentrations. Results showed that PO_4^-, NO_3-N, and TP concentrations decreased in surface flow through the VFS. Many surface outflow water samples from the VFS showed concentration reductions of as much as 75% for PO_4^- and 70% for TP. For subsurface outflow water samples through the drainage system, concentrations of PO_4^- and TP decreased but NO_3-N concentrations increased in comparison to concentrations in surface inflow samples. Soil samples that were collected from various depths in the VFS showed a minimal buildup of nutrients in the top soil profile but indicated a gradual buildup of nutrients at the depth of the subsurface drain. Results demonstrate that although a VFS can be very effective in reducing runoff and nutrients from surface flow, the presence of a subsurface drain underneath the VFS may not be environmentally beneficial. Such a combination may increase NO_3-N transport from the VFS, thus invalidating the purpose of the BMP.

1. Introduction

Water quality concerns are increasingly becoming an environmental priority not only in the United States but worldwide. About 66% of US population relies on surface water as its primary source of drinking water (EPA, 2008). The primary sources of contamination to surface waters are runoff from agricultural fields and livestock facilities. Runoff from animal production facility contains high amount of nutrients, solids and microorganism that can degrade surface water quality (Dillaha et al., 1989b; Chaubey et al., 1994). There is a substantial risk for disease transmission by water-borne pathogens present in runoff from animal production facility. One of the common management practices for controlling pollutant from agricultural runoff and livestock feedlots is Vegetative Filter Strips (VFS), which are bands of planted or indigenous vegetation situated down-slope of cropland or animal production facilities to prevent localized erosion and filter nutrients, sediment, and other pollutants (Dillaha et al., 1989a,b). A VFS can remove sediment and other pollutants from runoff and wastewater by filtration, deposition, infiltration, absorption, adsorption, decomposition, and volatilization, thereby reducing the amount of pollutants entering surface waters (EPA, 2005). Other pollutants including bacteria, pathogens, pesticides, organic matter, and solids can also be removed by a VFS. This best management practice (BMP) is a better economic option for handling runoff and removing waste from flow coming out of animal production facilities compared to other treatments that may be more expensive or complex. Improved water quality results in the general environmental benefit of healthier wildlife habitat and natural surroundings.

Many studies have evaluated the performance of VFS in removing sediment (Robinson et al., 1996), nutrients (Chaubey et al., 1994; Mendez et al., 1999; Abu-Zreig et al., 2003; Blanco-Canq

Abbreviations: BOD_5, 5 day Biochemical oxygen demand; COD, Chemical Oxygen Demand; FC, Fecal Coliform; FS, Fecal *Streptococcus*; *E. coli*, *Escherichia coli*; NH_3-N, Ammonia Nitrogen; NH_4-N, Ammonium Nitrogen; NO_3-N, Nitrate Nitrogen; TN, Total Nitrogen; fTKN, Filtered Total Kjeldahl Nitrogen; TKN, Total Kjeldahl Nitrogen; PO_4^-, Orthophosphorus; PO_4-P, Orthophosphate; TP, Total Phosphorus; TS, Total Solid; TSS, Total Suspended Solid.

* Corresponding author. Tel.: +1 217 333 0945; fax: +1 217 244 0323.
E-mail addresses: rbhatta2@illinois.edu (R. Bhattarai), pkalita@illinois.edu (P.K. Kalita).

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ui et al., 2004) and pathogens (Lim et al., 1998; Coyne et al., 1998; Williamson et al., 1999; Entry et al., 2000; Atwill et al., 2002; Tate et al., 2004; Trask et al., 2004; Tate et al., 2006; Mankin et al., 2006). Koelsch et al. (2006) provided a detailed review of about 40 vegetative filter system-related field trials and plot studies. Brief reviews of six previous studies are summarized in Table 1. This table provides details such as length, slope, and vegetation along with percent reduction of different nutrient and pathogen parameters on a concentration basis. The reductions were based on the monitoring of the VFS surface inflow and outflow concentrations.

Although many studies have evaluated the performance of VFS for sediment, nutrients and pathogen removal from surface runoff, the efficiency of a VFS combined with a subsurface drainage system has not been reported in the literature. Subsurface drainage is a common agricultural water management practice in areas with seasonally perched water tables or shallow groundwater areas as it provides a pathway for excess or drainable water to leave the soil. In the Midwestern United States, subsurface drainage is one of the most common agricultural management practices used for increased crop production. Illinois alone has a total drained area of approximately 4 million ha (Kalita et al., 2007). Subsurface drainage improves the productivity of poorly drained soils by lowering the water table, providing greater soil aeration, and enabling faster soil drying and warming in the spring. It also facilitates in planting fields earlier and allows other field operations to take place in a timely fashion. It can reduce soil compaction and provide a better environment for crop emergence and growth (Sands, 2001). For these reasons, subsurface-drained soils represent some or the most productive soils worldwide (Skaggs et al., 1994). Since infiltration of runoff water is the primary mechanism that reduces surface flow and nutrient concentrations in a VFS, one may perceive that presence of a subsurface drain beneath a VFS would further enhance infiltration, thus reducing total nutrient in outflow from a VFS and reduce the water quality impact on receiving waters. However, the effect of subsurface drain beneath a VFS in reducing nutrient transport to receiving water is unknown. The objective of this study was to evaluate the efficiency of a VFS with a subsurface drainage system installed underneath in removing nitrogen and phosphorus from feedlot runoff. To our knowledge, a study to evaluate the performance of a VFS with subsurface drainage in reducing nutrients has not been carried out yet.

2. Methodology

Field-scale experiments on a VFS were conducted at the University of Illinois South Farm during 2001–2004. The details of the experimental components have been described in the following sections.

2.1. Vegetated Filter Strip description

The VFS used in this study was first established in 1994 to reduce the pollution of runoff from a feedlot into the surrounding experimental areas. The VFS had the ability to treat runoff from a feedlot with 300 head of cattle. However, the feedlot had less than half that amount, 130 cattle at the most during the study period. Runoff from the feedlot was collected at a settling basin located between the feedlot and the VFS. After settlement of solids at the settling basin, collected runoff water was evenly distributed on to the filter strip with two distribution channels of 6 m length each from a riser (Fig. 1). These channels were made of 0.15 m PVC pipe cut in half with indented cutouts every 0.3 m. The holes allowed even distribution of the runoff across the width of VFS at the inlet.

The filter strip used in the study was 14 m wide and 113 m long. The VFS was reconstructed in the spring of 2001. During reconstruction, flow to this filter strip was blocked at the settling basin while the filter strip was repeatedly disk plowed to break down and eradicate the old vegetation. Then the filter strip was regraded with a 1.5% slope. A mixture of brome grass and annual rye grass was planted as the new vegetation. The annual rye grass served as a cover crop to prevent the brome grass from misplacement due to rain or wind. The drilling method was used to plant the annual rye grass seed while the brome grass was broadcasted with a hand spreader. The brome grass seed was applied at a rate of 50.4 kg/ha and the annual rye grass seed was applied at a rate of 16.8 kg/ha; 8.0 kg of brome grass and 2.7 kg of annual rye grass was applied to

Table 1
Percent reduction on a concentration basis from reviewed VFS studies.

Reference	Filter parameter			Percent reduction (concentration basis)														
	Length (m)	Slope (%)	Vegetation	BOD_5	COD	FC	FS	*E. coli*	NH_3-N	NH_4-N	NO_3-N	TN	fTKN	TKN	PO_4-P	TP	TS	TSS
Chaubey et al. (1994)	3.00	3.00	Fescue	–	74.6	93.5	–	–	82.2	–	10.7	78.5	–	–	79.5	80.9	–	80.4
	6.00	3.00	Fescue	–	78.3	81.1	–	–	93.0	–	14.3	88.2	–	–	89.1	89.3	–	81.5
	9.00	3.00	Fescue	–	82.0	89.0	–	–	98.4	–	14.3	95.2	–	–	95.3	94.9	–	85.9
	15.00	3.00	Fescue	–	66.2	90.0	–	–	99.5	–	23.2	94.2	–	–	97.0	96.4	–	84.6
	21.00	3.00	Fescue	–	84.7	86.8	–	–	99.7	–	21.4	94.6	–	–	97.5	96.9	–	91.4
Edwards et al. (1983)	30.00	2.00	Tall fescue	34.2	40.0	–	–	–	–	–	–	37.0	–	–	–	36.2	32.5	–
	60.00	2.00	Tall fescue	59.5	69.1	–	–	–	–	–	–	63.1	–	–	–	66.5	62.4	–
Lim et al. (1998)	6.10	3.00	Kentucky-31 "tall" fescue	–	–	100.0	–	–	–	–	–	–	–	79.8	75.8	77.5	30.2	71.9
	12.20	3.00	Kentucky-31 "tall" fescue	–	–	100.0	–	–	–	–	–	–	–	88.0	86.7	89.4	22.1	83.2
	18.30	3.00	Kentucky-31 "tall" fescue	–	–	100.0	–	–	–	–	–	–	–	90.1	82.0	83.8	24.6	91.9
Mendez et al. (1999)	4.30	18.00	Kentucky-31 "tall" fescue	–	–	–	–	–	–	58.4	50.8	–	47.8	56.0	–	–	–	83.0
	8.50	18.00	Kentucky-31 "tall" fescue	–	–	–	–	–	–	64.7	52.4	–	41.6	74.8	–	–	–	87.3
Vanderholm et al. (1979)	61.00	2.00	Fescue	–	–	–	–	–	71.5	–	–	71.1	–	–	–	–	63.1	–
	91.00	0.50	Mixed grasses	–	–	–	–	–	86.2	–	–	80.1	–	–	–	78.2	73.1	–
	148.00	0.25	Garrison grass	–	–	–	–	–	85.2	–	–	88.9	–	–	–	–	78.7	–
	229.00	0.25	Garrison grass	–	–	–	–	–	40.5	–	–	49.6	–	–	–	–	39.2	–
	305.00	0.25	Garrison grass	–	–	–	–	–	62.9	–	–	60.9	–	–	–	16.0	59.0	–
	381.00	0.25	Garrison grass	–	–	–	–	–	64.2	–	–	66.3	–	–	–	48.6	56.2	–
	533.00	0.25	Garrison grass	–	–	–	–	–	83.4	–	–	83.1	–	–	–	–	79.7	–
Williamson et al. (1999)	239.00	1.2	Brome Grass	–	–	78.9	–	79.3	–			61.5				28.6		
	427.00	0.75	Brome grass	–	–	76.5	–	78.2	–			63.7				56.8		
	213.00	2	Fescue	–	–	36.0	83.0	–	–			19.0				13.0		
	137.00	0.6	Brome grass	–	–	90.3	88.4	–	52.8			74.2						

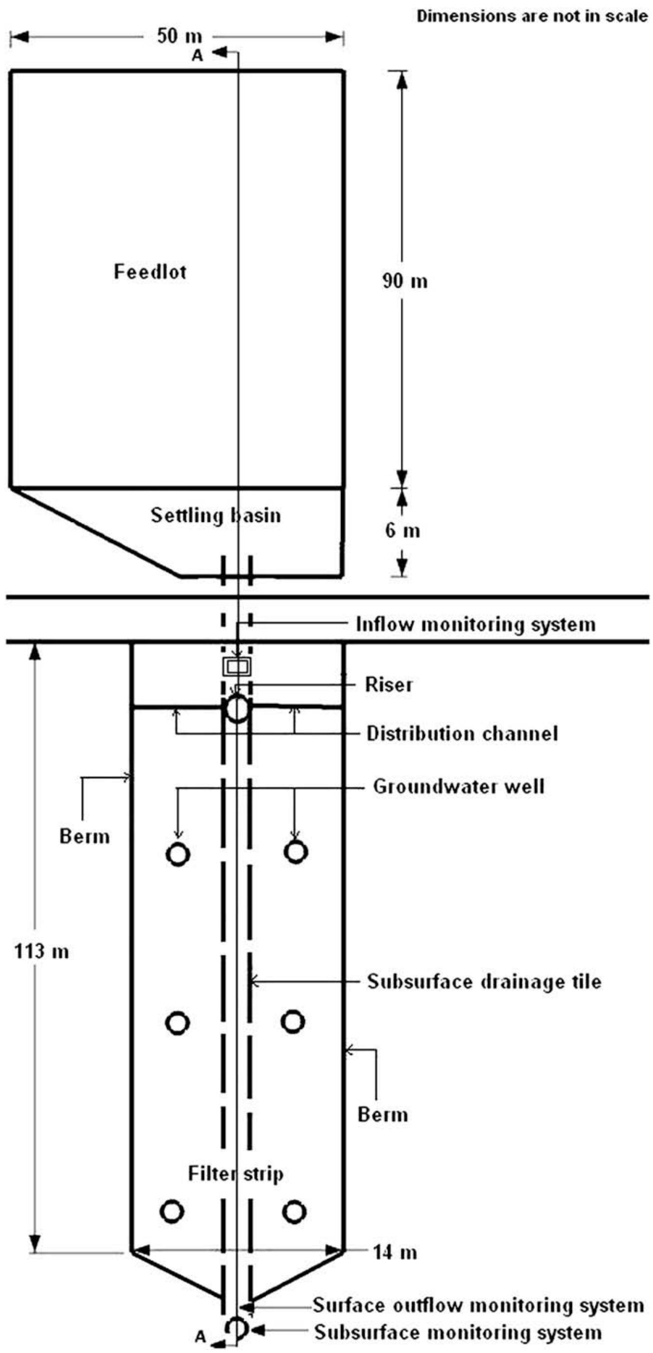

Fig. 1. Schematic diagram of VFS, feedlot, settling basin, groundwater wells and subsurface drainage tile (dimensions are in meter).

the VFS area of 0.16 ha. The VFS was established on Drummer silty clay loam soil (fine-silty, mixed, superactive, mesic Typic Endoaquoll). This taxonomic classification is a very typical of the wet, dark colored, prairie-derived soils of Illinois. The moist bulk density of the soil varied from 1.1 to 1.3 g/cm^3. Similarly, soil pH ranged from 5.6 to 7.8. The soil permeability varied from 0.015 to 0.05 m/h and soil organic matter measure ranged from 5 to 7%.

2.2. Subsurface drain system

A subsurface drainage pipe of diameter 0.1 m was installed at a depth of 1.22 m under the filter strips along the centerline of the VFS in 1994 (Fig. 1). The drainage pipe runs along the VFS length and discharges to a bigger drain pipe of 0.15 m diameter located at the end of the filter strip. The bigger drain pipe empties into a drainage ditch 312 m downstream of the end of the VFS.

2.3. Feedlot information

The feedlot area of the University of Illinois at South Farm is almost a century old with a capacity of approximately 300 cattle. Both heifers and steers of various breeds of cattle occupied this feedlot area. Their ages spanned from 4 months to 1.5 years while their weights ranged from 135 to 500 kg. The cattle stayed on the feedlot from late summer to late spring of the following year, spending most of their time on the actual feedlot. Their high-energy diet consisted of grains and ensilage that causes the cattle to produce liquid manure. Dimensions of the feedlot were approximately 90 m long by 50 m wide. Runoff from the feedlot occurs from natural rainfall events. The runoff was collected in a concrete settling basin with dimension of 20 m long and 6 m wide located at the end of feedlot. Two 0.15 m dual-wall corrugated polyethylene drain pipes were used to drain feedlot runoff from the settling basin to the filter strip (Fig. 1). The majority of the feedlot maintenance took place while it was uninhabited and the weather was suitable during the summer months. Maintenance included grading the feedlot and repairing the mound. Regular maintenance is important to prevent health problems for the animals. The health condition of the animals directly affects the quality and content of the liquid that is passed onto the VFS.

2.4. Flow rate and nutrient level monitoring system

Entering runoff flow rates to the VFS were measured with the inflow monitoring system. A closed stilling well and a trapezoidal flume were the two main components of the inflow monitoring system that were placed upstream of the riser. The downstream end of the flume was set up to allow the flow to discharge freely into the riser. The stilling well contained a weight and pulley system attached to a data logger to measure the water level within the stilling well every 15 min. Water samples for nutrient concentration measurement were collected from the downstream end of the flume after rainfall events. However, samples were also collected from the riser itself during periods when flows through the flume were too low.

Outgoing surface flow rates from the VFS were measured with the outflow surface monitoring system located at the end of the filter strip. The system comprised of a closed stilling well and an H-flume. The stilling well contained a weight and pulley system attached to a data logger to measure the water level within the stilling well every 15 min. The H-flume was placed at the end of the VFS. Runoff from the VFS passed into a small bucket that acted as a small retention basin. Water samples were taken from this small retention basin after rainstorm events. An H-flume has the ability of measuring a wide range of flow, accuracy of a sharp-crested weir, self-cleaning feature of a flume, and is simple in construction. Two metal borders having the dimensions of 0.2 m width and 2.5 m length guided surface runoff from the VFS into the H-flume.

The subsurface flow rates from the VFS were measured with the subsurface monitoring system located at the end of the filter strip. The system comprised of a closed stilling well and a Palmer–Bowlus flume. Water samples were taken directly from the upstream end of the flume following rainstorm events. The depth of flow in the Palmer–Bowlus flume was measured by an attached closed stilling well. The stilling well contained a weight and pulley system attached to a data logger to measure the water level within the stilling well every 15 min. The Palmer–Bowlus flume is designed to measure flow rates in manholes or open-round or rectangular channel bottoms and has been used extensively in research in east central Illinois for measuring tile drain flow (Mitchell et al., 2000; Cooke et al., 2002). Accuracy of measurement, low energy loss, minimal restriction to flow, and ease of installation into existing conduits are some of the advantages of this flume.

A tipping bucket rain gage was installed near surface monitoring system in order to measure rainfall. This rain gauge contained an HOBO event logger (Onset Corporation, USA), which was set to record the data in units of 0.254 mm. The schematic diagram of the VFS along with the feedlot and the nutrient monitoring system is shown in Fig. 1. Similarly, section A–A passing through Fig. 1 has been presented in Fig. 2.

2.5. Groundwater monitoring system

Six piezometers (groundwater monitoring wells) were installed for the measurement of water table depths and collection of groundwater samples from the VFS. The wells were placed evenly along the length of the VFS in 3 pairs, equidistant from the center of the VFS (Fig. 1). The construction of each well entailed boring a 1.3 m deep and 7.6 cm diameter hole by hand auger. Each well was made of PVC pipe with dimensions of 5 cm diameter and 1.6 m length. The pipes were perforated up to 0.5 m length at one end and the holes were covered with geo-textiles to prevent sediment flow into the well. The perforated end was placed into the hole and the area surrounding the pipe was filled with sand. The hole was sealed with Bentonite clay at the top in order to prevent the seepage of runoff into the wells. Water level depths were measured daily during the rainfall events and at least once in a week in dry periods.

2.6. Soil sampling system

Soil sampling was carried out to conduct soil nutrient analysis. Soil samples were collected from 18 fixed locations throughout the VFS, once a year, for three years. There were six sets of soil sampling sites, where each site had three sampling locations: one along the centerline of the VFS and two on either side of the centerline at 5 m distance. Soil sampling sites along the length of VFS were located at 0, 18, 37, 55, 77, and 100 m downstream of the inlet distribution pipe. This set up provided representative monitoring of the soil-nutrient levels throughout the filter strip. At each soil sampling location, soil samples were extracted from four different depths of 15, 30, 61, and 92 cm. Soil sampling started in 2001 before discharging feedlot runoff to the VFS to obtain a baseline nutrient concentration of the soil. Subsequent samplings were carried out in 2002 and 2003.

3. Data analysis

Water samples were collected from surface inflow (feedlot runoff), surface outflow runoff from VFS, subsurface outflow, and groundwater monitoring wells within the VFS. The water samples were analyzed for Nitrate Nitrogen (NO_3-N), Orthophosphorus (PO_4^-), and Total Phosphate (TP). Similarly, soil samples were analyzed for plant available Phosphorus (Bray P1) and Nitrate Nitrogen (NO_3-N) concentrations. The nutrient analysis was conducted in the Water Quality Laboratory of the Agricultural and Biological Engineering Department at the University of Illinois at Urbana-Champaign.

3.1. Soil samples analysis

A total of 72 soil samples were collected from different depths and locations of the VFS each year from 2001 to 2003 and analyzed for Bray P1 and NO_3-N concentration. For the analysis of Bray P1, 1 g of air dried, pulverized soil was mixed with a solution of Ammonium fluoride (NH_4F) and Hydrochloric acid (HCl) in a centrifuge tube. The mixture was swirled for 1 min at high speed. This solution was filtered and left to settle for 1 h. One ml aliquot was taken from the filtered solution and was diluted up to 10 ml with deionized water. The sample was analyzed for all forms of P. The analysis procedure for all form of P is described below.

For the analysis of NO_3-N, 40 g sample of soil was collected from the VFS and stored at 0 °C until analysis. After adding 10 g soil sample to 50 ml Potassium chloride (KCl), the mixture was shaken for 60 min and left to settle overnight. The soil extraction solution was filtered through a 0.7 μm glass filter. Samples were stored at 4 °C until the analysis of NO_3-N was carried out. The analysis procedure for NO_3-N is described below.

3.2. Water samples analysis

A 450 ml sample from each sampling bottle was collected and preserved with Sulfuric acid (H_2SO_4) at a concentration of 2 ml/L and stored at 4 °C until analysis. From preserved 450 ml water sample, 10 ml aliquot was used for each analysis. The analysis procedures for NO_3-N and all forms of P are described below.

3.3. NO_3-N analysis procedure

An alkaline solution of hydrazine sulfate that contained a copper catalyst was added to 10 ml aliquot of the sample. This allowed NO_3^- to be reduced to NO_2^-. Sulfanilamide was then used under acidic conditions to form a soluble azo dye and measured colorimetrically at 520 nm using a continuous flow Technicon Autoanalyzer II. Using known concentrations of NO_3-N, a standard curve was established. The output peaks generated by the samples from the autoanalyzer were measured and compared to the standard curve to determine sample concentrations (mg/L). This procedure followed the EPA Method 352.1, Standard Methods 4500-NO_3 and Technicon Method 696-82W (EPA, 1989).

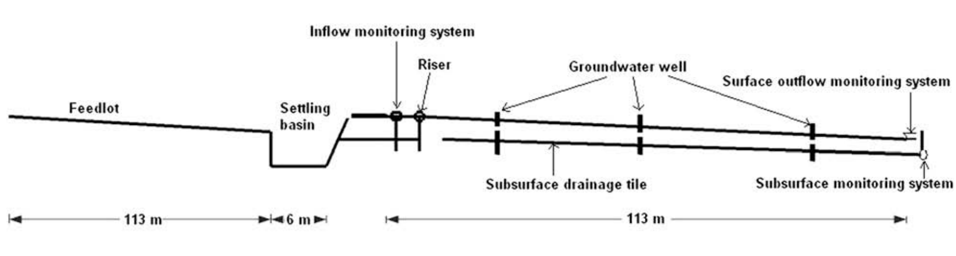

Fig. 2. Section A–A passing through Fig. 1.

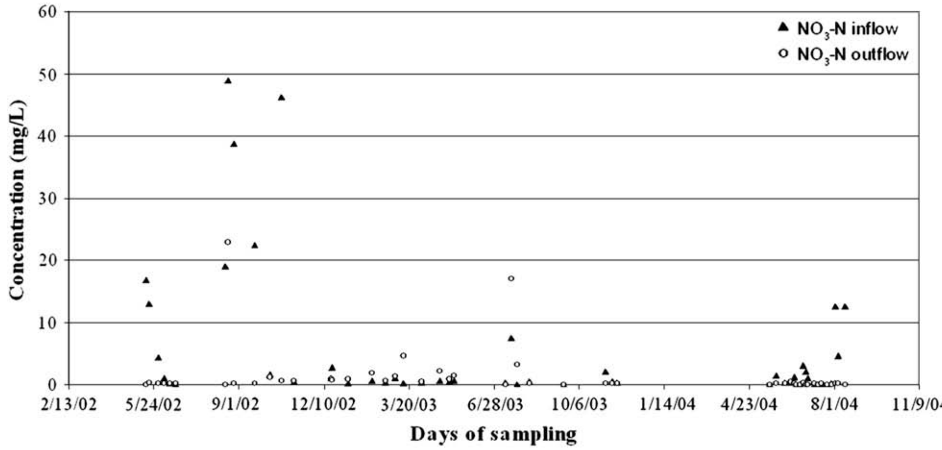

Fig. 3. Nitrate Nitrogen (NO_3-N) concentrations in surface inflow and outflow.

3.4. All forms of P analysis procedure

The EPA Method 365.1 (P in all forms), Standard Methods 4500-P F and Technicon Method 696-82W provided a method for Bray phosphorus, PO_4^-, and TP analysis (EPA, 1989). Using the same preserved 450 ml sample for the NO_3-N analysis, 10 ml aliquot was taken for analysis. Molybdate ion and antimony ion were added to the aliquot and reduced using ascorbic acid at an acidic pH forming a phosphomolybdenum complex (blue color). The complex was measured at 660 nm using a continuous flow Technicon Auto-analyzer II. Once again, known concentrations were used to form a standard curve, which was then compared to the measured peak outputs for each sample. Based on this comparison, PO_4^-, TP and Bray P1 concentrations in mg/L were determined for all samples.

4. Statistical analysis

Statistical analysis was carried out to check whether the concentrations of water quality parameters decreased significantly after the VFS treatment. Assuming that the observations follow a normal distribution, a statistical significance test was performed using samples before and after treatment as paired-dependent samples. Since the nutrient concentrations were measured for individual events, the effect of the VFS treatment can be considered as the average difference between the two measurements, before and after treatment. The nutrient concentrations at surface and subsurface outflow were related to the concentration of surface inflow to the VFS. In the analysis, the difference between measurements before and after the treatment for each event was calculated, and the mean of those differences was tested to determine if it was significantly different from zero. The null hypothesis for the test can be stated as follows: the difference in mean nutrient concentrations before and after treatment is not significantly different from zero. Let d_i denote the difference in nutrient concentration for an event *i*. The test static for dependent sample is:

$$t = \frac{\overline{d} - 0}{s_d/\sqrt{n}}$$

where $\overline{d}$ and s_d are the mean and standard deviation of the differences, and n is the number of samples. Although sample size varies from 21 to 41, the Student *t*-distribution is used for testing the hypothesis for consistency since the distribution of *t*-test tends to be normal as the sample size increases. Therefore, *t*-test can still be used for large samples even if the random variable is not normal (Kaps and Lamberson, 2004). The test static was compared with critical values for the Student *t*-distribution. The null hypothesis (nutrient concentration in samples before and after treatment are not significantly different) was rejected if the calculated test static was more than the critical value for a given degree of freedom and level of significance. The degree of freedom was taken as $(n - 1)$,

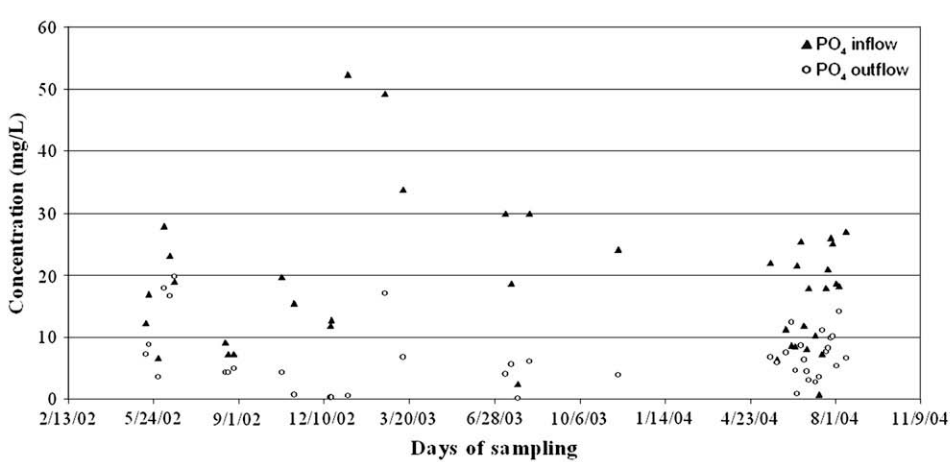

Fig. 4. Orthophosphorus (PO_4^-) concentrations in surface inflow and outflow.

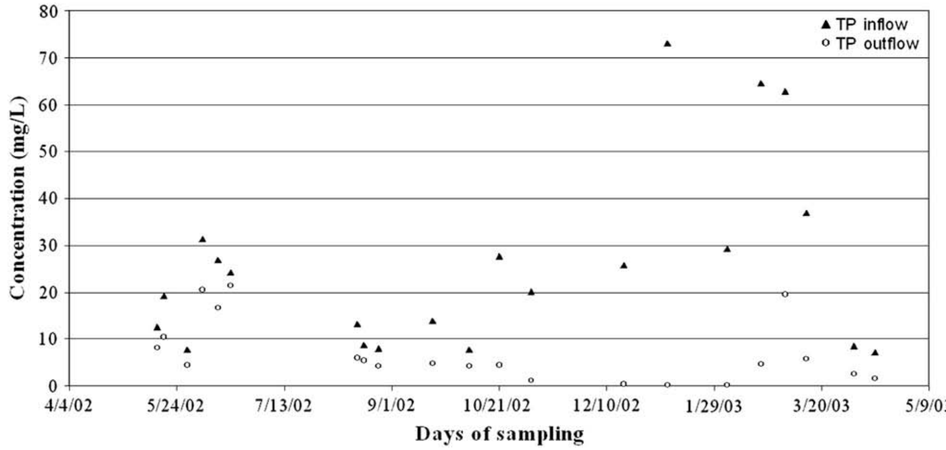

Fig. 5. Total Phosphate (TP) concentrations in surface inflow and outflow.

where n is the number of observations and the hypothesis was tested with $\alpha = 0.05$ level of significance.

5. Results and discussion

Looking into the water balance in the system, surface and subsurface hydrograph responses varied depending on nature of rainfall pattern (intensity and duration) and initial moisture condition of the soil within the VFS. Surface outflow hydrographs were influenced by surface inflow hydrographs and subsurface outflow hydrographs for all the events in terms of flow rate and volume. Subsurface outflow rates and volume outnumbered surface inflow for the rainfall events in wet conditions. For example, rainfall event of 7.56 cm on August 19, 2002 produced a higher subsurface outflow rate and volume compared to surface inflow. The excess subsurface outflow can be attributed to rain directly falling on the VFS. In case of rainfall events during dry condition, surface inflow rate and volume were higher than those of subsurface outflows. For example, surface inflow rate and volume were higher than those of the subsurface outflows during rainfall event of 1.2 cm on August 9, 2004.

5.1. Nutrient concentrations in inflow and outflow water samples

The results of the nutrient (NO_3-N, PO_4^- and TP) analysis in runoff at the surface inlet and surface outlet are provided in Figs. 3–5. Similarly, Figs. 6–8 show the results of NO_3-N, PO_4^- and TP analysis in runoff at the surface inlet and subsurface drain outlet.

It was observed that the VFS was able to not only reduce the quantity of runoff water, but also reduce NO_3-N, PO_4^- and TP concentrations in surface outflow compared to surface inflow as indicated in Figs. 3–5. The reduction NO_3-N values were very high in most cases. Few negative reduction values for NO_3-N were observed when surface inflow concentrations were very low. The bulk of these water samples was accumulated rainfall in the bucket from which the samples were collected. Additionally, surface outflows tended to be localized. Both NO_3-N and PO_4^- concentrations were relatively high when sampling began, as can be observed in Figs. 3–7. On June 5, 2002 the beef cattle were removed from the feedlot. Subsequently, concentrations of NO_3-N decreased but remained high for PO_4^-. NO_3-N concentrations increased rapidly, while PO_4^- concentration took some time to show a response after the cattle returned to feedlot on August 16 of that year. The PO_4^- concentrations on the filter strip were exceptionally high after few flow events. The higher concentrations can be attributed to two reasons – the accumulation of nutrients on the feedlot from previous storm event, and the transport of Orthophosphorus attached with soil particles from high levels of erosion on the feedlot. Some events showed that PO_4^- concentrations were as high as 70 mg/L coming from the feedlot, but were reduced to 0.52 mg/L in the surface outlet sample for the same event. In most cases, the reduction of Orthophosphorus was greater than 75% of

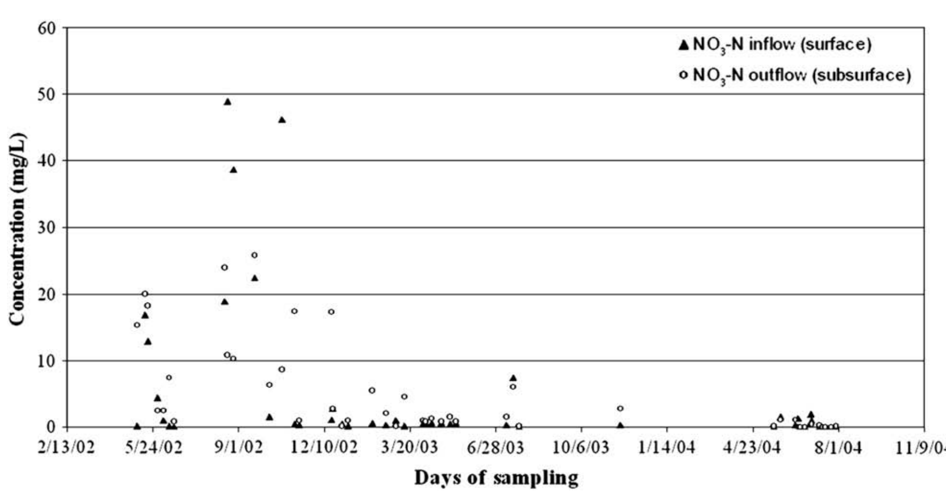

Fig. 6. Nitrate Nitrogen (NO_3-N) concentrations in surface inflow and subsurface outflow.

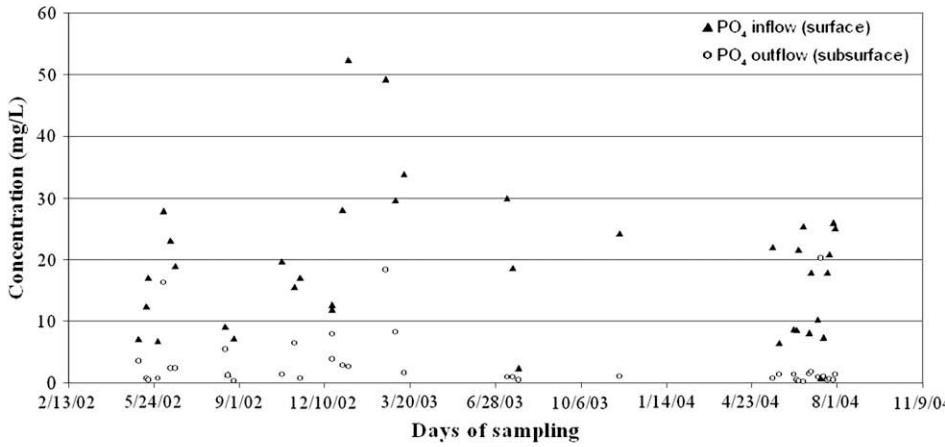

Fig. 7. Orthophosphorus (PO_4^-) concentrations in surface inflow and subsurface outflow.

the total surface inflow entering the VFS for the same rainfall event. The Orthophosphorus was most likely adsorbed in the soil organic matter or clay and became incorporated (immobilized). These results agree with past studies showing a reduction of PO_4^- on a filter strip (Chaubey et al., 1994; Lim et al., 1998; Young et al., 1980). Due to the length of the VFS (113 m), it is plausible that the reduction in concentration of P and N in surface water could be affected by factors other than infiltration and sorption of pollutants in the soil matrix including settling or dilution by rainfall over the VFS.

As can be seen in Fig. 6, the NO_3-N concentrations were rarely reduced in the subsurface outflow compared to the concentrations at the inflow station for the same event. In most cases, the concentrations in subsurface outflow increased. Increased nitrate concentrations may be attributed to part of the ammonia being converted to nitrate in the soil, the leaching of NO_3-N from upper soil profile or just due to high mobility of nitrate with soil water. As noted by Gilliam et al. (1999), the loss of N in drainage system can be attributed to mineralization of organic N, followed by nitrification of NH_4-N. However, the concentrations of PO_4^- and TP were reduced, unlike nitrate, as observed in Figs. 6 and 7. The PO_4^- and TP tend to strongly attach to soil particles and are less likely to percolate through soil profile. These results indicate that a subsurface outflow system in a VFS aids in the reduction of PO_4^- and TP, but it may negatively affect the environment, since high concentrations of NO_3-N may be discharged out to receiving waters.

5.2. Nutrient concentrations in water along the VFS length

Table 2 shows the nutrient concentrations in surface inflow, groundwater wells along the length of the VFS, surface outflow, and subsurface outflow for selected events during 2002 and 2003. The surface and subsurface outflow monitoring stations were located at a distance of 104 m and 113 m respectively, from surface inflow monitoring station.

It has been observed that PO_4^- and TP had a smooth trend of concentrations being reduced as the flow traveled down the length of the VFS, and concentrations at the surface and subsurface outflow being lesser than the inflow concentrations. The NO_3-N, however, did not follow the same trend. The NO_3-N level at the subsurface outflow monitoring system was higher than the NO_3-N concentration at the surface inlet. The averaged NO_3-N concentration in groundwater samples (collected at the same distance from inflow collection system) indicated lower values compared to the inflow concentrations for most of the events.

Since the study was more focused on looking into the effect of subsurface drainage on nutrient retention and transport through the VFS, the effect of VFS length, slope, soil and vegetation type on nutrient retention and transport through VFS could not be accessed. Chaubey et al. (1994) reported that NH_3-N, TKN, PO_4-P, TP and TSS were effectively removed by a fescue VFS whereas COD, FC and NO_3-N were not. They also found that there was no significant increase of VFS effectiveness beyond 3 m length for TSS and beyond

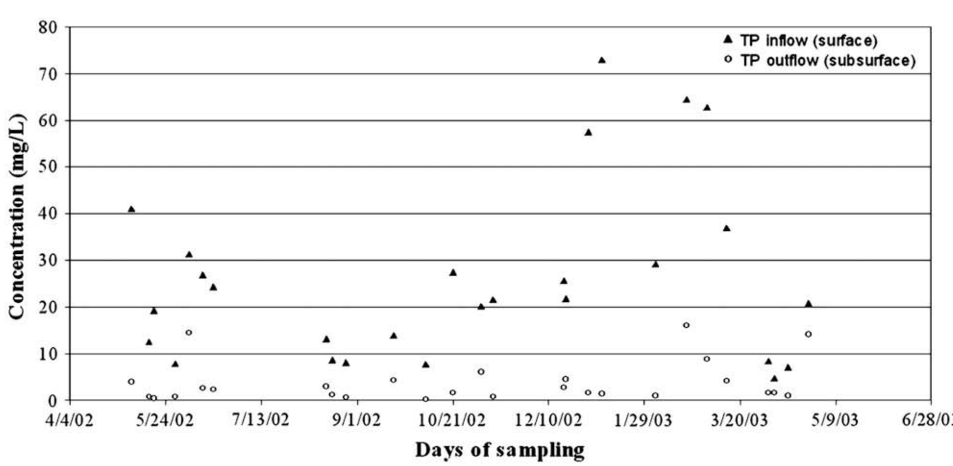

Fig. 8. Total Phosphate (TP) concentrations in surface inflow and subsurface outflow.

Table 2
Nutrient concentrations in water samples along the VFS for selected events.

Date	Nutrient	Inlet conc. (mg/L)	Decrease in concentration				
			Groundwater wells at distance			Surface outflow (%)	Subsurface outflow (%)
			5.5 m (%)	56.5 m (%)	99.4 m (%)		
5-Nov-2002	NO_3-N	0.38	−61.71	85.34	13.33	−60.00	−4527.42
11-Nov-2002	NO_3-N	0.28	10.26	72.73	−131.82	–	−263.64
18-Dec-2002	NO_3-N	1.05	−128.26	−119.57	65.22	13.04	−1553.46
19-Dec-2002	NO_3-N	2.77	84.40	80.29	78.65	73.72	−0.90
31-Dec-2002	NO_3-N	0.37	44.44	47.01	34.44	–	−2.50
7-Jan-2003	NO_3-N	0.12	−20.68	62.65	49.80	−574.30	−666.67
20-Feb-2003	NO_3-N	0.34	−50.00	34.25	−21.43	−71.43	−514.29
13-Mar-2003	NO_3-N	0.19	−37.50	32.37	25.80	25.00	−2250.00
5-Nov-2002	PO_4^-	15.52	63.71	97.14	99.21	95.62	58.42
11-Nov-2002	PO_4^-	17.08	53.60	99.03	99.44	–	95.74
18-Dec-2002	PO_4^-	11.87	45.52	79.72	94.62	96.27	76.84
19-Dec-2002	PO_4^-	12.66	53.16	89.04	99.67	94.94	64.14
31-Dec-2002	PO_4^-	28.06	77.24	98.49	98.71	–	94.23
7-Jan-2003	PO_4^-	52.38	88.78	97.92	97.86	99.69	97.33
20-Feb-2003	PO_4^-	49.27	59.51	87.39	81.48	65.50	62.86
13-Mar-2003	PO_4^-	33.84	56.86	89.04	96.10	79.91	95.29
5-Nov-2002	TP	20.18	51.56	95.16	99.66	93.99	69.42
11-Nov-2002	TP	21.49	56.25	99.38	99.44	–	96.67
18-Dec-2002	TP	25.63	52.38	87.12	95.38	98.63	85.02
19-Dec-2002	TP	21.86	43.73	93.87	99.73	98.76	63.82
31-Dec-2002	TP	57.48	76.33	99.13	99.89	–	95.04
7-Jan-2003	TP	73.00	82.97	95.36	99.90	99.29	96.48
20-Feb-2003	TP	64.51	66.69	95.41	85.17	92.82	74.98
13-Mar-2003	TP	36.93	58.41	89.68	97.15	84.21	88.80

9 m length for NH_3-N, TKN, PO_4-P and TP. Similarly, Coyne et al. (1998) found that the efficiency of VFS to remove sediment and bacteria from the runoff didn't increase significantly when the length of VFS was increased to 9 m from 4.5 m. Similarly, Abu-Zreig et al. (2003) found that increase in VFS length enhances phosphorus trapping in VFS.

5.3. Nutrient concentrations in soil samples at different depths

Soil samples were analyzed for Bray P1 and NO_3-N concentrations in order to monitor nutrient level at different soil depths during 2001–2003. The soil analysis results for Bray P1 and NO_3-N are presented in Table 3. As expected, the concentrations were higher in soil samples collected when the feedlot runoff was diverted to the filter strip in 2002. The Bray P1 concentrations during year 2002 and 2003 remained almost constant, with few exceptions. It appears that there was no built up of Bray P1 in the VFS, and the concentrations decrease with soil depth. Our observation supports the findings from Thomas et al. (1997) which reported significant leaching of P through a silty clay loam soil but little accumulation of P with depth. The presence of subsurface drainage also enhances the rapid mobility of dissolved phosphorus through the soil profile (Algoazany et al., 2007). Similarly, preferential flow through the soil macropores can play a significant role in subsurface transport of dissolved phosphorus (Simard et al., 2000). Accumulation of P in soil can have environmental consequences as it may turn into long-term diffuse sources of P discharge to water. The highest concentration of Bray P1 was found in the first 37 m of the VFS. The high concentration of Bray P1 in 2001 can be attributed to the fertilizer that was applied at the time of vegetation establishment.

In case of NO_3-N, there was an increase in concentrations after feedlot runoff was applied to the VFS in 2002 compared to the concentrations in samples collected in 2001. In overall, there was no substantial buildup of NO_3-N over the three-year period, and concentrations decreased slightly as the depth increased. An increase in concentration occurred near the inflow station for the 15 cm depth over the course of sampling period. The movement of nitrate towards the subsurface drain is typical, because the negatively charged nitrate anion is weakly bounded to the soil particles.

5.4. Statistical analysis results

For NO_3-N concentrations in surface inflow and subsurface outflow combination, the calculated test statistic, t was computed to be 0.163, while the critical value for the given degree of freedom and level of significance was 2.025. Therefore, the null hypothesis

Table 3
Bray P1 and NO_3-N concentrations in soil at different depths along the vegetated filter strip.

Depth (cm)	Distance (m)	Average Bray P1 concentration (mg/L)			Average NO_3-N concentration (mg/L)		
		2001	2002	2003	2001	2002	2003
15	0	18.96	15.46	41.75	1.01	1.81	2.85
	18	17.38	24.79	22.67	1.22	2.63	2.30
	37	4.66	14.26	13.89	0.68	2.2	2.67
	55	22.03	9.10	7.90	3.90	1.40	1.13
	77		3.05	7.89		0.95	1.33
	100		10.25	6.45		2.46	1.94
30	0	4.12	12.72	8.43	6.19	0.31	0.42
	18	4.52	13.53	8.67	1.67	1.24	1.70
	37	1.86	5.68	9.93	0.37	0.74	1.21
	55	15.04	2.63	2.41	2.02	0.84	0.62
	77		1.26	1.48		0.77	0.89
	100		3.55	4.49		1.56	1.61
61	0		1.53	5.23		0.25	0.50
	18		8.08	6.18		0.51	1.02
	37		1.58	1.14		0.43	0.71
	55		0.75	1.88		0.54	0.08
	77		0.40	0.90		0.30	0.34
	100		3.27	4.78		1.21	1.24
92	0		1.52	0.94		0.17	0.02
	18		5.31	5.45		0.42	0.88
	37		1.96	0.23		0.37	0.93
	55		1.00	0.91		0.40	0.40
	77		0.19	0.55		0.35	0.04
	100		0.58	2.63		0.19	0.24

Table 4
Summary of statistical analysis.

Combination	Sample size (n)	Computed test statistic (t)	Critical value for $\alpha = 0.05$ and $(n-1)$ degree of freedom	Decision on null hypothesis
NO3-N in surface inflow and surface outflow	41	2.686	2.021	Rejected
PO_4^- in surface inflow and surface outflow	41	6.586	2.021	Rejected
TP in surface inflow and surface outflow	21	2.553	2.086	Rejected
NO3-N in surface inflow and subsurface outflow	39	0.163	2.025	Accepted
PO_4^- in surface inflow and subsurface outflow	39	7.926	2.025	Rejected
TP in surface inflow and subsurface outflow	27	6.447	2.056	Rejected

was accepted for the combination. This indicates that NO_3-N concentrations in subsurface outflow were not significantly different from those in surface inflow. For all other combinations (NO_3-N in surface inflow and subsurface outflow, PO_4^- in surface inflow and surface outflow, PO_4^- in surface inflow and subsurface outflow, TP in surface inflow and surface outflow, and TP in surface inflow and subsurface outflow), the null hypotheses were rejected. The statistical analysis reflects that nutrient concentrations were reduced in both surface and subsurface outflow from the VFS compared to those at surface inflow except NO_3-N in subsurface outflow. The summary of statistical analysis is presented Table 4.

6. Conclusion

Vegetative Filter Strips are very effective in reducing the amount of nutrients and sediment in agricultural runoff, especially during shallow, uniform flow, as opposed to concentrated flow. The results from this study show that a VFS is effective in reducing flow volume, and consequently, reduces NO_3-N, PO_4^-, and TP concentrations in surface outflow runoff. Although the subsurface drainage system under a VFS helps in removing a higher volume of water in a shorter period of time and prevents inundation of the vegetation, it can provide a path for NO_3-N to be transported off site quickly. The results of the data from subsurface outflow samples show that NO_3-N is being transported out of the VFS and possibly to receiving waters. The subsurface outflow samples indicate that combining a VFS with underlying subsurface outflow drainage system may negatively harm the surrounding environment.

Overall, these results show that a VFS can be used as a BMP for controlling nutrients from feedlot. The VFS reduced all nutrient concentration from surface runoff. However, placement of a subsurface drainage system may need more careful consideration. For the VFS to be effective, proper care must be given in its design to ensure that surface flow is uniform and there are no preferential flow paths. A VFS without any subsurface drainage system may be an effective BMP, if lowering groundwater table quickly is not a prime issue.

References

Abu-Zreig, M., Ramesh, P.R., Hugh, R.W., Manon, N.L., Narinder, K.K., 2003. Phosphorus removal in vegetated filter strips. Journal of Environmental Quality 32, 613–619.

Algoazany, A.S., Kalita, P.K., Czapar, G.F., Mitchell, J.K., 2007. Phosphorus transport through subsurface drainage and surface runoff from a flat watershed in east central Illinois, USA. Journal of Environmental Quality 36 (3), 681–693.

Atwill, E.R., Hou, L., Karle, B.M., Harter, T., Tate, K.W., Dahlgren, R.A., 2002. Transport of *Cryptosporidium parvum* oocysts through vegetated buffer strips and estimated filtration efficiency. Applied Environmental Microbiology 68, 5517–5527.

Blanco-Canqui, H., Gantzer, G.J., Anderson, S.H., Alberts, E.E., Thompson, A.L., 2004. Grass barrier and vegetative filter strip effectiveness in reducing runoff, sediment, nitrogen, and phosphorus loss. Soil Science Society of America Journal 68, 1670–1678.

Chaubey, I., Edwards, D.R., Daniel, T.C., Moore Jr., P.A., Nichols, D.J., 1994. Effectiveness of vegetative filter strips in retaining surface-applied swine manure constituents. Transactions of the ASAE 37 (3), 845–850.

Cooke, R.A., Nehmelman, J.E., Kalita, P.K., 2002. Effect of Tile Depth on Nitrate Transport from Tile Drainage Systems. ASAE Paper No. 022017. American Society of Agricultural Engineers, 2950 Niles Rd. St. Joseph, MI. 49085.

Coyne, M.S., Vilalba, A.Z., Gilfillen, R.A.Z., 1998. Fecal bacteria trapping by grass filter strips during simulated rain. Journal of Soil and Water Conservation 53 (2), 140–145.

Dillaha, T.A., Sherrard, J.H., Lee, D., 1989a. Long-term effectiveness of vegetative filter strips. Water and Environment Technology 1 (3), 419–421.

Dillaha, T.A., Reneau, R.B., Mostaghimi, S., Lee, D., 1989b. Vegetative filter strips for agricultural nonpoint source pollution control. Transactions of the ASAE 32 (2), 513–519.

Environmental Protection Agency (EPA), 1989. Method 352.1, standard methods 4500-NO_3, and Technicon method 696-82W. In: Methods for Chemical Analysis of Water and Wastes. National Technical Information Service, Springfield, VA EPA/600/4-79/020.

Environmental Protection Agency (EPA), 2005. National management measures to protect and restore wetlands and riparian areas for the abatement of nonpoint source pollution. In: National Management Measures to Protect and Restore Wetlands and Riparian Areas for the Abatement of Nonpoint Source Pollution, pp. 61–80. EPA 841-B-05-003.

Environmental Protection Agency (EPA), 2008. Ground water & drinking water: frequently asked questions. http://www.epa.gov/OGWDW/faq/faq.html (accessed 17.04.08).

Edwards, W.M., Owens, L.B., White, R.K., 1983. Managing runoff from a small, paved beef feedlot. Journal of Environmental Quality 12 (2), 281–286.

Entry, J.A., Hubbard, R.K., Thies, J.E., Fuhrmann, J.J., 2000. The influence of vegetation in riparian filterstrips on coliform bacteria. I. Movement and survival in water. Journal of Environmental Quality 29, 1206–1214.

Gilliam, J.W., Baker, J.L., Reddy, K.R., 1999. Water quality effects of drainage in humid regions. In: Agricultural Drainage. Agronomy Monograph No. 38. American Sdociety of Agronomy, Crop Science Society of America, Soil Science Society of America, Madison WI.

Kalita, P.K., Cooke, R.A.C., Anderson, S.M., Hirschi, M.C., Mitchell, J.K., 2007. Subsurface drainage and water quality: the Illinois Experience. Transactions of ASABE 50 (5), 1651–1656.

Kaps, M., Lamberson, W., 2004. Biostatistics for Animal Science. CABI Publishing. 72–79.

Koelsch, R.K., Lorimor, J.C., Mankin, K.R., 2006. Vegetative treatment systems for management of open lot runoff: review of literature. Applied Engineering in Agriculture 22 (1), 141–153.

Lim, T.T., Edwards, D.R., Workman, S.R., Larson, B.T., Dunn, L., 1998. Vegetated filter strip removal of cattle manure constituents in runoff. Transactions of the ASAE 41 (5), 1375–1381.

Mankin, K.R., Barnes, P.L., Harner, J.P., Kalita, P.K., Boyer, J.D., 2006. Field evaluation of vegetative filter effectiveness and runoff quality from unstocked feedlots. Journal of Soil and Water Conservation 61 (4), 209–221.

Mendez, A., Dillaha, T.A., Mostaghimi, S., 1999. Sediment and nitrogen transport in grass filter strips. Journal of American Water Resources Association 35, 867–875.

Mitchell, J.K., McIsaac, G.F., Walker, S.E., Hirschi, M.C., 2000. Nitrate in river and subsurface drainage flows from an east central Illinois watershed. Transactions of the ASAE 43 (2), 337–342.

Robinson, C.A., Ghaffarzadeh, M., Cruse, R.M., 1996. Vegetative filter strip effects on sediment concentration in cropland runoff. Journal of Soil and Water Conservation 50 (3), 227–230.

Sands, G., 2001. Subsurface Drainage – Soil Water Concepts. University of Minnesota Extension Service BU-07644-GO.

Simard, R.R., Beauchemin, S., Haygarth, P.M., 2000. Potential for preferential pathways of phosphorus transport. Journal of Environmental Quality 29, 97–105.

Skaggs, R.W., Breve, M.A., Gilliam, J.W., 1994. Hydrologic and water quality impacts of agricultural drainage. Critical Reviews in Environmental Science and Technology 24 (1), 1–32.

Tate, K.W., Gracas, M.D., Pereira, C., Atwill, E.R., 2004. Efficacy of vegetated buffer strips for retaining *Cryptosporidium parvum*. Journal of Environmental Quality 33, 2243–2251.

Tate, K.W., Atwill, E.R., Bartolome, J.W., Nader, G., 2006. Significant *Escherichia coli* attenuation by vegetative buffers on annual grasslands. Journal of Environmental Quality 35, 795–805.

Thomas, D., Heckrath, G., Brookes, P.C., 1997. Evidence of phosphorus movement from Broadbalk soils by preferential flow. In: Tunney, H. (Ed.), Phosphorus Loss from Soil to Water. CAB International, Wallingford, pp. 369–370.

Trask, J.R., Kalita, P.K., Kuhlenschmidt, M.S., Smith, R.D., Funk, T.L., 2004. Overland and near-surface transport of *Cryptosporidium parvum* from vegetated and nonvegetated surfaces. Journal of Environmental Quality 33, 984–993.

Vanderholm, D.H., Dickey, E.C., Jackobs, J.A., Elmore, R.W., Spahr, S.L., 1979. Livestock Feedlot Runoff Control by Vegetative Filters. EPA-600/2-79-143. U.S. Environmental Protection Agency, Ada, Oklahoma.

Williamson, T.S., Kalita, P.K., Harner, J.P., Marchin, G.L., Rice, C.W., 1999. Coliform reduction by filter strips in Kansas watersheds. In: ASAE Annual Int. Meeting, Toronto, 18–21 July. Am. Soc. Agric. Eng., St. Joseph, MI Paper 99–2076.

Young, R., Huntrods, A.T., Anderson, W., 1980. Effectiveness of vegetated buffer strips in controlling pollution from feedlot runoff. Journal of Environmental Quality 9 (3), 483–487.

Attachment 13:

Vegetative Filter Treatment of Livestock Feedlot Runoff
(Dickey and Vanderholm 1981)

12. Reddy, C. N., and W. H. Patrick, Jr. 1979. Distribution of added labelled ($^{15}NH_4)_2SO_4$ in a flooded soil as influenced by redox potential and pH. p. 607–617. *In* Isotopes and radiation in research on soil-plant relationships. IAEA/FAO, Vienna.
13. Rittenberg, S. C., K. O. Emergy, and W. L. Orr. 1955. Regeneration of nutrients in sediments of marine basins. Deep-Sea Res. 3: 23–45.
14. Serruya, C., M. Edelstein, U. Pollingher, and S. Serruya. 1974. Lake Kinneret sediments: nutrient compaction osition in the pore water and mud water exchanges. Limnol. Oceanogr. 19:489–508.
15. Theis, T. L., and P. C. Singer. 1974. Complexation of iron (II) by organic matter and its effect on iron (II) oxygenation. Environ. Sci. Technol. 8:569–573.
16. Watanabe, F. S., and S. R. Olsen. 1965. Test of an ascorbic acid method for determining phosphorus in water and $NaHCO_3$ extracts from soil. Soil Sci. Soc. Am. Proc. 29:677–678.
17. Whelan, T., III, J. T. Ishmael, and W. S. Bishop. 1976. Long-term chemical effects of petroleum in south Louisiana wetlands -1. Organic carbon in sediment and waters. Mar. Pollut. Bull. 7(8):150–156.

Vegetative Filter Treatment of Livestock Feedlot Runoff[1]

ELBERT C. DICKEY AND DALE H. VANDERHOLM[2]

ABSTRACT

Four vegetative filters were installed on feedlots in central and northern Illinois. Two configurations were used: channelized flow and overland flow. After settling for partial solids removal, runoff was applied directly to the filters and allowed to flow from the inlet to the outlet section. Results from measurement analyses and sampling of influent, effluent, and surface flow at intermediate points were reported.

Most runoff events were infiltrated completely, resulting in no filter discharge. Runoff from larger events was partially discharged. Filters removed as much as 95% of nutrients and oxygen-demanding materials from the applied runoff on a weight basis, and 80% on a concentration basis. Removal was directly related to flow distance or contact time with the filter. Channelized flow with greater flow depths required greater contact time or flow distance than shallow overland flow to achieve the same level of treatment.

Additional Index Words: **nutrients, water quality, land application, pollution.**

Dickev, E. C., and D. H. Vanderholm. 1981. Vegetative filter treatment of livestock feedlot runoff. J. Environ. Qual. 10:279–284.

Many livestock feedlots are not subject to the National Pollutant Discharge Elimination System (NPDES) permit program. While most are small feedlots, some have a potential water pollution problem because of uncontrolled runoff from open lot areas. Installation of a zero-discharge runoff-control system is one method of solving this pollution threat. But this approach is economically prohibitive for many small operations,[3] even though the zero-discharge system is required in several states. An alternative is a vegetative filter system which adequately controls runoff so that violations of water quality standards will not occur during storm runoff. This alternative has the advantage of controlling runoff at lower cost than conventional zero-discharge systems, and requires less management.

Vegetative filters are systems in which a vegetative area such as pasture, grassed waterway, or even cropland is used for treating feedlot runoff by settling, filtration, dilution, absorption of pollutants, and infiltration. Mather (1969) reported removal of biochemical oxygen demand (BOD) from cannery wastes of 94–99% during overland flow in a disposal area, although Bendixen et al. (1969) reported only 66% BOD removal. Nitrogen removals of 61–94% and phosphorus removals of 39–81% were also reported in these two studies.

McCaskey et al. (1971) found a renovating effect for livestock waste water traveling over a grassed surface in a thin layer, but did not determine the effect on a quantitative basis. Edwards et al. (1971) measured significant reductions in the nutrient content of feedlot runoff after the runoff traversed a grassed waterway. Reduction was attributed to deposition of solids in the waterway and to dilution of feedlot runoff by surface water from nearby cropland. Kramer et al. (1974) indicated that possibly spray-runoff was satisfactory for removal of BOD and total suspended solids from beef feedlot runoff, but that nutrient levels could still be too high for discharge to be practical.

Sievers et al. (1975) used a grassed waterway filter to treat anaerobic swine lagoon effluent. Willrich and Boda (1976) also treated swine lagoon effluent with sloping grass strips. Open feedlot runoff-treatment systems have been reported by Sutton et al. (1976) and Swanson et al. (1975). Most early systems were designed on the premise that all or most of the feedlot runoff from storms would infiltrate into the soil, with the uninfiltrated runoff being adequately treated so that it could enter surface watercourses. However, no uniform design criteria has been developed, and variable performance has made environmental authorities hesitant to give blanket approval to this concept.

A study was begun in 1975 to further evaluate vegetative filter systems. The study was conducted year-round for over 2 years. Its objective was to determine whether or not vegetative filters are feasible alternatives for management of feedlot runoff.

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[2] Assistant Professor, Dep. of Agric. Eng., Univ. of Nebraska, Lincoln; and Associate Professor, Dep. of Agric., Univ. of Illinois, Urbana, respectively.

[3] D. Lybecker. 1977. Comparative surface runoff control system investment and operating costs for six Illinois demonstration-research sites. Paper presented at Southern Illinois Univ. Liquid Livestock Waste Disposal Field Day, Dep. of Agric. Ind. Southern Illinois Univ., Carbondale.

MATERIALS AND METHODS

Channelized flow and overland flow systems were studied. Channelized flow systems have various configurations such as a graded terrace channel or grassed waterway, and are systems in which flow is concentrated in a relatively narrow channel. One channelized flow system was a graded terrace that traversed a hillside several times in a serpentine fashion. The other channelized flow system had one section of graded terrace channel followed by a section of grassed waterway. In overland systems, flow occurs as sheet flow generally <30 mm deep, with widths ranging from 5 to 30 m.

Four feedlots were selected in which vegetative filters were well-adapted to the physical situation and appeared to have a reasonable chance for managing feedlot runoff. At all locations, the basic system consisted of a settling facility, a distribution component, and one of the two types of vegetative filter illustrated in Fig. 1. No storage unit for runoff was involved. Runoff from storms went directly to the filter area. Similar concrete settling basins were used at each location, but each vegetative filter was quite different. One system was installed on the University of Illinois dairy farm, and the other three systems were at commercial livestock production facilities.

At the University of Illinois dairy facility (System 1), effluent from the settling basin was pumped by an automatic pump (controlled by the water level) through a gated irrigation-pipe distribution system, spreading the effluent on three field plots, each 12 by 91 m and with a slope of about 0.5%. One grass species was seeded on each plot. Species used were reed canarygrass (*Phalaris arundinacea* L.) smooth bromegrass (*Bromus inermis* Leyss.), and orchardgrass (*Dactylis glomerta* L.). Each plot was surrounded by a berm to prevent any outside drainage water from entering and any applied effluent and rainfall from discharging, except at the controlled plot outlet. A control plot, planted to smooth bromegrass, received no effluent applications. The flow over the plots was intended to approximate sheet or overland flow. The ratio between the vegetative filter area and feedlot area was about 1:1.

System 2 was also an overland flow type and was installed to control the runoff from a beef feedlot holding about 450 cattle. The facility obtained an NPDES permit, which allowed use of the vegetative filter area. This was a gravity-flow system, with runoff distributed across the upper end of a sloping vegetated area. Initially, runoff was distributed through a perforated plastic pipe 15.2 cm in diameter. Later, a rigid plastic pipe was split to form a weir. The vegetative filter area was seeded to a fescue (*Festuca arundinacea* Schreb.) and alfalfa (*Medicago sativa* L.) mixture. Since the soil was sandy, a filter area to lot area ratio of 0.7:1 was used. The constructed filter, 27 by 61 m, had a slope of about 2%.

System 3 was on a beef feedlot holding 500 cattle. Runoff was directed to a channelized flow vegetative filter (graded terrace) patterned after the serpentine waterway system studied by Swanson et al. (1975). The terrace channel was about 564 m long and had a parabolic cross-section with a top width of 8.5 m and a depth of 0.9 m. The channel slope was 0.25%.

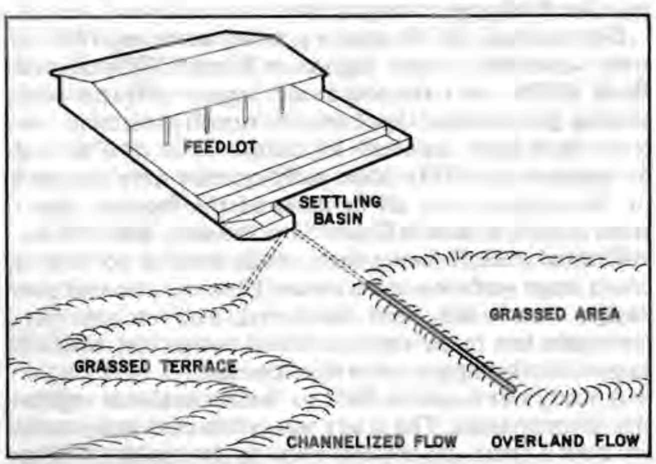

Fig. 1—Alternative configurations for vegetative filters used as a treatment for feedlot runoff.

System 4 was on an uncovered swine-finishing facility holding 480 animals. Runoff entered a vegetated terrace channel seeded with garrison creeping foxtail (*Alopecurus arundinaceus* Poir). Runoff traversed 152 m of terrace channel and 457 m of grassed waterway before reaching a defined watercourse. The terrace channel slope was 0.25% and the waterway was 2%.

Experimental Procedures

A recording rain gauge was used to collect rainfall data at each site. For System 1, the quantity of runoff applied to plots was calculated from records of elapsed pumping time and pump calibration curves. Applied runoff in System 3 was measured with an H-type flume and a water-stage recorder at the channel inlet. Applied runoff quantities were estimated for Systems 2 and 4 by using rainfall data and previously developed rainfall-runoff relationships for feedlots in Illinois (Dickey and Vanderholm, 1977).

Each site was equipped with automatic samplers capable of taking 24 discrete 550-ml samples. In addition, three composite type automatic samplers were used at System 1. At each automatic sampler location H-type flumes with stage recorders were used to measure the flow rate. Samplers and flumes were located at each filter outlet and also at intermediate points on System 3. All samplers were flow-activated, and usually set to take a 500-ml sample at 45-min intervals. Automatic samples were augmented by grab sampling along the flow length. Grab samples of runoff entering the filters were taken periodically. At System 3, the sampler location during 1976 was 305 m downslope from the settling basin discharge. In 1977, two samplers were positioned at 229 and 381 m from the basin discharge until midsummer, after which the sampler at 229 m was moved to 533 m.

Samples were analyzed for ammonia and Kjeldahl-N according to Bremner and Keeny (1965), and solids, conductivity, chloride chemical oxygen demand (COD), BOD, and total P and K, according to *Methods for Chemical Analysis of Water and Waste* (USEPA, 1974). Filter influent and effluent samples from System 1 were analyzed for fecal coliform and fecal streptococcus according to *Standard Methods* (APHA, 1975).

RESULTS AND DISCUSSION

Average concentrations in the filter effluent at System 1 represented a reduction of about 80% from concentrations in the settling-basin effluent (Table 1). Both COD and BOD levels were reduced to 85% of those in the basin effluent. The filter discharge had an average BOD concentration of 165 mg/liter, but only a limited number of BOD measurements were obtained. However, filter effluent volume for the sampling period was considerably less than basin effluent volume because infiltration occurred in the filter area. The filter effluent volume was 413 m³ while the filter area received 2,453 m³ of feedlot runoff. On a weight basis, an average of

Table 1—Constituent concentrations and constituent retention on a weight basis for vegetative filter System I.

Constituent	Settling basin effluent	Vegetative filter effluent†	Concentration reduction	Constituent retention (weight basis)
	mg/liter		%	
NH_3-N	134	18.5	86.2	97.7
Total Kjeldahl-N	300	59.6	80.1	96.7
Total solids	3,700	996	73.1	95.5
COD	4,220	616	85.4	97.5
P	64.1	14	78.2	96.3
K	665	168	74.7	95.7
Effluent volume	2,453 m^3	413 m^3		83.2

† Average concentrations in samples taken at equal time intervals during discharge events.

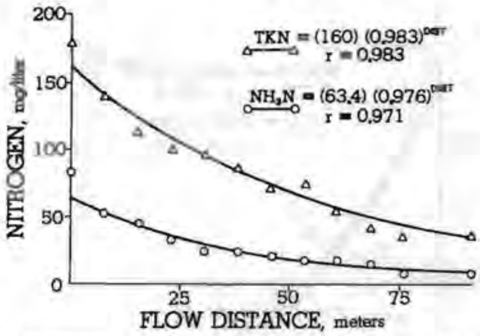

Fig. 2—Nitrogen concentration changes with overland flow (System 1).

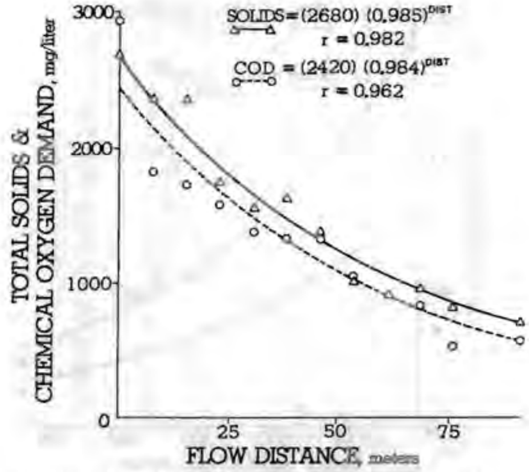

Fig. 3—COD and total solids concentration changes with overland flow (System 1).

about 96% of constituents applied were retained by the filter. Ammonia-N had the greatest reduction, showing a removal of 97.7%; total solids had the least reduction, a removal of 95.5%.

Samples from System 1 averaged 5.75×10^5 fecal coliforms 100 m^{-1} in discharge from the control plot that received no waste, 1.05×10^7 100 ml^{-1} in treated plot discharge, and 1.25×10^7 100 ml^{-1} in applied feedlot runoff. Fecal streptococcus averaged 1.8×10^3 100 ml^{-1} from the control plot, 1.1×10^5 100 ml^{-1} in the treated-plot discharge, and 1.6×10^6 100 ml^{-1} in applied runoff. While some differences were indicated, the number of bacterial analyses was not large enough to analyze statistically. Bacteria levels were high in both the treatment and control plots, but the data were consistent with a previous study by Dornbush et al. (1974).

Figures 2 and 3 clearly show decreases in constituent concentrations as basin effluent traversed the vegetative filter at System 1. Data points on Fig. 2 and 3 are averages of grab samples obtained during seven different runoff events. The figures indicate that constituent concentrations approached background levels (and the stream standards) asymptotically as vegetative filter length increased, and that excessive flow lengths would be required to meet standards unless further dilution occurred.

While the filters were effective in removing pollutants, the effluent still had sufficiently high pollutant levels to cause a violation of stream water quality standards in some instances. Measured discharge rates from System 1 were low, averaging 1.70 liters sec^{-1}, with a maximum observed discharge of 10.8 liters sec^{-1}. This flow rate is quite small relative to many receiving stream flow rates during the storms.

Relatively high constituent concentrations were found in the filter effluent from System 2, as compared to System 1 (Table 2). System 1 was a dairy, cleaned daily when possible, but the beef feedlot in System 2 was only cleaned every 3 or 4 months. System 2 also had an animal density about 7 times that of System 1. Thus, there were much higher constituent concentrations in the feedlot runoff entering the settling basin at System 2 than in System 1. The settling basin at System 2 was cleaned infrequently, which meant a loss of settling capacity during many storms. These factors contributed to high concentrations of constituents in the settling basin effluent for System 2. As a result, the upper end of the vegetative filter at System 2 became a shallow but effective settling area, trapping large amounts of manure solids.

Table 2—Estimated pollutant removal in System 2 filter based on System 3 basin effluent concentrations.

Constituent	Settling basin effluent	Vegetative filter effluent	Constituent reduction
	mg/liter		%
NH_4-N	608	173	71.5
Total Kjeldahl-N	1,122	324	71.1
Total solids	12,777	4,710	63.1
COD	14,288	2,691	81.2

Representative samples of the settling basin effluent at System 2 were not obtained. Consequently the effluent from the settling basin at System 2, after traversing the first few meters of filter, was assumed to be similar to the settling basin effluent at System 3, a beef feedlot similar in size, stocking density, and management. Constituent concentrations in the vegetative filter effluent of System 2 generally represent about a 70% reduction of the concentrations in the settling basin effluent.

Using the relationships between concentrations and distances developed for System 1 (Fig. 2 and 3) and the 61-m flow distance of System 2, the projected concentration reduction for constituents in the settling basin effluent after traversing System 2 would be about 65%. This is close to the observed 70% reduction after 61 m of flow (Table 2). The comparison between the concentration reductions at System 1 and 2 indicates comparable and fairly consistent performance, although the flow distance of System 2 was considered inadequate to achieve an acceptable pollutant reduction.

The amount of nutrients removed by System 2 was not calculated, but most rainfall events of < 25 mm had no vegetative filter discharge. This indicated that retention of constituents as calculated on a weight basis would be greater than the 70% reduction on a concentration basis.

Average constituent concentrations in flow samples from System 3 are shown in Fig. 4 and 5. The concentration reductions at the System 3 sampling points are

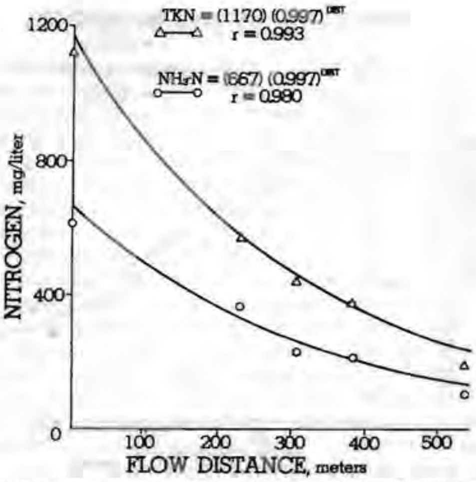

Fig. 4—Nitrogen concentration changes with channelized flow (System 3).

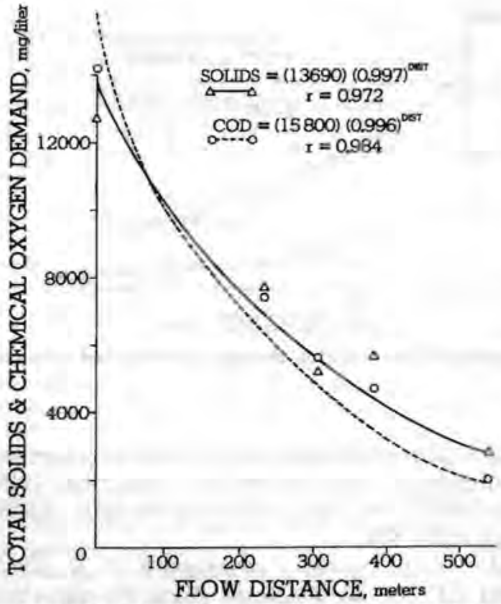

Fig. 5—COD and total solids concentration changes with channelized flow (System 3).

listed in Table 3. Comparing these reductions with those in System 1 and 2 (Tables 1 and 2) shows that a vegetative filter with channelized flow must be much longer than an overland flow system to achieve the same reduction. For example, overland flow systems have about a 70% concentration reduction after 90 m of flow, while channelized flow systems require about 427 m of flow distance to achieve a similar reduction.

Curvilinear regressions were used to develop relationships between constituent concentrations and flow length. The equations developed (Fig. 2, 3, 4, and 5) and r values exceeding 0.95. As with System 1, the data from System 3 also indicated that constituent concentrations approached background levels asymptotically.

Assuming that the filter discharge should meet current Illinois stream quality standards (1.5 mg liter^{-1} NH_3-N and 1,000 mg liter^{-1} solids), the required filter length based on equations developed would be 154 m for System 1 and 2,030 m for System 3. Even though Systems 1 and 3 had nutrient retentions exceeding 90%, in order to meet stream standards these filters should have had flow lengths 1.7 and 3.6 times longer, respectively. This procedure does not consider dilution potentials of receiving streams or additional runoff from surrounding areas.

During the 17-month study period (May 1976–October 1977) 10 storms resulted in discharges from System 3. Mass-balance studies were conducted for three rainfall events totaling 17.4 cm. Using the average concentrations presented in Fig. 4 and 5 and the flow volumes measured for each storm, mass balances were calculated for four constituents (Table 4). About 30% of the constituents were removed in the first 229 m of flow, with the next 152 m removing an additional 50%. The last 152 m of vegetative filter removed about 12% of the constituents. The resulting total constituent removal for System 3 was about 92% on a weight basis. For the three events, only 15.4 kg of ammonia-N was discharged from the filter. Assuming this measured quantity was representative of the other seven rainfall events (which were of about the same magnitude), the total ammonia-N discharged from System 3 would be 51.3 kg.

Low removal rates at the upper end of filter 3 reflected an inherent problem with a parabolic channel filter. Flow width in the waterway seldom exceeded 1.5 m, primarily because of the controlled outflow from the settling basin. Grass in the waterway bottom was killed in a 0.3- to 0.9-m width for about 9 m. Vegetation was stunted for another 150 m beyond the killed area. Nutrients, solids, and water from most small runoff events were deposited or infiltrated in the waterway segment where vegetation was killed or stunted. Waterways with larger flow widths (such as flat-bottomed) apparently distributed basin effluent more evenly and might have alleviated the vegetation kill resulting from excessive nutrients and water in the narrow channel bottom.

The channelized filter, System 4, performed better than System 3. Average constituent concentration re-

Table 3—Reduction in constituent concentration in the basin effluent at various locations in the vegetative filter System 3.

Constituent	Distance from basin discharge, m 229	305	381	533
	concentration reduction, %			
NH_3-N	40.5	62.9	64.2	83.4
Total Kjeldahl-N	49.6	60.9	66.3	83.1
Total solids	39.2	59.0	56.2	79.7
COD	49.2	60.4	67.4	86.0
P	–	16.0	48.6	–

Table 4—Constituent retention on a weight basis by vegetative filter System 3; average for three storms.

Constituent	Distance from basin discharge, m 229	381	533
	constituent retention, %		
NH_3-N	24.3	80.0	92.3
Total Kjeldahl-N	35.8	81.2	92.2
Total solids	23.4	75.6	90.7
COD	34.0	81.8	93.5

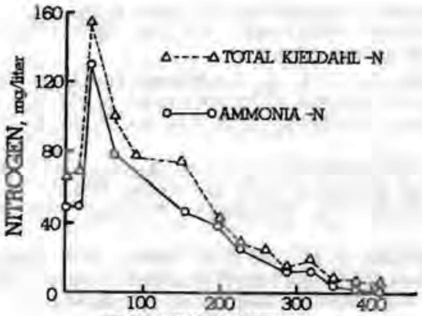

Fig. 6—Nitrogen concentration changes with channelized flow for an individual storm (System 4).

duction after 148 m of flow distance was about 86% (Table 5). Total solids were reduced 78.7% in the same distance. Higher pollutant removals than this are desirable and in this instance were achieved since the graded terrace discharged into an existing grass waterway. Figures 6 and 7 show constituent concentration along the filter of System 4 immediately after a 56-mm rainfall. Sampling immediately after rather than during the rainfall event probably resulted in the lower constituent concentration at the upper end of the terrace channel. The data for Systems 3 and 4 show that equivalent treatment requires longer flow lengths with channelized flow than with overland flow.

Results from monitoring soils, crops, and ground water in the filter areas studied are contained in a final project report (Vanderholm et al., 1979). The final report and an associated paper (Vanderholm and Dickey, 1978) also contain recommended design criteria and management practices.

CONCLUSIONS

Vegetative filters reduced nutrients, solids, and oxygen-demanding materials from feedlot runoff over 80% on a concentration basis and over 90% on a weight basis. Degree of pollutant removal was dependent upon type of flow (overland or channelized) and length of flow. Channelized flow systems were less effective than overland flow systems, and required much greater flow lengths for a similar degree of treatment. Constituent concentrations approached background levels asymptotically as flow length increased. Even though vegetative filters studied retained over 90% of the measured constituents, discharge concentrations did not meet stream quality standards. Using constituent concentration and flow-length relationships developed, the flow length required to meet standards would be two

Table 5—Constituent concentrations in System 4 settling basin and vegetative filter effluent after a flow distance of 148 m.

Constituent	Settling basin effluent	Vegetative filter effluent	Constituent reduction
	mg/liter		%
NH_4-N	478	70.6	85.2
Total Kjeldahl-N	1,081	120	88.9
Total solids	7,010	1,492	78.7
COD	11,063	871	92.1

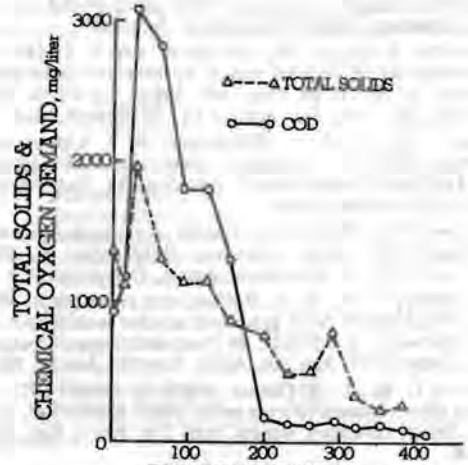

Fig. 7—COD and total solids concentration changes with channelized flow for an individual storm (System 4).

to four times longer than those evaluated. However, the relationships developed did not consider dilution potentials of receiving streams or additional runoff from surrounding areas.

Bacteria levels in feedlot runoff were not greatly reduced by vegetative filters. Fecal coliform levels of 1.05×10^7 100 ml^{-1} in the filter discharge, and 5.75×10^5 100 ml^{-1} in the control-plot discharge receiving no feedlot runoff, were observed. Both of these values were high in relation to current stream standards, which range from 10^2 to 10^3 100 ml^{-1} depending upon location and stream use. Additional research is needed to accurately define bacterial quality for agricultural runoff and to aid in assessing the practicality of current stream standards.

To prevent damage to vegetation and reduced filter effectiveness, settling should be used to remove solids from feedlot runoff before application to filter areas.

Discharge from adequate size vegetation filters occurs only during large runoff events, which coincide with periods of high stream flows. The overall impact of multiple vegetative filter systems on receiving streams appeared to be negligible, but needs to be evaluated in more detail before these can be widely recommended and used. Vegetative filters can provide a satisfactory alternative to zero-discharge systems and result in reduced pollution problems associated with feedlot runoff.

LITERATURE CITED

1. American Public Health Association. 1975. Standard methods for the examination of water and wastewater. 14th ed. APHA, New York.
2. Bendixen, T. W., R. D. Hill, F. T. DuByne, and G. G. Robeck. 1969. Cannery waste treatment by spray irrigation—runoff. J. Water Pollut. Control Fed. 41:385-391.
3. Bremner, J. M., and D. R. Keeney. 1965. Steam distillation of Ammonium, nitrate, and nitrite. Anal. Chim. Acta 32:485-495.
4. Dickey, E. C., and D. H. Vanderholm. 1977. Feedlot runoff holding ponds—nutrient levels and related management aspects. J. Environ. Qual. 6:307-312.
5. Dornbush, J. N., J. R. Andersen, and L. L. Harms. 1974. Qualification of pollutants in agricultural runoff. U.S. Environmental

Protection Agency. Rep. no. EPA-660/2-74-005. U.S. Government Printing Office, Washington, D.C.

6. Edwards, W. M., F. W. Chichester, and L. L. Harrold. 1971. Management of barnlot runoff to improve downstream water quality. p. 48–50. *In* Proc. Int. Symp. Livestock Wastes. 19–22 Apr. 1971. Am. Soc. Agric. Eng., St. Joseph, Mich.
7. Kramer, J. A., D. E. Eisenhauer, R. I. Lipper, and H. L. Manges. 1974. A spray-runoff system with recirculation of treating beef cattle feedlot runoff. Am. Soc. Ag. Eng. Paper no. MC-74-301. St. Joseph, Mich.
8. Mather, R. J. 1969. An evaluation of cannery waste disposal by overland flow spray irrigation. Publications in Climatology 22:2(1–73). C. W. Thornwaite Assoc., Centerton, N.J.
9. McCaskey, T. A., G. H. Rollins, and J. A. Little. 1971. Water quality of runoff from grassland applied with liquid, semiliquid and dry waste. p. 239–242. *In* Proc. Int. Symp. Livestock Waste. 19–22 Apr. 1971. Am. Soc. Agric. Eng., St. Joseph, Mich.
10. Sievers, D. M., G. B. Garner, and E. E. Picket. 1975. A lagoon-grass terrace system to treat swine waste. p. 541–543. *In* Proc. 3rd Int. Symp. Livestock Wastes. Am. Soc. Agric. Eng., St. Joseph, Mich.
11. Sutton, A. L., D. D. Jones, and M. C. Brumm. 1976. A low cost settling basin and infiltration channel for controlling runoff from an open swine feedlot. Am. Soc. Agric. Eng. Paper no. 76-4516. St. Joseph, Mich.
12. Swanson, N. P., C. L. Linderman, and L. N. Mielke. 1975. Direct land disposal of feedlot runoff. p. 255–257. *In* Proc. 3rd Int. Symp. Livestock Wastes. Am. Soc. Agric. Eng., St. Joseph, Mich.
13. U.S. Environmental Protection Agency. 1974. Methods for chemical analysis of water and wastes. USEPA Rep. no. EPA-625/6-74-00. U.S. Government Printing Office, Washington, D.C.
14. Vanderholm, D. H., and E. C. Dickey. 1978. Design of vegetative filters for feedlot runoff treatment in humid area. Am. Soc. Agric. Eng. Paper no. 78-2570. St. Joseph, Mich.
15. Vanderholm, D. H., E. C. Dickey, J. A. Jacobs, R. W. Elmore, and S. L. Spahr. 1979. Livestock feedlot runoff control by vegetative filters. U.S. Environmental Protection Agency Rep. no. EPA-600/2-79-143. U.S. Government Printing Office, Washington, D.C.
16. Willrich, T. L., and J. O. Boda. 1976. Overland flow treatment of swine lagoon effluent. Am. Soc. Agric. Eng. Paper no. 76-4515. St. Joseph, Mich.

Utilization Efficiency of Nitrogen from Sewage Effluent and Fertilizer Applied to Corn Plants Growing in a Clay Soil[1]

A. FEIGIN, SALA FEIGENBAUM, AND HEDVA LIMONI[2]

ABSTRACT

Effects of irrigation with secondary municipal sewage effluents on N availability in a fertilized soil were studied in a greenhouse experiment using ^{15}N as a tracer. Corn (*Zea mays*) was grown in a clay soil with ammonium-N added as solid fertilizer, sewage effluent, and a mineral solution.

Between 55 and 69% of the ammonium sulfate-^{15}N was taken up by the corn plants. Between 21 and 32% of the fertilizer-N was recovered as organic-N in the soil after 43 days, while negligible amounts of exchangeable-NH_4 and NO_3 were detected. Losses of ammonium sulfate-N applied to the soil before seeding, probably through denitrification, ranged between 6 and 15%. Similar results were obtained whether the fertilized soil was irrigated with demineralized water, sewage effluent, or a mineral solution simulating the mineral composition of the sewage effluent.

About 61% of the tagged ammonium-N applied as sewage effluent was taken up by the corn plants, and 14% was immobilized in the organic fraction of soil. About 24% of the effluent-tagged-ammonium-N was lost, apparently through both denitrification and volatilization. The corresponding loss from the mineral-solution-tagged-N was about 17%. The simultaneous application of C and N by sewage effluents was probably responsible for the increased losses of N through denitrification found in the effluent-tagged-ammonium-N treatment.

Recovery of N, in plant and soil, from ammonium sulfate incorporated into the soil before planting was somewhat greater than that of sewage effluent ammonium-N, and was not affected by irrigation with sewage effluent.

Additional Index Words: **wastewater-N, ^{15}N.**

Feigin, A., S. Feigenbaum, and H. Limoni. 1981. Utilization efficiency of nitrogen from sewage effluent and fertilizer applied to corn plants growing in a clay soil. J. Environ. Qual. 10:284–287.

Partially treated sewage effluents contain considerable amounts of N, mainly—and sometimes almost solely—as ammonium (Lance, 1972; Bouwer and Chaney, 1974). These secondary effluents supply available-N to plants (Feigin et al., 1978). The efficient use of effluent-N by plants means smaller outlays on fertilizers and a reduced pollution hazard for ground water. Attainment of this objective is subject to a basic understanding of N transformations in effluent-irrigated fields.

Effluents contain various organic compounds (Hunter and Kotalyk, 1974) and elements (e.g., NH_4-N and PO_4-P) that do not regularly appear in irrigation water. Moreover, sodium, chloride, and bicarbonate than in irrigation water (Feigin et al., 1978). Therefore, it may be anticipated that irrigation with sewage effluents could probably influence the N cycle in soils, including N uptake by the plants and N losses from the soil by denitrification due to the organic-C that from the soil by denitrification due to the organic-C that can be used as an energy source for denitrifying bacteria (Bouwer and Chaney, 1974). Losses by volatilization of ammonia are also to be expected (Lance, 1972).

The purpose of this work was to study: (i) the effect of effluent irrigation on the utilization of fertilizer-N by plants and on N losses from the soil; and (ii) the fate of effluent-applied NH_4-N and its uptake by plants.

[1] Contribution of the Agric. Res. Org., The Volcani Center, Bet Dagan, Israel. No. 202-E, 1980 Series. Received 11 July 1980.

[2] Soil Scientists, Inst. of Soils and Water, A.R.O., and Agronomist, formerly with the Ministry of Agric., Ext. Serv., Qiryat Malakhi, Israel, respectively.

Attachment 14:

Vegetative Treatment Systems for
Management of Open Lot Runo
Review of Literature
(Koelsch et al. 2006)

Vegetative Treatment Systems for Management of Open Lot Runoff: Review of Literature

Richard K. Koelsch* Jeffrey C. Lorimer† Kyle R. Mankin‡

*University of Nebraska - Lincoln, rkoelsch1@unl.edu
†Iowa State University, Ames
‡Kansas State University, Manhattan, Kansas

VEGETATIVE TREATMENT SYSTEMS FOR MANAGEMENT OF OPEN LOT RUNOFF: REVIEW OF LITERATURE

R. K. Koelsch, J. C. Lorimor, K. R. Mankin

ABSTRACT. *Runoff from open lot livestock systems (beef and dairy) defined as Concentrated Animal Feeding Operations (CAFO) must be controlled by systems designed and managed to prevent the release of manure-contaminated runoff for storms equal to or less than a 25-yr, 24-h design storm. This performance standard has been attained for open lot systems with some combination of clean water diversion, settling basins, runoff collection ponds, and irrigation systems (baseline system).*

An alternative approach is to rely on overland flow and infiltration into cropland with perennial forage or grasses for treatment of open lot runoff. Such vegetative systems have been researched since the late 1960s. This article reviews the research literature on vegetative treatment systems (VTS) for managing open lot runoff summarizing available science on system performance, design, and management.

Based upon this review of the literature, the following conclusions are drawn about the application of VTS to manage runoff from open lot livestock production systems:

(1) Substantial research (approximately 40 identified field trials and plot studies) provides a basis for understanding the performance of VTS. These performance results suggest that a vegetative system consisting of a settling basin and VTA or Vegetative Infiltration Basin (VIB) has the potential to achieve functional equivalency to conventional technologies.

(2) The existing research targeting VTS is confined to non-CAFO applications, likely due to past regulatory limits. Unique challenges exist in adapting these results and recommendations to CAFO applications.

(3) The pollutant reduction resulting from a VTS is based upon two primary mechanisms: 1) sedimentation, typically occurring within the first few meters of a VTS, and 2) infiltration of runoff into the soil profile. Systems relying primarily on sedimentation only are unlikely to perform equal to or better than baseline technologies. System design based upon sedimentation and infiltration is necessary to achieve a required performance level for CAFO application.

(4) Critical design factors specific to attaining high levels of pollutant reduction within a VTS include pre-treatment, sheet flow, discharge control, siting, and sizing. Critical management factors include maintenance of a dense vegetation stand and sheet flow of runoff across VTA as well as minimization of nutrient accumulation.

Keywords. *Vegetative Treatment Systems, Vegetative Infiltration Basin, Feedlot, Runoff.*

Runoff from open lot livestock production systems continues to be a contributor to surface and groundwater impairment. Vegetative Treatment Systems (VTS) applied to open lot systems represent an alternative technology that may potentially achieve significant pollutant reduction. [The terms VTS and VTA will both be used. Vegetative Treatment Area (VTA) applies to a cropped area with perennial grass or forage specifically designed to manage runoff from an open lot livestock facility. VTS will refer to the combination of treatment components including a VTA or Vegetative Infiltration Basin (VIB) and other possible treatment components (e.g. solids settling).]

The United States Environmental Protection Agency (USEPA), National Pollutant Discharge Elimination System (NPDES) establishes a technology-based standard that defines the acceptable performance for runoff control on permitted facilities. A VTS has the potential for providing control of pollution from feedlot runoff that is “functionally equivalent” to the conventional impoundment and land application system for Concentrated Animal Feeding Operations (CAFO).

The 2003 final federal rule for the NPDES Permit Regulation and Effluent Limitation Guidelines (ELG) and Standards for CAFOs (Federal Register, 2003) sets the 25-yr, 24-h storm technology standard for baseline systems (runoff holding facilities dewatered by irrigation systems). The federal rule also opens the door for alternative technologies (such as a VTS) if they can be documented to achieve equal or better pollutant control performance as the baseline technology. A “site-specific comparison” provision within these regulations places the burden of proof on the individual producer for comparing the baseline and alternative technology for individual farms.

The objective of this article is to summarize the knowledge base for VTS. This literature review provided a foundation for the development of a USDA Natural Resource Conservation Service guidance document on VTS siting, design, and management. At the time this review was

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The authors are **Richard K. Koelsch, ASABE Member Engineer,** Livestock Environmental Engineer, Associate Professor, Department of Biological Systems Engineering, University of Nebraska, Lincoln, Nebraska; **Jeffery C. Lorimor, ASABE Member Engineer,** Extension Waste Management Specialist, Associate Professor Emeritus, Department of Agricultural and Biosystems Engineering, Iowa State University, Ames, Iowa; and **Kyle R. Mankin, ASABE Member Engineer,** Associate Professor, Department of Biological & Agricultural Engineering, Kansas State University, Manhattan, Kansas. **Corresponding author:** Richard K. Koelsch, Department of Biological Systems Engineering, University of Nebraska-Lincoln, 213 L. W. Chase Hall, Lincoln, NE 68583-0726; phone: 402-472-4051; fax: 402-472-6338; e-mail: rkoelsch1@unl.edu.

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prepared, a draft of this guidance document was available at http://www.heartlandwq.iastate.edu/manure.

Performance Models for VTS

An Iowa State University VTS software modeling tool has been designed to predict the performance of a site-specific VTS to meet the Voluntary Alternative Performance Standards (see Introduction) of the new EPA CAFO rules (Wulf et al., 2003). The VTS model performs site-specific modeling using daily weather inputs to estimate the performance of site-specific feedlots and VTS designs. The model uses weather data for 25-yr period to compare performance of the alternative VTS (median outflow for 25-yr period times pollutant concentration) with baseline containment system performance for the same site. It follows procedures outlined by the Voluntary Alternative Performance Standards provisions of the CAFO regulations (Federal Register, 2003). At the time this literature review was prepared, a peer review process for the model was completed and the Iowa Department of Natural Resources has accepted model results as acceptable documentation for an NPDES permit application.

Another systematic model was develop by a collaboration of several Minnesota agencies to identify appropriate applications of VTSs to feedlot runoff (Brach, 2003; Minnesota Pollution Control Agency, 2003). They developed a standard identifying five levels of control (including VTA) and appropriate application of those five levels to individual situations based upon farm size and proximity to water. The team has developed a model, FLEVAL: An Evaluation System to Rate Feedlot Pollution Potential, to objectively evaluate feedlot pollution potential (http://www.bwsr.state.mn.us/outreach/engineering/fleval.html). Overcash et al. (1981) describes an additional model for predicting performance of a vegetative system located down-gradient from a manured land application site.

In-Field VTS Performance

Literature review of performance data from 16 research citations reporting 40 sets of performance data under field conditions are listed in table 1. An additional 16 research citations reporting 58 sets of performance data under simulated conditions are included in table 2. Results are for both VTAs and Vegetative Infiltration Basins (VIB). The preponderance of the performance data is for a VTA. VTA efficiency was estimated from the literature by comparing the reduction of pollutant concentration and/or mass entering and leaving the VTA. Pollutants of concern in livestock runoff include solids, nitrogen, phosphorus and pathogens.

VTAs provide an opportunity for reduction of pollutants in runoff through two primary mechanisms: 1) sedimentation, typically occurring within the first few meters of a VTA, and 2) infiltration of runoff into the soil profile (Pope and Stolenberg, 1991). The soil system also provides a physical structure and biological environment for treatment of pollutants including filtration (e.g., restricting movement of most protozoa and bacteria), immobilization (e.g., soil cations immobilizing ammonium), aerobic processes (e.g., conversion of organic compounds to water and carbon dioxide), and anaerobic process (e.g., conversion of nitrates to nitrogen gas). The VTA also allows the uptake of nutrients by plants (Fajardo et al., 2001).

Type of VTA

Ikenberry and Mankin (2000) defined a VTA as a band of planted or indigenous vegetation situated down-slope of cropland or animal production facilities that provides localized erosion protection and contaminant reduction. Planted or indigenous vegetation is defined as pasture, grassed waterways, or cropland that is used to treat runoff through settling, filtration, adsorption, and infiltration. Murphy and Harner (2001) identified four primary approaches used in plant-based treatment systems:

- Grass filters can be designed with a 1% to 4% slope and 61 m (200 ft) of filtering length per 1% slope. Total area should be designed to match crop nitrogen uptake with estimated N in runoff. Sheet flow across filtering slope is necessary, typically requiring laser-guided land leveling equipment.
- Constructed wetlands have been applied to open lot runoff. Design and management is challenged by the intermittent flow from open lots. The authors suggest that seasonal open lots used for winter livestock housing and empty during the summer may be a preferred application for constructed wetlands.
- Infiltration basins are a containment type of system with a 30- to 60-cm (12- to 24-in.) berm placed around the vegetated area. They can be designed as discharging or non-discharging systems. A vegetative area necessary to infiltrate design runoff within 30 to 72 h must be considered in the sizing of an infiltration basin.
- Terraces, similar to infiltration basins, have been used to contain runoff on sloped areas. Both overflow and cascading terraces have been used. Overflow terraces move runoff from one terrace to an adjacent terrace at a lower elevation by cascading of runoff over the terrace top or by plastic tile drains. Serpentine terraces move runoff back and forth across the face of a slope. In both situations, the upper terrace is typically used for solids settling.

Flow within VTA

VTAs can be classified as either channelized or sheet flow (Dickey and Vanderholm, 1981a). Their work showed that "the channelized flow system required a flow length over five times longer than the overland flow systems to achieve a similar concentration reduction." Dillaha et al. (1988) studied concentrated flow effects on removal efficiencies and found that lower removal efficiencies occurred in VTAs with concentrated flows than in VTAs with shallow, sheet flow.

Channelized surface flow in VTAs results in non-uniform nutrient and hydraulic loading of VTA thereby reducing system performance and increasing soil erosion. Sheet flow systems allow a uniform loading of runoff (across the width of the VTA) at a relatively shallow depth (<4 cm). Uniform flow results in a slower velocity, which allows sediment and nutrients to be trapped by the vegetation and adsorbed by the soil. Dickey and Vanderholm (1981b) showed progressively better removal of TKN and ammonium (NH_4^+) with VTA length for a 100-head dairy and 500-head beef lot (fig. 1). Lim et al. (1997) and Chaubey et al. (1995) demonstrated that a first-order exponential relationship better described the interaction between VTA length and pollutant transport.

Table 1. Summary of VTA performance (no pre-treatment performance included in values) on commercial or research livestock facilities. This table was originally developed by Ikenberry and Mankin (2000) and updated with additional references. Reductions are either in concentration or mass for individual studies as indicated by the last column.

Study Description			VTA Information							Percent Reduction													
Reference	Summary	Study Period	Pollutant Source	Settling Basin	Length (m)	AR (1)	Slope (%)	Vegetation	Soil	TS	TSS	BOD_5	COD	Total N	TKN	NH_4-N (2)	NO_3-N (3)	Total P	Ortho-P	FC	FS	E. Coli	(4)
Adam et al., 1986	Settling basin and vegetative filter operated below 75 head feedlot in Quebec cold climate.	70 mm on 9/27/86	75 hd beef feedlot	Y	108	2.3	0.75%	Kentucky blue grass	Sandy loam	99.5			99.9		99.9	99.9		<0.01 kg	<0.01 kg				m
			For 21 additional snow and rainfall feedlot runoff events between September 1985 and April 1986, 100% treatment efficiency occurred with no VTS runoff observed.																				
Barker and Young, 1984	Milking center wastewater and open lot runoff from a 54 cow dairy was directed to settling basin and VTA. Four earthen berms located at 9 m intervals were designed to create a cascading type system. System was monitored over two years	5/82 – 5/84	Milking Center wastewater only	Yes	91 Width =23	--	10	Orchard grass and foxtail at upper end. Hairy crabgrass in drier areas.	VTA only	90	--	--	96	97	97	99	82	98	--	--	--	--	c
									VTA+ Basin	95	--	--	98	99	99	98	81	98	--	--	--	--	c
									VTA only	99	--	--	100	100	100	100	99	100	--	--	--	--	m
									VTA+ Basin	100	--	--	100	100	100	100	99	100	--	--	--	--	m
			Milking center waste water and paved dairy lot runoff	Yes	91 Width =23	--	10	Orchard grass and foxtail at upper end. Hairy crabgrass in drier areas.	VTA only	45	--	--	56	46	46	55	-68	68	--	--	--	--	c
									VTA+ Basin	65	--	--	65	60	60	40	-17	68	--	--	--	--	c
									VTA only	97	--	--	98	97	97	98	92	98	--	--	--	--	m
									VTA+ Basin	98	--	--	98	98	98	97	94	98	--	--	--	--	m
Dickey & Vanderholm, 1981a	4 different VTA systems after settling basins at actual feedlots	17 months	Dairy farm	Yes	91	1.00	0.5	reed canary, brome grass, and orchard grass	--	73.1	--	--	85.4	--	80.1	86.2	--	78.2	--	--	--	--	c
	*Influent concentrations estimated from a similar site		450 head beef feedlot	Yes	61	0.70	2	fescue alfalfa mix	sandy	63.1	--	--	81.2	--	71.1	71.5	--	--	--	--	--	--	c
	*Channelized flow VTA (serpentine terrace channel)		500 head beef feedlot	Yes	533	--	0.25	--	--	79.7	--	--	86	--	83.1	83.4	--	--	--	--	--	--	c
	*Vegetated terrace channel and grassed waterway		480 head swine finisher	Yes	148	--	0.25	garrison creeping foxtail	--	78.7	--	--	92.1	--	88.9	85.2	--	--	--	--	--	--	c
Fausey et al., 1988	Infiltration basin used with 56 head of beef cattle on concrete lot	3 year study	56 head beef feedlot	Yes	27.5 Width =6m	0.7	1	Reed canary grass	Silt loam					Org N									
								1) Drain tile with slope		61-81	--	--	69-87	69-85	--	69-92	(5)	62-91	73-93	--	--	--	c
								2) Drain tile across slope		55-83	--	--	59-86	59-87	--	56-89	(5)	63-89	67-90	--	--	--	c
Edwards et al., 1986	Infiltration basin used with 56 head of beef cattle on concrete lot	3 year study	56 head beef feedlot	Yes	27.5 Width =6m	0.7	1	Reed canary grass	Silt loam					Org N									
								1) VTA and Settling		82	--	--	85	80		50	-643	--	80	--	--	--	c
								Basin		80	--	--	83	78		50	-940	--	74	--	--	--	m
								2) VTA only		66	--	--	69	70		73	-733	--	77	--	--	--	c
										61	--	--	65	66		72	-1150	--	70	--	--	--	m
Harner and Kalita, 1999	300-head feedlot runoff is directed to settling basin and VTA.	2 years	300-head beef feedlot	Yes	427	0.97	0.3-4	Brome	silty clay loam	--	65	--	--	26	--	44	2	14	18	--	--	--	c
Keaton, 1998										--	76	--	--	50	--	63	34	42	45	--	--	--	m
	300-head beef feedlot discharge to settling basin and VTA	2 years	350-head beef feedlot	Yes	239	0.23	0.5-2	Brome	sandy loam	--	78	--	--	73	--	74	95	71	64	--	--	--	c
										--	83	--	--	59	--	74	87	52	44	--	--	--	m
Komor and Hansen, 2003	Settling basin and VTA were placed below two cattle feedlots and monitored for seven storm events	1995-96	200 head capacity lot (35 cattle during test)	Yes	79	0.2	1.2	Grass	Silt loam	1.5 cm rainfall on 5/14/96					85	61	--	82	25	62	--	--	m
			225 head feedlot	Yes	58	0.2	0.5	Grass	loam	9.1, 3.6, and 0.6 cm rainfalls on7/27/96, 6/2/96, & 6/27/98					35-75	35-80	--	Susp. P 25-75	15-75	20-80	--	--	m

Table 1 (continued). Summary of VTA performance when placed on commercial or research livestock facilities.

Study Description				VTA Information						Percent Reduction													
Reference	Summary	Study Period	Pollutant Source	Settling Basin	Length (m)	AR (1)	Slope (%)	Vegetation	Soil	TS	TSS	BOD_5	COD	Total N	TKN	NH_4-N (2)	NO_3-N (3)	Total P	Ortho-P	FC	FS	E. Coli	(4)
Lorimor et al., 2003	Runoff from concrete open lot beef facility is directed to settling basin, totally bermed infiltration basin (IB), and constructed wetland (CW)	1997 to present – data based upon five years	380 head concrete beef cattle facility	Yes	108	0.18	0	IB: Reed canary grass. CW: Common cattails	Loam														
									IB:	65	--	--	--	80	--	81	-87	77	--	--	--	--	c
									IB+CW:	71	--	--	--	85	--	83	-43	83	--	--	--	--	c
									IB+CW:	93	--	--	--	97	--	98	86	95	--	--	--	--	m
										Most mass flow reduction occurred in infiltration basin.													
Mankin and Okoren, 2003	300 head heifer feedlot with runoff directed to settling basin (1[st] stage) and VTA (2[nd] stage).	5/01-5/02	300 head dairy heifer feedlot	Yes	150	--	2	Fescue	Silt Loam Mass Reductions at:			TDS											
									30 m	--	93	74	--	77	--	--	--	84	--	84	--	91	m
									150 m	--	95	68	--	81	--	--	--	79	--	85	--	90	m
Paterson et al., 1980	Milking center waste and barnyard runoff from 70 cow dairy studied for five year period	5 years	Natural rainfall	Yes	36	--	3.4	Tall fescue	silt loam	--	71	42	--	--	--	38	incr.	7	--	--	--	--	c
			Snow melt							--	84	77	--	--	--	78	-40	32	--	--	--	--	c
			Perched water table							--		99	--	--	--	97	incr.	98	--	--	--	--	c
Schellinger & Clausen, 1992	Runoff from paved dairy lot to detention pond then VTA subject to natural rainfall	18 months	Dairy barnyard	Yes	22.9	0.27	2	fescue, bluegrass, and ryegrass mix	--	--	33	--	--	--	18	15	--	12	6	--	--	--	m
Williamson, 1999	Describes and compares design and performance of 4 VTA in Kansas for feedlot	5 months 5/98	350 head beef feedlot	Yes	239	0.23	1.2	brome grass	sandy loam	--	--	--	--	61.5	--	--	--	28.6	--	78.9	--	79.3	c
*Same study, different VTA location and design		11/98 for all sites	300 head beef feedlot	Yes	427	0.97	0.75	brome grass	silty clay loam	--	--	--	--	63.7	--	--	--	56.8	--	76.5	--	78.2	c
*Same study, different VTA location			300 head beef feedlot	Yes	213	0.36	2	fescue	silt loam	--	--	--	--	19	--	--	--	13	--	36	83	--	c
*Same study, different VTA location			200 head beef feedlot	Yes	137	0.59	0.6	brome grass	loam	--	--	--	--	52.8	--	--	--	74.2	--	90.3	--	88.4	c
Woodbury et al., 2002; Woodbury et al., 2003a; Woodbury et al., 2003b	Settling basin and VTA collects open lot runoff from beef cattle facility	1997-2003	600 head beef feedlot	Yes	200	3	0.5	brome grass	--	No observed discharge of water below root zone for two years or as surface water from VTA for 5 years.													m

1. AR = Area Ratio = (VTA Area) / (Feedlot Drainage Area).
2. The label NH_4–N is used to represent the sum of ammonium (NH_4) nitrogen and ammonia (NH_3) nitrogen.
3. Negative nitrate values indicate an increase in nitrate concentration.
4. m = reductions calculated on a mass basis, c = reductions calculated on a concentration basis
5. NO_3-N before VTA less than 1 ppm, NO_3-N after VTA is 76 and 64 ppm for drain tile laid with and across slope, respectively.

Table 2. Summary of VTA performance (no pre-treatment performance included in values) under simulated conditions[1]. Reductions are in concentration or mass for individual studies as indicated by the last column.

Study Description						VTA Information		Percent Reduction													
Reference	Summary	Intensity	Length (m)	AR (2)	Slope (%)	Vegetation	Soil	TS	TSS	BOD_5	COD	Total N	TKN	NH_4-N (3)	NO_3-N (4)	Total P	Ortho-P	FC	FS	E. Coli	(5)
Coyne et al., 1998	4 VTA plots placed after poultry manure amended pasture area	64 mm/hr	4.5	0.25	9	Tall fescue and Kentucky blue grass	silt loam	96	--	--	--	--	--	--	--	--	--	75	68	--	c
		64 mm/hr	9	0.66	9	fescue bluegrass mix	silt loam	98	--	--	--	--	--	--	--	--	--	91	74	--	c
Chaubey et al., 1994	Swine manure applied to VTA subject to simulated rainfall	50 mm/hr	3	1.00	3	fescue	silt loam	--	--	--	--	--	65	71	--	67	65	--	--	--	m
			6	2.00				--	--	--	--	--	69	83	--	71	71	--	--	--	m
			9	3.00				--	--	--	--	--	89	96	--	87	89	--	--	--	m
			15	5.00				--	--	--	--	--	86	99	--	91	93	--	--	--	m
			21	7				--	--	--	--	--	87	99	--	92	94	--	--	--	m
Chaubey et al., 1995	Poultry manure applied to VTA subject to simulated rainfall	50 mm/hr	3	1.00	3	fescue	silt loam	--	--	--	--	--	39	47	--	40	39	--	--	--	m
			6	2.00				--	--	--	--	--	54	70	--	58	55	--	--	--	m
			9	3.00				--	--	--	--	--	67	78	--	74	71	--	--	--	m
			15	5.00				--	--	--	--	--	76	94	--	87	85	--	--	--	m
			21	7				--	--	--	--	--	81	98	--	91	90	--	--	--	m
Dillaha et al., 1988; Dillaha et al., 1986	Simulated feedlot and rainfall	50 mm/hr	4.6	0.25	11	orchard grass	silt loam	--	87	--	--	61	64	34	-36	63	-20	--	--	--	c
			9.1	0.50	11			--	95	--	--	77	80	69	4	80	30	--	--	--	c
			4.6	0.25	16			--	76	--	--	67	69	-21	3	52	-108	--	--	--	c
			9.1	0.50	16			--	88	--	--	71	72	-35	17	57	-51	--	--	--	c
	*concentrated flow		4.6	0.25	5			--	31	--	--	0	1	1	-82	2	-3	--	--	--	c
	*concentrated flow		9.1	0.50	5			--	58	--	--	7	9	-11	-158	19	31	--	--	--	c
Edwards et al., 1983	VTA test plots after settling basin, natural rainfall, 56 head of beef cattle on concrete lot	--	30	Width =2 m	2	fescue	silt loam	87	--	81	89	83	--	--	--	84	--	--	--	--	m
Fajardo et al., 2001	Plot study comparing fallow vs. vegetated filter strip	17 mm/hr for fallow 110 mm/hr for VTA	30	--	4.3-5.1	tall fescue	fine silt	--	--	--	--	--	--	94– 99	--	--	--	No change	--	--	c
Goel et al., 2004	A dairy slurry and water mix was applied to upper end of three lengths of VTA and three vegetative covers were tested	1.2 L/s applied to upper end of filter strip	5	Width =1.2 m	3	Perennial rye	Guelph loam	--	86	--	--	91	--	--	--	88	50	61	--	66	c
			10					--	86	--	--	90	--	--	45	88	44	53	--	36	c
			5			Mixed grass species		--	87	--	--	87	--	--	25	87	44	15	--	-26	c
			10					--	91	--	--	84	--	--	16	86	48	52	--	58	c
			5			Kentucky blue grass		--	89	--	--	92	--	--	13	89	50	68	--	-130	c
			10					--	91	--	--	95	--	--	35	92	58	74	--	77	c
			5			Perennial rye		--	90	--	--	94	--	--	3	91	64	71	--	67	m
			10					--	94	--	--	95	--	--	67	95	77	77	--	64	m
			5			Mixed grass species		--	91	--	--	89	--	--	49	90	66	56	--	58	m
			10					--	95	--	--	91	--	--	52	92	75	75	--	82	m
			5			Kentucky blue grass		--	97	--	--	98	--	--	75	97	85	91	--	39	m
			10					--	99	--	--	100	--	--	96	100	97	99	--	99	m
Hawkins et al., 1998	WW pumped from swine lagoon to VTA; runoff and percolate analyzed	--	6.1	--	5	Bermuda and ryegrass mix	loamy sand	14	--	--	52	--	3	1	47	22	--	--	--	--	c
								5	--	--	81	--	60	58	54	75	--	--	--	--	m
				--	11			-557	--	--	14	--	33	33	-834	-11	--	--	--	--	c
								37	--	--	92	--	93	93	-59	92	--	--	--	--	m

Table 2 (continued). Summary of VTA performance under simulated conditions[1].

Study Description							VTA Information						Percent Reduction								
Reference	Summary	Intensity	Length (m)	AR (2)	Slope (%)	Vegetation	Soil	TS	TSS	BOD_5	COD	Total N	TKN	NH_4-N (3)	NO_3-N (4)	Total P	Ortho-P	FC	FS	E. Coli	(5)
Lim et al., 1997	Simulated pasture and rainfall	10 cm/hr	6.1	0.50	3	fescue	silt loam	23.6	70	--	--	--	78	18.6	-498.2	76.1	74.5	100	--	--	m
			12.2	1.00				40.8	89.5	--	--	--	89.5	52.8	-140.1	90.1	87.8	100	--	--	m
			18.3	1.50				69.8	97.6	--	--	--	95.3	68	-96.7	93.6	93	100	--	--	m
Prantner et al., 2001	Lab scale study of raw swine manure applied to soil infiltration areas	--	--	--	--	No vegetation	Clarion loam soil	1998 Undiluted swine manure					94	--	Inc.	85	--	--	--	--	c
								3 parts manure + 1 part water					87	--	from 1	78	--	--	--	--	c
								1999 Undiluted swine manure					96	--	to 77	98	--	--	--	--	c
								3 parts manure + 1 part water					94	--	mg/l	97	--	--	--	--	c
Sanderson et al., 2001	Manure application to grassland with VTA down gradient.	--	16.4	1.0	1	Switch grass	Fine sandy loam	--	--	--	25-44	--	--	--	--	--	47-76	--	--	--	m
Schwer & Clausen, 1989	VTA test plot, natural rainfall, milk house waste water pumped to VTA	--	26	--	2	fescue, ryegrass, bluegrass mix	sandy loam	--	92	--	--	--	83	46	--	86	82	--	--	--	c
								--	97	--	--	--	93	70	--	92	90	--	--	--	c
	*Same VTA, subsurface flow analysis	Hi Rate: 20 to 27 cm/week	--	--	--		Loam (surface) and clay loam (sub-surface)	Turbidity				--									
						Fescue		31	--	31	15	--	--	26	--	14	--	13	--	--	c
		Low Rate: 6 to 16 cm/week				Low Rate			--	--	67	--	--	62	--	62	--	--	--	--	m
						High Rate			--	--	50	--	--	43	--	46	--	--	--	--	m
Srivastava et al., 1996	Nine control VTA plots placed after manure amended pasture		3.1-18.3	3/1	3	Fescue	Silt loam	--	--	--	30	--	67	75	--	66	--	--	--	--	m
				1/1				--	--	--	11	--	44	39	--	36	--	--	--	--	m
				1/3				--	--	--	13	--	21	27	--	26	--	--	--	--	m
											(6)										
Young et al., 1980	Rainfall simulator applied 25-year, 24-hour storm to VTA plots containing corn, orchard grass, sorghum-Sudan grass mix over 2 year test period.	6.35 cm/hr for 71 minutes					Runoff volume reduction:	Sediment												Total Coli-forms	
			27	2	4	Corn	98%	93	--	--	--	--	98	98	95	98	100	--	--		c
			27	2	4	Orchard grass	81%	66	--	--	--	--	69	65	9	76	77	55	72	53	c
			27	2	4	Sorghum-Sudan grass mix	61%	82	--	--	--	--	50	47	-81	48	42	83	68	81	c
			21	1.6	4	Corn	66%	81	--	--	--	--	79	78	-441	74	41	--	--	71	c
			21	1.6	4	Oats	41%	75	--	--	--	--	45	33	-1130	50	-3	--	--	70	c
Willrich & Boda, 1976	VTA test plots, natural rainfall, swine lagoon effluent pumped to VTA	--	30.5	--	3	fescue	clay loam	--	--	31	15	--	--	26	--	--	14	31	--	--	c
			6.1	--	5	Bermuda and ryegrass mix	loamy sand	59	--	--	81	--	60	58	54	75	--	--	--	--	--
	*Same source of wastewater pumped to VTA with different slope		6.1	--	11	Bermuda and ryegrass mix	sandy loam	-557	--	--	14	--	33	33	-834	-11	--	--	--	--	--
			6.1	--	11	Bermuda and ryegrass mix	sandy loam	37	--	--	92	--	93	93	-59	92	--	--	--	--	--

1. Simulated conditions refer to studies conducted under simulated rainfall conditions and/or studies where the VTA is a bench scale or small plot scale facility.
2. AR = Area Ratio = (VTA Area) / (Feedlot Drainage Area).
3. The label NH_4–N is used to represent the sum of ammonium (NH_4) nitrogen and ammonia (NH_3) nitrogen.
4. Negative nitrate values indicate an increase in nitrate concentration.
5. m = reductions calculated on a mass basis, c = reductions calculated on a concentration basis.
6. Data represents total organic carbon as measured by Srivastava et al., 1996.

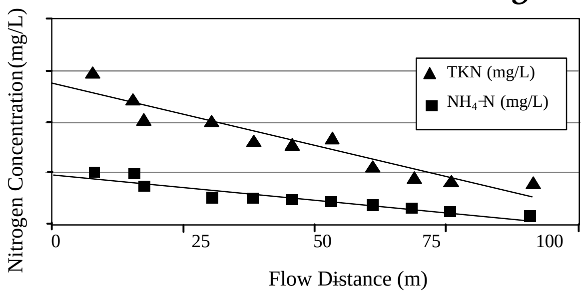

Figure 1. Effect of VTA length on TKN and ammonium-N reduction (Dickey and Vanderholm, 1981a).

Solids Removal

Extensive research has been conducted on solids removal by VTA. Total solids are commonly reduced by 70% to 90% (tables 1 and 2). Variations occur due to site-specific conditions such as vegetation, slope, soil type, size and geometry of VTA, and influent solids concentration. When receiving runoff directly from a feedlot, VTAs remove most solids within the first few meters of the filter strip. Coyne et al. (1998) found most reductions in concentration occurred in the first 4.5 m. Chaubey et al. (1995) showed improved P removal effectiveness from swine lagoon effluent with increased VTA length up to 9 m (30 ft). Solids reduction would likely perform in a similar manner. Chaubey et al. (1995) noted that removal of total suspended solids and chemical oxygen demand in VTA increased for lengths up to 3.1 m. This quick reduction can be attributed to a significant reduction in flow velocity resulting in settling of solids.

Nitrogen Removal

The most common gauges of nitrogen content in surface runoff include total nitrogen (TN), total Kjeldahl nitrogen (TKN), ammonium nitrogen (NH_4-N) [The term ammonium nitrogen (NH_4-N) is used to represent the sum of ammonium (NH_4) nitrogen and ammonia (NH_3) nitrogen], and nitrate (NO_3) (Ikenberry and Mankin, 2000). Removal of TN, TKN, and NH_4-N by VTA, has been shown to attain or exceed 80%. Chaubey et al. (1995) noted that removal of ammonium nitrogen and TKN in VTA increased for lengths up to 15.2 and 9.2 m, respectively. Overall properly designed and managed VTAs are very effective, averaging approximately 70% nitrogen removal (Ikenberry and Mankin, 2000). Some VTA performance results have suggested 100% reduction in situations where soil infiltration of runoff prevented any effluent from leaving the vegetative area. Nitrate (NO_3) removal has typically been much lower. In some studies NO_3 increased from near-zero levels typical of most anaerobic feedlot runoff levels to concentrations commonly less than the 10-ppm drinking water standard during flow through the VTA. However, test results illustrating an increase in concentration of nitrate can be accompanied by total nitrate mass reductions due to reduction in runoff volume resulting from soil infiltration (Barker and Young; 1984).

The authors have standardized the results of multiple studies over the past 25 plus years (tables 1 and 2) to show the relationships of total N and P reduction to the ratio of VTA area to feedlot drainage area (DA). As much as an 80% reduction in total N and P was observed (fig. 2). At smaller VTA to DA ratios, reported performance levels appear to be more highly variable with multiple performance results producing less than 50% reductions in N and P. For results to consistently exceed a 50% reduction, a VTA to DA ratio of 2 or greater was necessary.

Phosphorous Removal

Because the majority of the phosphorous in feedlot runoff is adsorbed to solids particles, total phosphorous removal is directly related to solids removal efficiencies. Phosphorous removal rates have ranged from 7% to 100% (table 1), averaging about 70%. Chaubey et al. (1995) also noted that removal of dissolved and total phosphorus in VTA increased for lengths up to 15.2 and 9.2 m, respectively. The authors have standardized the multiple studies for P removal in figure 2.

Pathogen Removal

Research on fecal coliform (FC) removal by VTAs provides a less clear picture of performance. Reported values vary greatly and few studies have been conducted on large-scale VTAs. Fajardo et al. (2001) report FC removal rates between 64% and 87% when using small-scale simulated runoff events with stockpiled manure. Lim et al. (1997) found that all fecal coliforms were removed in the first 6.1 m of a VTA used to treat runoff from a simulated pasture. Average FC removal in the studies reported was 76.6% (Ikenberry and Mankin, 2000). A model for describing fecal pathogens in vegetative filter strips was being assembled by Zhang et al. (2001) and linked to an existing model of VTA hydrology and sediment transport, although data were not available to test the model at the time this research article was prepared.

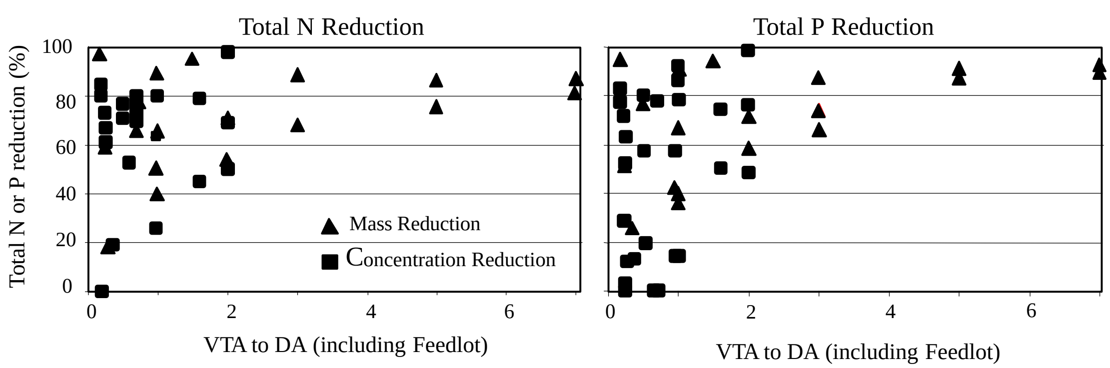

Figure 2. Nutrient removal by VTA based upon VTA to drainage area ratio for references listed in tables 1 and 2.

Vegetative Infiltration Basin (VIB)

Vegetative infiltration basins are VTS systems with additional berms that force infiltration of runoff through a soil filter and prevent surface-water discharges. As runoff infiltrates the soil, aerobic nitrification occurs, converting ammonium to nitrate by the aerobic bacteria *Nitrosomonas* and *Nitrobacter* (Prantner et al., 2001). In addition, phosphorus complexes with minerals (i.e. Ca, Mg, Fe) in the soil bound in the profile. Field drainage tile is commonly used to intercept the filtrate and carry it to an additional treatment system, such as a constructed wetland or VTA (Lorimor et al., 2003; Fausey et al., 1988). A VIB has a smaller surface area (1/6 to 1/12 of most standard VTA designs) and no direct surface-water discharge. Infiltration basins also slow the flow rate exiting the infiltration basin during the storm event and delay much of the discharge until after the event, which enhances the potential for successful treatment in later treatment components, such as a VTA (Lorimor et al., 2003). Preferential flow through the soil filter may be a potential concern over time. Reduction in infiltration due to potential sealing has not been observed after more than five years of operation (Lorimor et al., 2003).

Using a lab-scale VIB to treat liquid swine manure, Prantner et al. (2001) showed over 93% reduction in NH_4-N and 89% reduction in P. Lorimor et al. (2003) and Yang and Lorimor (2000) reported operation of a bermed infiltration area that allowed discharges only through subsurface drain tiles placed 1.8 m (6 ft) below the surface of this basin. After five years of experience, soil P levels did not show signs of buildup (Lorimor et al., 2003). Yang and Lorimor (2000) reported average reductions of 81% for suspended solids, 83% for TKN, 85% for NH_4-N, and 78% for P. Nitrate levels increased by 87%. Edwards et al. (1986) and Fausey et al. (1988) reported operation of an infiltration basin below a small open-lot cattle facility with similar decreases in organic and ammonium nitrogen and significant increases in nitrate N. These studies suggest a need for nitrate utilization or treatment downstream of an infiltration system (Lorimor et al., 2003;. Edwards et al., 1986).

Infiltration basins based upon soil filters are limited to sites conducive to tile drainage where a restrictive soil layer exists below the surface to minimize contaminant (especially nitrate) movement to ground water. Alternative infiltration systems, such as a constructed infiltration bed of sand, biosolids, and wood-chip mixtures laid over a gravel layer with a tile drain used to treat runoff from paved parking lots (Culbertson and Hutchinson, 2004) or a wood chip bed (Murphy and George, 1997), may have application to livestock runoff.

Overall VTS Performance

By coupling various combinations of treatment technologies, including VTA and/or VIB, the quality of feedlot runoff can be significantly improved to the point of achieving “functional equivalency” to baseline technologies to complete elimination of surface water runoff. Although the particular combination of treatments selected for any feedlot will be site specific, essentially all should begin with solids removal. Table 3 shows a summary of the anticipated contaminant reductions discussed previously plus common performance levels for constructed wetlands. A combination of a settling basin with a VTA or a VIB has the potential for achieving functional performance equivalency to runoff holding ponds designed to manage a 25-yr, 24-h storm plus normal precipitation runoff based upon results reported by Anschutz et al. (1979), Koellicker et al. (1975), and Wulf et al. (2003).

Table 3. Summary of typical contaminant concentration reductions for various treatment components associated with a dairy or beef open lot facility.[a]

	Total Solids (%)	TKN (%)	Ammonium N (%)	Total P (%)	BOD[b] (%)
Settling basin[b]	60	80	80	80	−−
VTA	60	70	70	70	75
VIB	80	80	85	80	−−

[a] Reductions for two or more components can be estimated by multiplying remaining contaminants (1 − reduction) for each component. A settling basin and VIB will reduce concentration by 92% or $\{1-[(1-0.6)\times(1-0.8)]\}\times 100$. Caution: These values are the author’s best estimates of typical performance for well designed and managed treatment systems. Individual conditions may result in lower performance.
[b] Biochemical Oxygen Demand.
[c] USDA NRCS (2005). Chapters 4 and 9 review performance of settling basins.

VTA Design

The literature provided illustrations of a number of critical design considerations for VTAs (table 4). Based upon this literature, there are several design considerations that are generally accepted for VTAs:

Pre-Treatment

A need exists for some degree of pretreatment. Solids settling is commonly used with VTAs to minimize solids accumulation at the front end of a VTA. This pre-treatment minimizes vegetation damage and reduces the potential for channel flow paths developing where runoff first enters the VTA.

Sheet Flow

Sheet flow of liquid is essential for optimum VTA performance. Design of VTA inlets and headlands is critical to initiating sheet flow. Field management is critical to minimizing concentrated flow. Even with the best inlet design and management, concentrated flow is likely to occur within a VTA and may require additional structures and ongoing maintenance to redistribute flow.

Discharge Control

For VTS on CAFOs, minimizing potential for discharge will be critical for achieving equal or better performance than baseline technologies. Combinations of treatment components into systems, attention to sizing, and modification of hydrograph of flow into a VTA are important considerations for minimizing discharge potential.

Siting Criteria

Siting criteria is critical to the appropriate application of VTAs. Iowa Department of Natural Resources has established nine evaluation criteria used to initially judge a site. These included available area, soil permeability, depth to water table, subsoil and geology, slope, spreaders for uniform distribution, berming for inflow water protection, flooding potential, and proximity to waters of the state (Iowa Department of Natural Resources, 2004).

Sizing Criteria

Multiple approaches have been suggested for VTA sizing:

- Dickey and Vanderholm (1981a) recommended a minimum VTA width of 61 m (200 ft) and a length adequate to completely infiltrate the feedlot runoff and rainfall from a 1-yr, 2-h storm. They calculated minimum flow lengths to provide 2-h contact times. Based on their model, minimum lengths varied from 91 m (300 ft) for a 0.5% slope up to 262 m (860 ft) for a 4% slope.
- Nienaber et al. (1974) suggested a disposal area of one-half hectare per hectare of feed lot is needed. Data in figure 2 suggest that a ratio of 1 to 1 (disposal to feedlot area) or greater is necessary to achieve peak performance. Lorimor et al. (2003) has achieved high contaminant removal rates with a ratio of 1 to 6 (infiltration basin to feedlot area) for a bermed infiltration area that allows discharges only through subsurface drain tiles.
- A design procedure was developed by NRCS in Pennsylvania suggesting that the VTA be designed for the peak discharge resulting from a 2-yr, 24-h storm event at a maximum flow depth of 1.3 cm with a minimum flow through time of 15 minutes (Murphy and Bogovich, 2001). A design procedure based upon a sheet flow equation was proposed:

$$T = 0.29\ (n\ L)^{0.8} / (P_2^{\ 0.5} \times s^{0.4}) \qquad (1)$$

  where T represents travel time (h), n represents Manning's roughness coefficient (0.24 for dense grass), L equals flow length (m), P_2 equals 2-yr, 24-h storm (cm), and s equals land slope (m/m). Schellinger and Clausen (1992) used this USDA SCS design standard for Vermont applications and observed poor performance results. Additional design criteria have been assembled by other USDA NRCS state offices including the Montana Supplement to Chapter 10 of the Agricultural Waste Management Field Handbook (Montana NRCS, 2003). All of these practice standards have typically targeted non-CAFO units. For example, the Montana practice standard states that "final designs for feedlots larger than 3 acres (about 600 cattle) should not be designed with the Simplified Method (Montana practice standard)."
- Murphy and Harner (2001) suggested sizing a VTA area based upon normal nitrogen runoff balanced against nitrogen removal as harvested hay. Procedures for estimating mass of nitrogen runoff from the feedlot and example design calculations are provided by this resource.
- Overcash et al. (1981) proposed a design equation based on influent and effluent concentrations.

$$C_X = C_B + (C_O - C_B) \times e^{\{[1/(1-D)] \times \ln[1/(1+K)]\}} \qquad (2)$$

  This procedure requires knowledge of the influent contaminant concentrations, C_O, to the VTA. A desired VTA effluent concentration, C_X, can then be selected. C_B represents the background concentration, D is the ratio of infiltration to runoff, and K is the ratio of VTA length to waste area length. Once C_X, C_B, C_O, and D have been determined, the equation must be solved for K to size the filter strip. This calculation should be made for all contaminants of concern, and filter strip length be selected based on the limiting contaminant.

VTA Maintenance

Several maintenance issues are critical in VTA function (table 4):

- A good stand of dense vegetation is needed. Dickey and Vanderholm (1981a) noted that dormant residues are effective for filtering and settling pollutants. Management practices that contribute to strong fall growth and well-established winter vegetative cover are critical. Regular harvesting (including hay removal), prevention of channel flow, and minimizing solids accumulation in the VTA are of value in achieving dense fall vegetation. Soil testing to determine fertilization will be of value.
- Sheet flow conditions are essential to VTA performance. Minimizing animal traffic and limiting vehicle traffic to dry conditions are critical to sheet flow maintenance.
- Minimization of nutrient accumulation in VTA is important. Regular harvesting with crop removal to encourage a balance of nutrients is necessary. Mechanical harvesting and animal grazing have been used for harvesting vegetation. Grazing results in low nutrient removal rates and potential nutrient accumulation concerns.
- Higher nutrient deposition is anticipated in the first few meters of the VTA suggesting a potential for nitrate leaching and increased soil P. Regular soil testing for residual soil nitrates and phosphorus may be necessary at the upper end of the VTA.

Conclusions

Based upon this review of the literature, the following conclusions are drawn about the application of vegetative treatment areas to runoff from open lot livestock production systems:

- Substantial research (approximately 40 identified field trials and plot studies) provides a basis for understanding the performance of VTS. These performance results suggest that a vegetative system consisting of a settling basin and VTA or VIB has the potential to achieve functional equivalency to conventional technologies.
- The existing research targeting VTS is confined to non-CAFO applications, likely due to past regulatory limits. Unique challenges exist in adapting these results and recommendations to CAFO applications.
- The pollutant reduction resulting from a VTS is based upon two primary mechanisms: 1) sedimentation, typically occurring within the first few meters of a VTS, and 2) infiltration of runoff into the soil profile. Systems relying primarily on sedimentation only are unlikely to perform equal to or better than baseline technologies. System design based upon sedimentation and infiltration is necessary to achieve a required performance level for CAFO application.
- Critical design factors specific to attaining high levels of pollutant reduction within a VTS include pre-treatment, sheet flow, discharge control, siting, and sizing. Critical management factors include maintenance of a dense vegetation stand and sheet flow of runoff across VTA as well as minimization of nutrient accumulation.

Table 4. Summary of design and management recommendations for VTA for past research and field demonstration projects.

Reference	Type of System	Design Recommendations	Management Recommendations
Barker and Young (1984)	Milking center wastewater and open lot runoff from a 54 cow dairy was directed to settling basin and VTA. Four earthen berms located at 30-ft intervals were designed to create a cascading type system.	Initial seeding of fescue, ye and reed canary grass was used due to tolerance to wet conditions. Four distribution points at upper end of VTA proved inadequate to create sheet flow. Later expansion to seven distribution points reduced problems of channel flow.	At conclusion of study, orchard grass and foxtail grass were dominant species at upper end of filter strip and hairy crabgrass dominated in drier areas. Four grass cuttings were made per year with an attempt to hold grass height near 6 to 12 in. high.
Dickey and Vanderholm (1981a); Dickey and Vanderholm (1981b)	Papers review design and performance of four VTA, two functioning as overland flow (100 cow dairy and 450 beef feedlot) and additional two as channelized flow (500 head beef feedlot and 480 swine operation)	Solids settling in advance of a VTA minimize vegetation damage and maintain VTA effectiveness. Overland or sheet flow within VTA. Minimum recommend contact time for runoff with a VTA is 2 h. Overland VTA does not require longer contact time as lots increase in size. Infiltration area should be designed to allow infiltration for all runoff from a 1-yr, 2-h storm. Additional area provides little improvement. Slope and soil infiltration rate are important considerations in VTA sizing. Channelized flow systems will require: 1 Flow distances at least 10 times greater that sheet flow design; 2 One additional hour of contact time beyond the 2 h minimum for each 465 m^2 (5,000 ft^2) of open lot greater than 929 m^2 (10,000 ft^2); 3 Large areas for open lots of more than 0.4 ha (1 acre);	Dormant residues in VTA have proven to be an effective filter and settling mechanism. Management practices that contribute to a strong fall growth and well-established dormant residue through winter have value in pollutant removal from winter precipitation and snowmelt runoff.
Dillaha et al. (1988); Dillaha et al. (1986)		Effectiveness of VTA is dependent upon design and management measures that create shallow sheet flow and prevent concentrated flow. VTA site selection should target flat areas and avoid hilly terrain.	See first bullet under design recommendations.
Edwards et al. (1983)	VTA test plots after settling basin, natural rainfall, 56-head of beef cattle on concrete lot. Two grass filter cells were used in series, each representing approximately 50% of the concrete lot area.		The grass filter strip was more effective when basin release was actively managed and slowly drained one day following a storm event and after settling of solids.
Ikenberry and Mankin (2000)	Review of literature		Key management considerations recommended: 1 Soil testing to determine fertilization requirement at time of planting of vegetation; 2 Reseeding and fertilization to maintain dense stand; 3 Repairing of gullies soon after their development, 4 Regular moving and harvesting of plant material to remove nutrients and maintain dense vegetation stand; 5 Restriction of field traffic and grazing during wet periods to avoid development of ruts leading to channel flow and damage to vegetation.
Lorimor et al. (2003)	Runoff from concrete open lot beef facility is directed to settling basin, totally bermed infiltration basin, and constructed wetland	Infiltration basin was bermed to provide total containment fo 25-yr, 24-h storm. Infiltration basin was size to provide a land area that was 1/6 th of the drainage area of the concrete open lot. Three parallel buried tile lines ran the length of the infiltration basin to move filtrate from the basin to a constructed wetland.	

Table 4 (continued). Summary of design and management recommendations for VTA for past research and field demonstration projects.

Reference	Type of System	Design Recommendations	Management Recommendations
Murphy and Bogovich (2001)	Summarizes NRCS design recommendations for application of VTA to open lot dairies in Pennsylvania for handling runoff and milking center effluent.	Determines hydarulic characteristics that provide a minimum 15-min flow through time for sheet flow at depths of 1.3 cm and less for various flow rates and slopes. Pretreatment settling basin volume was recommended to be 2-yr peak flow times 15 min.	Checking of pre-treatment facilities on a routine basis, after major rainfall events, and before winter.
Nienaber et al. (1974)	Settling basin, holding pond, sprinkler irrigation on grassed treatment area. Fresh water application compared with beef feedlot runoff.	$\text{VTA size} = \dfrac{\text{Annual Feedlot Runoff (acre}-\text{in.)}}{\text{Max. annual crop water tolerance} - \text{Annual precipitation (in.)}}$	Applied effluent to a grassed disposal area planted with a mixture of nine cool and warm season grasses. Brome grass and intermediate wheat grass became the dominant species, not necessarily due to effluent application. Grazing cattle did not discriminate between areas receiving effluent and area receiving only water for irrigation.
Norman and Edwards (1978)	Ohio NRCS recommendations for sizing of buffer strip dimensions for cattle feedlots.	Travel time should be proportional to BOD concentration.	
Paterson et al. (1980)	Milking center waste and barnyard runoff from - dairy was directed through settling basin (1st stage), holding tank with lift pump, and VTA (2nd stage).	Distribution lines longer than 30 m created challenges with sheet flow. Filter area designed for flow of 4.5 L/m^2 VTA/day was a safe load for high rainfall and snowmelt events. Discharge from VTA was common.	Daily application of waste resulted in tall fescue being replaced by barnyard grass in early season and crab grass later in the season. Mechanical harvesting and removal of grass on a monthly basis was preferable to pasturing. Duplicate VTA area was needed to allow soil drying and harvesting due to daily effluent additions. High rate "dosing" with a pump was found to be preferable for even distribution and to avoid freeze up problems during winter operation.
Murphy and Harner (1999); Harner and Kalita (1999)	VTA established on several open lot beef systems in three watersheds, three of which were monitored for performance.	VTA should be located at least 3 m (10 ft) above groundwater or seasonal perched water table and 30 m (100 ft) from wells. Sedimentation structure must preceed VTA. 61 m (200 ft) of length minimum per 1% slope. For finishing cattle, 1 ha of VTA is suggested per 200 head. For calves confined for 150 days per year, 1 ha of VTA is suggested per 1000 head	Quality of vegetation impacts nutrient removal of vegetation. Establishment procedures and harvesting frequency is important to establishing lush forage growth.
Murphy and Harner (2001)		VTA systems should be sized by matching normal nutrient runoff and crop nutrient utilization.	
Scheilinger and Clausen (1992)	Runoff from dairy barn yard is directed through a detention pond and then to a VTA	USDA-SCS design specification to pass the peak discharge of a 2-yr, 24-h storm at a maximum flow depth of 1.3 cm with a detention time of 15 min was inadequate.	Preferential flow path from the lip spreader through the VTA was another identified cause of poor performance.
Woodbury et al. (2002); Woodbury et al.(2003a); Woodbury et al. (2003b)	Runoff from eight open lot beef cattle pens (about 600 cattle) moved from the pens through a grass approach, settling basin (created by a 300-m long terrace below the pens), and a 6-ha VTA).	A mean hydraulic retention time of 5 to 8 min within the settling basin was used for peak runoff rates. Earth bottom settling basin was designed to be cleaned with front-end loader. For wet years, a settling basin slope (6 to 1) was selected to allow box scraper to be backed into settling basin while keeping tractor on dry ground. Settling basin drainage to minimize liquid depth was recommended to minimize seepage below the basin. Settling basin outlets were installed to place and maintain all outlets on an equal elevation (reinforced concrete pads set outlet elevation. Settling basin drain pipes (separate from normal outlets) were installed to allow complete basin drainage and solids drying prior to solids removal.	Cross drainage across lots should be avoided to prevent one area of settling basin collecting most solids. Berms or wooden planks at the fence line between pens were suggested. Solids accumulation at the bottom end of the pens (due to animal traffic and solids settling) created problems with uneven flow into the settling basin. Periodic solids removal from under the fence line at the lower end of the feedlot is needed. One to two harvests per year of brome grass was considered adequate. Herbicides were used for broadleaf weed control on the VTA and settling basin berm.

REFERENCES

Anschutz, J. A., J. K. Koelliker, J. J. Zovne, T. A. Bean, and M. J. Peterson. 1979. Sizing components on open feedlot runoff control systems. *Transactions of the ASAE* 22(4): 803-808.

Adam, R., R. Lagace, and M. Vallieres. 1986. Evaluation of beef feedlot runoff treatment by a vegetative filter. ASAE Paper No. NAR 66208. St. Joseph, Mich.: ASAE.

Barker, J. C., and B. A. Young. 1984. Evaluation of a vegetative filter for dairy wastewater treatment in southern Appalachia. North Carolina State University research report. U.S. Department of Commerce, National Technical Information Service.

Brach, J. C. 2003. Feedlot runoff control, the Minnesota approach. ASAE Paper No. 032310. St. Joseph, Mich.: ASAE.

Chaubey, I., D. R. Edwards, T. C. Daniel, P. A. Moore, and D. J. Nichols. 1994. Effectiveness of vegetative filter strips in retaining surface applied swine manure constituents. *Transactions of the ASAE* 37(3): 845-850.

Chaubey, I., D. R. Edwards, T. C. Daniel, P. A. Moore, Jr., and D. J. Nichols. 1995. Effectiveness Of vegetative filter strips in controlling losses of surface-applied poultry litter constituents. *Transactions of the ASAE* 38(6): 1687-1692.

Coyne, M. S., R. A. Gilfillen, A. Villalba, Z. Zhang, R. Rhodes, L. Dunn, and R. L. Blevins. 1998. Fecal bacterial trapping by grass filter strips during simulated rain. *J. of Soil and Water Conserv.* 53(2): 140-145.

Culbertson, T. L., and S. L. Hutchinson. 2004. Bioretention cell. *Resource: Engineering & Technology for a Sustainable World* 11(2): 9-10.

Dickey, E. C., and D. H. Vanderholm. 1981a. Performance and design of vegetative filters for feedlot runoff treatment. In *Livestock Waste: A Renewable Resource, Proc. of the 4th International Symposium on Livestock Wastes*, 257-260. St. Joseph, Mich.: ASAE.

Dickey, E. C., and D. H. Vanderholm. 1981b. Vegetative filter treatment of livestock feedlot runoff. *J. Environ. Qual.* 10(3): 279-284.

Dillaha, T. A., J. H. Sherrard, D. Lee, S. Mostaghimi, V. O. Shanholtz, and W. L. Magette. 1986. Use of vegetative filter strips to minimize sediment and phosphorus losses from feedlots: Phase I. Experimental Plot Studies. Virginia Water Resources Research Center, Virginia Polytechnic Institute and State University. Bulletin 151. Blacksburg, Va.

Dillaha, T. A., J. H. Sherrard, D. Lee, S. Mostaghimi, and V. O. Shanholtz. 1988. Evaluation of vegetative filter strips as a best management practice for feed lots. *J. of the Water Pollution Control Federation* 60(7): 1231-1238.

Edwards, W. M., L. B. Owens, and R. K. White.1983. Managing runoff from a small, paved beef feedlot. *J. Environ. Qual.* 12(2): 281-286.

Edwards, W. M., L. B. Owens, R. K. White, and N. R. Fausey. 1986. Managing feedlot runoff with a settling basin plus tiled infiltration bed. *Transactions of the ASAE* 29(1): 243-247.

Fajardo, J. J., J. W. Bauder, and S. D. Cash. 2001. Managing nitrate and bacteria in runoff from livestock confinement areas with vegetative filter strips. *J. of Soil and Water Conserv.* 56(3): 185-191.

Fausey, N. R., W. M. Edwards, L. B. Owens, and R. K. White. 1988. Subsurface drained infiltration beds for feedlot runoff management. *Transactions of the ASAE* 31(1): 98-101.

Federal Register. 2003. 40 CR Parts 9, 122, 123, and 412, National Pollution Discharge Elimination System Regulation and Effluent Limitation Guidelines and Standards for Concentrated Animal Feeding Operations; Final Rule. 12 February 2003. 68(29): 7176-7274.

Goel, P. K., R. P. Rudra, B. Gharabaghi, S. Das, and N. Gupta. 2004. Pollutants removal by vegetative filter strips planted with different grasses. ASAE Paper No. 042177. St. Joseph, Mich.: ASAE.

Hawkins, G. L., D. T. Hill, E. W. Rochester, and C. W. Wood. 1998. Evaluation of vegetative filter strips for swine lagoon wastewater. *Transactions of the ASAE* 41(3): 639-643.

Harner, J. P., and P. K. Kalita. 1999. Vegetative filters for improving environmental quality. In *Cattlemen's Days 1999 Conference Proceedings*, 44-47. Manhattan, Kans.: Kansas Agricultural Experiment Station, Kansas State University.

Ikenberry, C. D., and K. R. Mankin. 2000. Review of vegetative filter strip performance for animal waste treatment. ASAE Paper No. MC00128. St. Joseph, Mich.: ASAE.

Iowa Department of Natural Resources. 2004. Siting criteria: non-basin technology for waste treatment systems for open feedlots. Shared by Gene Tinkler, IDNR, by personal communications, July 2004.

Keaton, M.R., P. K. Kalita, J. P. Harner, and S. K. Starrett. 1998. Evaluation of vegetative filter strips for nutrient removal from feedlot runoff. ASAE Paper No. 982014. St. Joseph, Mich.: ASAE.

Koelliker, J. K., H. L. Manges, and R. I. Lipper. 1975. Modeling the performance of feedlot-runoff control facilities. *Transactions of the ASAE* 18(6): 1118-1121

Komor, S. C., and D. S. Hansen. 2003. Attenuation of runoff and chemical loads in grass filter strips at two cattle feedlots, Minnesota, 1995-98. U. S. Geological Survey. Water-Resources Investigations Report 03-4036. Denver, Colo.

Lim, T. T., D. R. Edwards, S. R. Workman, and B. T. Larson. 1997. Vegetated filter strip length effects on quality of runoff from grazed pastures. ASAE Paper No. 972060. St. Joseph, Mich.: ASAE.

Lorimor, J. C., L. Wulf, and P. Jaranilla. 2003. An infiltration-wetland system for treating open feedlot runoff. In *Proc. of the 9th International Symposium, Animal, Agricultural, and Food Processing Wastes*, 405-410. St. Joseph, Mich.: ASAE.

Mankin, K. R., and C. G. Okoren. 2003. Field evaluation of bacteria removal in a VHS. ASAE Paper No. 032150. St. Joseph, Mich.: ASAE

Minnesota Pollution Control Agency. 2003. Vegetated infiltration areas and filter strips to treat feedlot runoff: interim guidelines. www.pca.state.mn.us/hot/feedlots.

Montana NRCS. 2003. Analysis procedures for ag-waste collection basin/VFS. Montana Supplement to Chapter 10 of the *Agricultural Waste Management Field Handbook*. Bozeman, Mont.

Murphy, J. P., and J. P. Harner. 1999. Runoff compliance for Kansas cattle feedlots. Cattlemen's Days 1999 conference proceedings. Kansas Agricultural Experiment Station. Kansas State University.

Murphy, P., and J. P. Harner. 2001. Open lot runoff management options. Livestock Poultry Environmental Stewardship curriculum, Lesson 22. http://www.LPES.org.

Murphy, T. J., and W. M. Bogovich. 2001. Vegetated filter areas for agricultural wastewater treatment. ASAE Paper No. 012296. St. Joseph, Mich.: ASAE.

Murphy, T. J., and J. C. George. 1997. Performance of an organic filter fed and constructed wetland for the treatment of veal wastewater. ASAE Paper No. 974110. St. Joseph, Mich.: ASAE.

Nienaber, J. A., C. B. Gilbertson, T. M. McCalla, and F. M. Kestner. 1974. Disposal of effluent from a beef cattle feedlot runoff control holding pond. *Transactions of the ASAE* 17(2): 375-378.

Norman, D. A., and Edwards, W. M. 1978. Design criteria for grass filter areas. ASAE Paper No. 782573. St. Joseph, Mich.: ASAE.

Overcash, M. R., S. C. Bingham, and P. W. Westerman. 1981. Predicting runoff pollutant reduction in buffer zones adjacent to land treatment sites. *Transactions of the ASAE* 24(2): 430-435.

Paterson, J. J., J. H. Jones, F. J. Olsen, and G. C. McCoy. 1980. Dairy liquid waste distribution in an overland flow vegetative-soil filter system. *Transactions of the ASAE* 23(4): 973-978.

Pope, R. O., and D. E. Stoltenberg. 1991. A review of literature related to vegetative filter strips. Agronomy Department publication. Ames, Iowa: Iowa State University.

Prantner, S. R., R. S. Kanwar, J. C. Lorimor, and C. H. Pederson. 2001. Soil infiltration and wetland microcosm treatment of liquid swine manure. *Applied Engineering in Agriculture* 17(4): 483-488.

Sanderson, M. A., R. M. Jones, M. J. McFarland, J. Stroup, R. L. Reed, and J. P. Muir. 2001. Nutrient movement and removal in a switchgrass biomass-filter strip system treated with dairy manure. *J. Environ. Qual.* 30(1): 210-216.

Schellinger, G. R., and J. C. Clausen. 1992. Vegetative filter treatment of dairy barnyard runoff in cold regions. *J. Environ. Qual.* 21(1): 40-45.

Schwer, C. B., and J. C. Clausen. 1989. Vegetative filter treatment of dairy milkhouse wastewater. *J. Environ. Qual.* 18: 446-451.

Srivastava, P., D. R. Edwards, T. C. Daniel, P. A. Moore, Jr. and T. A. Costello. 1996. Performance of vegetative filter strips with varying pollutant source and filter strip lengths. *Transactions of the ASAE* 39(6): 2231-2239.

USDA NRCS. 2005. Vegetative treatment systems for open lot runoff. Waiting to be printed and posted to web. Contact William Boyd at 336-370-3334 or william.boyd@gnb.usda.gov.

Williamson, T. S. 1999. Effectiveness of vegetative filter strips in reducing fecal coliform from surface runoff. Unpublished Masters Thesis. Biological and Agricultural Engineering. Kansas State University. Manhattan, Kans.

Willrich, T. L., and J. O. Boda. 1976. Overland flow treatment of swine lagoon effluent. ASAE Paper No. 764515. St. Joseph, Mich.: ASAE.

Woodbury, B. L., J. A. Nienaber, and R. A. Eigenberg. 2002. Operational evaluation of a passive beef cattle feedlot runoff control and treatment system. *Applied Engineering in Agriculture* 18(5): 541-545.

Woodbury, B. L., J. A. Nienaber, and R. A. Eigenberg. 2003a. Sustainability of a passive feedlot runoff control system using a vegetative filter strip for nutrient control. ASAE Paper No. 032269. St. Joseph, Mich.: ASAE.

Woodbury, B. L., J. A. Nienaber, and R. A. Eigenberg. 2003b. Nitrogen management of a feedlot runoff control system with vegetative filter strip. In *Proc. of the 9th International Symposium, Animal, Agricultural and Food Processing Waste*, 372-376. St. Joseph, Mich.: ASAE.

Wulf, L. W., J. C. Lorimor, and S. W. Melvin. 2003. Modifications to feedlot runoff containment systems in Iowa. In *Animal, Agricultural and Food Processing Wastes IX. Proc. of the Ninth International Symposium,* 387-396. St. Joseph, Mich.: ASAE.

Yang, P., and J. Lorimor. 2000. Physical and chemical analysis of beef cattle feedlot runoff before and after soil infiltration and wetland treatment. In *Proc. of the 8th International Symposium, Animal, Agricultural, and Food Processing Wastes,* 203-209. St. Joseph, Mich.: ASAE.

Young, R. A., T. Huntrods, and W. Anderson. 1980. Effectiveness of vegetated buffer strips in controlling pollution from feedlot runoff. *J. Environ. Qual.* 9(3): 483-487.

Zhang, Q., C. G. Okoren, and K. R. Mankin. 2001. Modeling fecal pathogen transport in vegetative filter strips. ASAE Paper No. 012194. St. Joseph, Mich.: ASAE.

Attachment 15:

Photograph of livestock waste application field next to an Illinois river

Attachment 16:

Complaint, *People of the State of Illinois v. Kenneth W. Fehr, d/b/a Fehr Brothers Swine Farm*

IN THE CIRCUIT COURT FOR THE ELEVENTH JUDICIAL CIRCUIT

WOODFORD COUNTY, ILLINOIS

COPY

PEOPLE OF THE STATE OF ILLINOIS,)
ex rel. LISA MADIGAN, Attorney)
General of the State of Illinois)
)
Plaintiff,)
) No. 11 CH 106
v.)
)
KENNETH W. FEHR, d/b/a)
FEHR BROTHERS SWINE FARM)
)
Defendant)

ORIGINAL FILED
DEC 2 2 2011
WOODFORD COUNTY CIRCUIT COURT

COMPLAINT FOR INJUNCTIVE AND OTHER RELIEF

The PEOPLE OF THE STATE OF ILLINOIS, by Lisa Madigan, Attorney General of the State of Illinois, complain of Defendant Kenneth W. Fehr, d/b/a Fehr Brothers Swine Farm, as follows:

COUNT I

WATER POLLUTION

1. This Count is brought on behalf of the People of the State of Illinois, by Lisa Madigan, Attorney General of the State of Illinois, on her own motion and at the request of the Illinois Environmental Protection Agency ("Illinois EPA") pursuant to Sections 42(d) and (e) of the Illinois Environmental Protection Act ("Act"), 415 ILCS 5/42(d), (e).

2. The Illinois EPA is an agency of the State of Illinois created by the General Assembly in Section 4 of the Act, 415 ILCS 5/4, and which is charged, inter alia, with the duty of enforcing the Act.

3. Defendant Kenneth W. Fehr, d/b/a Fehr Brothers Swine Farm ("Fehr") owns a swine farrowing and gestation facility, maintains sows at a second facility, and has multiple swine finishing facilities in Woodford County. Defendant Fehr operates this swine production

business with his four sons. Better known to the Defendant, the sons may have ownership interest in the various production facilities.

4. One of the Defendant's finishing facilities is known as the Red Finisher Swine Facility. It is located in the SE 1/4, Section 9, T27N, R2E (Panola Township) in Woodford County. It is operated by Jake Fehr. Another one of the Defendant's finishing facilities is known as the Jake Fehr Swine Facility, and it is also operated by Jake Fehr. It is located in the NW 1/4, Section 15, T27N, R2E (Panola Township) Woodford County. The address of the Jake Fehr Swine Facility is 2939 County Road 1500 N, El Paso, IL 61738.

5. The Kenneth Fehr Home Farm is a 700 sow gestation and farrowing facility operated by Kenneth Fehr and is located north of Eureka at 1217 State Route 117, Eureka, IL. Kenneth Fehr also owns approximately 750 sows that are maintained at a sow operation in Woodford County known as the Mark Schmidgall swine farm.

6. Another one of the finishing facilities is known as the Neisler Swine Facility. It is located about 4 miles southeast of Minonk, along County Road 2900 E, in the northeast corner of Woodford County. The legal description is NW 1/4, Section 34, T28N, R2E (Minonk Township), Woodford County. The Neisler Swine Facility is located in the watershed of Panther Creek. Panther Creek is tributary to the Mackinaw River which is tributary to the Illinois River. Panther Creek is a perennial stream.

7. Toby's Place is another one of the Defendant's swine finishing facilities. It is located approximately 2.5 miles southwest of Roanoke. The address is 1660 County Road 1150 North, Eureka, IL 61530 and the legal description if NE 1/4, Section 33, T27N-R1W (Roanoke Township) Woodford County. Toby's Place is a total confinement swine facility at which the manure is contained in 8 foot deep pits under the buildings. The facility has capacity for 1800 finishing hogs and 500 nursery piglets. There are five total confinements buildings on-

site: one finishing building with a capacity of 1200 head, three smaller finishing buildings each with a capacity of 200 head and one small nursery building that has a capacity of 500 head. The facility is a wean to finish operation.

8. Section 3.165 of the Act, 415 ILCS 5/3.165, provides the following definition:

> "CONTAMINANT" is any solid, liquid, or gaseous matter, any odor, or any form of energy, from whatever source.

9. Section 3.545 of the Act, 415 ILCS 5/3.545, contains the following definition:

> 'WATER POLLUTION' is such alteration of the physical, thermal, chemical, biological, or radioactive properties of any waters of the State, or such discharge of any contaminant into any waters of the State, as will or is likely to create a nuisance or render such water harmful or detrimental or injurious to public health, safety or welfare, or to domestic, commercial, industrial, agricultural, recreational, or other legitimate uses, or to livestock, wild animals, birds, fish, or other aquatic life.

10. Section 3.550 of the Act, 415 ILCS 5/3.550, contains the following definition:

> 'WATERS' means all accumulations of water, surface and underground, natural, and artificial, public and private, or parts thereof, which are wholly or partially within, flow through, or border upon this State.

11. Section 12 of the Act, 415 ILCS 5/12, provides, in pertinent part, as follows:

No person shall:

a. Cause or threaten or allow the discharge of any contaminants into the environment in any State so as to cause or tend to cause water pollution in Illinois, either alone or in combination with matter from other sources, or so as to violate regulations or standards adopted by the Pollution Control Board under this Act;

12. The land application fields and confinement buildings Defendant uses for his swine operation are point sources of discharge.

13. Section 302.212 of the Illinois Pollution Control Board's Water Pollution Regulations, 35 Ill. Admin. Code 302.212, provides, in pertinent part:

> Total Ammonia Nitrogen
>
> a) Total ammonia nitrogen (as N: STORET Number 00610) must in no case exceed 15 mg/L.

14. Section 304.120 of the Board's Water Pollution Regulations, 35 Ill. Adm. Code 304.120, provides, in pertinent part, as follows:

> Deoxygenating Wastes
> Except as provided in 35 Ill. Adm. Code 306.Subpart C, all effluents containing deoxygenating wastes shall meet the following standards:
>
> a) No effluent shall exceed 30 mg/L of five day biochemical oxygen demand (BOD_5) (STORET number 00310) or 30 mg/L of suspended solids (STORET number 00530), except that treatment works employing three stage lagoon treatment systems which are properly designed, maintained and operated, and whose effluent has a dilution ratio no less than five to one or who qualify for exceptions under subsection (c) shall not exceed 37 mg/L of suspended solids.

February 15-16 2011 Land Application Field Release

15. Beginning on or about January 24, 2011 and continuing through February 2, 2011, Defendant Fehr land applied 65 semi-truck loads, representing 400,000 gallons, of swine waste on a 92-acre field of frozen farm ground located in the SW ¼, Section 19, T27N, R2E (Panola Township) in Woodford County ("application site"). The application site is within the Panther Creek watershed.

16. Defendant Fehr stopped applying waste on February 2, 2011 due to blizzard conditions.

17. Within two weeks of the land application event, ambient air temperatures rose and swine waste that had been in a frozen state on the application site thawed and ran off site into waters of the State. On the evening of February 14, 2011, a neighbor complainant

observed manure run off the application site into the road ditch. The complainant observed manure drainage in the waterway that passes beneath County Road 2600 E approximately 1/4 mile from the complainant's residence. The complainant indicated that foam in the waterway at County Road 2600 E was several feet thick the night of February 14, 2011. This waterway flows northwest into Panther Creek.

18. From the application site, drainage moves first to the northeast section of the field where it enters a field tile, crosses County Hwy 4, and moves to the north west toward Panther Creek in an unnamed tributary of Panther Creek.

19. On February 15, 2011, the Illinois EPA conducted an inspection of the application site in response to the neighbor complaint. At the time of the inspection, the Illinois EPA inspector observed swine waste foam in the unnamed tributary.

20. The field tile/waterway conveying water from the application site to Panther Creek culminates in a submerged outlet. At the outlet, the inspector observed that ice had melted around the outlet and foaming was occurring due to the presence of livestock waste. The water in Panther Creek was turbid. The inspector examined Panther Creek at the point where it flows beneath County Highway 4, approximately 1 ½ miles west of the application site. The location is several stream miles downstream from the application site. The inspector walked upstream approximately 100 yards from the bridge to a point where the stream was partially open. Panther Creek was slightly turbid and discolored at this location.

21. At the time of the February 15, 2011 inspection, the Illinois EPA inspector observed swine manure on the surface of the application site. A portion of the field was snow-covered. The inspector observed liquid draining off the northeast portion of the application site and into the south road ditch of County Highway 4. The liquid was dark colored, with foam and a swine waste odor. The runoff entered the road ditch and flowed east. Flow in the ditch was

estimated at approximately 200 gallons per minute. A liquid sample was collected at this location. Analytical results of this sample indicate the following parameter levels: ammonia, 59.9 mg/l; biochemical oxygen demand ("BOD"); 207 mg/l.

22. There are two culverts beneath County Highway 4 at the north end of the application site. The western culvert is a corrugated metal pipe approximately 24 inches in diameter. The eastern culvert is a concrete box culvert. Runoff from the application site drained north, off the field and into the south road ditch of County Highway 4. The liquid then turned east and drained to the 24-inch diameter culvert and passed north, beneath County Highway 4. Flow exited the north end of the 24-inch diameter culvert and turned east, flowed approximately 20 feet and entered the north end of the concrete box culvert and flowed back south under County Highway 4. At the time of the February 15, 2011 inspection, a liquid sample was collected from the waterway on the north (downstream) side of County Highway 4, about 50 yards downstream from where runoff from the application site was entering the south road ditch. The liquid at this location was turbid, dark color and had a swine waste odor. Analytical results of this sample indicate the following parameter levels: ammonia, 59.1 mg/l; BOD, 229 mg/l.

23. After entering the concrete box culvert, the flow of liquid drained south, beneath County Highway 4 and exited the box culvert. At the south end of the box culvert, the inspector observed a small vortex with liquid pooling in the area and draining into the ground. It was apparent that a tile existed beneath the box culvert. This tile evidently had experienced a blow-out whereby surface liquid was able to enter directly into the tile. Liquid in this tile flows to the northwest, following the grass waterway to Panther Creek. A liquid sample was collected from the grass waterway to Panther Creek, approximately 1/4 mile downstream of the application site. Liquid in the waterway was turbid with a swine waste odor. Analytical results of this

sample indicate the following parameter levels: ammonia, 76.9 mg/l; BOD, 366 mg/l; total suspended solids ("TSS"), 202 mg/l.

24. The Illinois EPA inspector observed a strong odor of swine waste in the air while standing along County Highway 4 on the north side of the application site and in the vicinity.

25. Defendant Fehr's Red Finisher Swine Facility and Jake Fehr Swine Facility were the source of the livestock waste land applied on the application site. On February 15, 2011, the Illinois EPA conducted an inspection at the Red Finisher Swine Facility. At the time of the inspection, the facility livestock waste lagoon contained approximately 1 foot of freeboard and was ice covered.

26. On February 15, 2011, the Illinois EPA also conducted an inspection at the Jake Fehr Swine Facility. At the time of the inspection, Jake Fehr told the Illinois EPA inspector that approximately 60 to 65 semi-tanker loads of liquid pit manure were hauled to the application site. The Defendant finished hauling to the application site on February 1, 2011, when a blizzard occurred. Jake Fehr indicated that manure had been hauled to the application site the entire week prior to February 1, 2011. Approximately 10 to 15 loads of liquid swine waste came from the manure storage pits at the Red Finisher facility. The remainder of the loads of liquid manure came from the manure storage pit(s) at the Jake Fehr Swine Facility. Kenneth, Zach and Jake Fehr conducted the hauling and application. When asked why they hauled the manure, Jake Fehr indicated they had no choice because the manure pits were full and about to run over. The Red Finisher has small pits, only 4 feet deep and 4 feet wide. At the time of the inspection, the Red Finisher facility housed 1150 head of finishing swine and approximately 1,000 pigs in its nursery. The Jake Fehr Swine Facility had 750 head of finishing swine.

27. At the time of the February 15, 2011 inspection, Jake Fehr was advised to stop the discharge of wastewater leaving the application site, impound/contain the wastewater, and

report the release. Defendant reported the release to the Illinois Emergency Management Agency on February 15, 2011 at 1:46 p.m.

28. On February 16, 2011, the Illinois EPA inspector conducted a follow-up inspection of the application site. Defendant Fehr and his sons had constructed a dam consisting of construction silt fencing and hay bales to slow the runoff from the field and retain as much runoff as possible. A large accumulation of liquid waste existed on the application site. Defendant Fehr and his sons were pumping the wastewater into semitrucks and hauling it to storage structures at their facilities.

29. At the time of the February 16, 2011 inspection wastewater from the impoundment in the application site continued to pass through the silt fence and flow north into the south road ditch of County Highway 4. The liquid in the south road ditch of County Highway 4 was dark colored and turbid with a swine waste odor. The liquid flowed to the east at an estimated rate of 150 gallons per minute and entered the south end of a 24-inch diameter metal culvert located beneath County Highway 4. The odorous liquid flowed north, beneath County Highway 4, exited the culvert and continued flowing north in a grass waterway. The Illinois EPA inspector also observed liquid flowing north through the concrete box culvert located just east of the metal culvert. Liquid was discharging from the north end of the box culvert and flowing north in the grass waterway leading to Panther Creek at an estimated rate of several hundred gallons per minute. The liquid had a swine waste odor.

30. At the time of the February 16, 2011 inspection, surface liquid samples were collected from four locations. One sample was collected from the wastewater impoundment on the north boundary of the application site. At this location, an estimated 100,000 gallons of liquid waste existed. The liquid waste was turbid and dark colored with a swine waste odor. Analytical results indicated the following parameter levels: ammonia, 120 mg/l; phosphorus,

16.5 mg/l; BOD, 337 mg/l; TSS, 202 mg/l.

31. At the time of the February 16, 2011 inspection, a liquid sample was collected from the south road ditch of County Highway 4 at the northeast portion of the application site. Drainage from the field was flowing into the ditch and then flowed east. Liquid in the ditch was turbid, dark colored and contained a swine waste odor. Analytical results indicated the following parameter levels: ammonia, 67.6 mg/l; BOD, 129 mg/l; TSS, 612 mg/l.

32. At the time of the February 16, 2011 inspection, a liquid sample was collected from the grass waterway northwest of the application site. The waterway drains to Panther Creek. The sample location is approximately 1/4 mile downstream from the application site. Liquid in the waterway was turbid with a swine waste odor. Analytical results indicated the following parameter levels: ammonia, 43.1 mg/l; BOD, 129 mg/l; TSS, 62 mg/l.

33. At the time of the February 16, 2011 inspection, a liquid sample was collected from the waterway on the north (downstream) side of County Highway 4, just downstream from the wastewater impoundment in the application site. The sample was collected from liquid draining out of the concrete box culvert. Flow at this location contained a swine waste odor and was estimated to be flowing at several hundred gallons per minute. Analytical results indicated the following parameter levels: ammonia, 9.11 mg/l; BOD, 48 mg/l; TSS, 68 mg/l.

34. At the time of the February 16, 2011 inspection, swine farm representatives told the Illinois EPA inspector that they hauled wastewater away from the site though the night. Based on the information provided, the Illinois EPA inspector calculated that the facility had hauled 21,000 gallons from the application site. The recovered swine wastewater was returned to a manure storage pit at Jake Fehr's Swine Facility located northeast of Panola.

35. At the time of the February 16, 2011 inspection, the Illinois EPA inspector observed that snow melt and run off from a soybean field directly west of the application site

was flowing through a culvert onto the application site. The Defendant's representatives were advised to temporarily divert clean surface water from this soybean field away from the application site.

36. At the time of the February 16, 2011 inspection, Jake Fehr told the Illinois EPA inspector that he did not have a nutrient management plan for either his swine facility or the Red Finisher Swine Facility, both of which were the source of the manure applied to the application site.

37. On February 24, 2011, the Illinois EPA conducted a reconnaissance inspection. At the time of the inspection, nothing was in place to divert clean water flowing from the soybean field to the west from draining onto the application site.

February 24, 2011 discharges from Neisler Swine Facility

38. On February 24, 2011, the Illinois EPA conducted an inspection of the Neisler Swine Facility. The Neisler Swine Facility is a total confinement swine operation with numerous buildings and a swine waste lagoon. The buildings were originally equipped with a gutter flush system that directed swine waste below ground via pipes to a pumping station. From that station, swine waste was pumped into a large, anaerobic lagoon.

39. The Neisler Swine Facility consists of six swine confinement buildings positioned side by side, extending east, away from County Road 2900 E. The long axis of each building is oriented north/south. Swine waste from the confinement buildings drains to the east to enter the collection pit. The lagoon is east of the collection pit. At the time of the February 24, 2011 inspection, the western confinement building existed on the site in a demolished condition. The concrete floor and manure pits remained in place and exposed to the elements. A seventh confinement building had been added to the site, south of the other buildings, along the south side of the gravel access lane. It is oriented east/west, perpendicular to the original

confinement buildings. The seventh building has a shallow manure storage pit. Swine waste from this pit is directed via pull plugs to a separate pumping station and then transferred to the lagoon.

40. The Neisler Swine Facility is used as a wean to finish operation. Approximately 1800 weaned piglets are delivered to the site every 9 weeks. At the time of the February 24, 2011 visit, the facility contained 1800 pigs. The south swine confinement building was not in use at the time of the inspection. Pigs raised to market weight at this facility come from the Mark Schmidgall swine farm.

41. At the time of the February 24, 2011 inspection, the Illinois EPA inspector observed the Neisler facility swine waste lagoon. The lagoon contained 12 to 14 inches of freeboard. Wind and wave action had deteriorated the integrity of the earthen berms and there was significant erosion on the inner slope of the earthen berms. The earthen berms also contained small trees and thick, tall weed growth.

42. The Neisler Swine Farm confinement buildings are spaced approximately 40 feet apart and are connected by an alley, allowing workers and/or hogs to move between buildings without going outside. A series of pipes are buried in an east/west orientation beneath the alleys. The pipes are designed to provide a conduit for swine waste to drain east from each building. The original design was to allow the waste to flow east in the pipes by gravity and collect in a concrete collection pit located beneath the loading/sorting building. This buried piping system beneath the allies has historically proven unreliable. Plugging occurs in the pipes and waste is not able to drain to the collection pit as designed.

43. At the time of the February 24, 2011 inspection, the Illinois EPA inspector observed overflows of swine waste discharging from the following locations: (1) the second confinement building to the east, (2) out of the alley of the third confinement building from the

east, and (3) the manure pit at the far east confinement building prior to where waste should enter the collection pit beneath the sorting building. In response to the plumbing ineffectiveness, the Defendant was attempting to pump waste from a makeshift access port at the manure storage pit beneath the far east confinement building. This pit overflow drained away from the pit in a southeasterly direction, around the west and south sides of the swine waste lagoon.

44. At the time of the February 24, 2011 inspection, the Illinois EPA inspector collected surface liquid samples. He collected a sample of liquid waste drainage created by waste overflow from the manure storage pit beneath the eastern most swine confinement building. Analytical results indicated the following parameter levels: ammonia, 1290 mg/l; phosphorus, 418 mg/l; BOD, 9210 mg/l; TSS, 20,200 mg/l; fecal coliform, 50,000 cfu/100 ml.

45. At the time of the February 24, 2011 inspection, the Illinois EPA inspector collected a sample of liquid waste from a drainage channel leading away from the swine confinement buildings on the northeast side of the swine buildings. Wastewater from the pipe located beneath the alley of a swine confinement building created this channel of drainage. The liquid in the drainage path was grey colored and turbid. Analytical results indicated the following parameter levels: ammonia, 3400 mg/l; phosphorus, 866 mg/l; BOD, 9380 mg/l; TSS, 24,600 mg/l, fecal coliform; 60,000 cfu/100 ml.

46. At the time of the February 24, 2011 inspection, the Illinois EPA inspector collected a sample of liquid waste from the south side of the swine waste lagoon and downstream of the swine confinement buildings. Analytical results indicated the following parameter levels: ammonia, 5520 mg/l; phosphorus, 33.9 mg/l; BOD, 1660 mg/l; TSS, 830 mg/l, fecal coliform; 21,000 cfu/100 ml.

47. At the time of the February 24, 2011 inspection, the Illinois EPA inspector

observed a location on an unnamed tributary to Panther Creek at a point approximately 1/4 to ½ mile south of the Neisler Swine Farm. At this location a corrugated metal pipe discharges to the unnamed tributary. The pipe outlets on the west side of County Road 2900E. At this location, the inspector observed a slight swine waste odor and some foam in the discharge.

48. At the Neisler Swine Farm, a waterway exists on the east side of the swine waste lagoon. The waterway is directly adjacent to the lagoon. This waterway flows from north to south and is tributary to Panther Creek. At the time of the February 24, 2011 inspection, the waterway was severely eroded along the northeast corner of the lagoon.

49. At the time of the February 24, 2011 inspection, the Illinois EPA inspector observed numerous dead swine at various locations along the north end of the swine confinement buildings. It was obvious from the disintegrating condition of the multiple swine carcasses that the animals had been dead for weeks. Some of the carcasses were partially eaten. The Defendant indicated to the inspector that the small field north of the confinement buildings is used as a burial site for dead swine. At the time of the inspection, the Illinois EPA inspector observed numerous mounds of dirt in the field and he also observed at least two exposed areas, piled with swine carcasses.

November 1 and 3, 2011 Toby's Place land application release

50. On November 1, 2011, at approximately 6:00 P.M., Todd and Jared Fehr were land applying waste from the Defendant's facility known as Toby's Place when a fitting failed at a booster pump causing the release of approximately 6,000 gallons of manure onto land that was adjacent to an unnamed tributary to West Branch Panther Creek. Waste entered the unnamed tributary.

51. On November 2, 2011 at 5:56 P.M., Ken Fehr called the Peoria Regional Office of the Illinois EPA and reported the release. The message was not received by Illinois EPA

personnel until the morning of November 3, 2011.

52. On November 3, 2011, the Illinois EPA conducted an inspection of the site of the release and the land application field. Todd and Jared Fehr utilized a hose drag system to land apply waste. At the time of the November 1, 2011 release, Todd Fehr was located at the tractor injection end of the application. Jared Fehr was in charge of starting the main pump at Toby's Place as well as the booster pump located approximately .5 miles east of Toby's Place. When the application was initiated at approximately 6:00 P.M. on November 1, 2011, it became apparent the waste was not reaching the injection end. It took Jared Fehr 5 to 10 minutes to reach the booster pump where he discovered the failed fitting.

53. In reviewing the conditions that resulted in the release the following circumstances were identified. A kink in the application hose caused increased pressure in the system, which ruptured the fitting at the booster pump. A failure to inspect and straighten the hose before starting the pumps as well as a failure to utilize a sufficient number of personnel to properly supervise and operate the pumps contributed to the cause of the release.

54. At the time of the November 3, 2011 inspection, the Illinois EPA inspectors observed the field where waste has been applied from Toby's Place. The field is located approximate .75 miles southeast of the spill area. Liquid waste was pooled in the northwest corner of the 61-acre manure application field. The liquid waste was dark colored and contained a swine waste odor. At the time of the inspection, the liquid waste was draining off the northwest corner of the field and entering a tile inlet. Foam was observed forming inside the tile inlet. The discharge from the application field was estimated to be 5 gallons per minute ("gpm"). The tile is tributary to an unnamed tributary to West Branch Panther Creek south of the application field.

55. At the time of the November 3, 2011 inspection, the Illinois EPA inspectors

collected samples at the release site and at the land application field. All three samples collected at the release site had a strong smell of swine manure, were turbid and dark colored. The first sample was collected at the location of the release, an area of pooled waste approximately 100 yards wide in a harvested field. Analytical results of this sample indicated the following parameter levels: ammonia, 37.0 mg/l; BOD, 194 mg/l; TSS, 5880 mg/l. The second sample was collected from the unnamed tributary to West Branch Panther Creek approximately 50 feet downstream from where the release entered the stream. Analytical results of this sample indicated the following parameter levels: ammonia, 300.00 mg/l; BOD, 1,350 mg/l; TSS, 730 mg/l. The third sample was collected from the unnamed tributary to West Branch Panther Creek approximately 100 yards downstream from the point where the release entered the stream. Analytical results of this sample indicated the following parameter levels: ammonia, 149.0 mg/l; BOD, 625 mg/l; TSS, 1020 mg/l. An additional sample was collected where the liquid waste runoff was entering the tile inlet at the land application field. The liquid waste had a slight swine waste odor, was turbid and dark colored. Analytical results of this sample indicated the following parameter levels: ammonia, 60.1 mg/l; BOD, 159 mg/l; TSS, 450 mg/l.

56. The Defendant caused, allowed or threatened the discharge of contaminants to waters of the State so as to cause or tend to cause water pollution in Illinois or to violate the Board's regulations or standards through the discharge of livestock waste from its facility to Panther Creek.

57. The discharges of contaminants from the Defendant's facilities have caused, threatened or allowed water pollution in that such discharges have likely rendered the waters of the State harmful or detrimental or injurious to public health, safety or welfare, or to agricultural, recreational, or other legitimate uses, or to livestock, wild animals, birds, fish or other aquatic

life and have likely created a nuisance.

58. By causing, allowing or threatening the discharge of contaminants to waters of the State so as to cause or tend to cause water pollution in Illinois or to violate the Board's regulations or standards, the Defendant has violated Section 12(a) of the Act, 415 ILCS 5/12(a).

59. By causing or allowing a point source discharge from the land application of livestock waste with ammonia levels that exceed the State's water quality standards, the Defendant has violated Section 12(a) of the Act, 415 ILCS 5/12(a), and 35 Ill. Admin. Code 302.212.

60. By causing or allowing a point source discharge from the land application of livestock waste with BOD_5 and TSS levels that exceed the State's effluent limits, the Defendant has violated Section 12(a) of the Act, 415 ILCS 5/12(a), and 35 Ill. Adm. Code 304.120.

PRAYER FOR RELIEF

WHEREFORE, the Plaintiff, PEOPLE OF THE STATE OF ILLINOIS, respectfully requests that this Court grant the following relief:

A. Find that the Defendant Kenneth W. Fehr, d/b/a Fehr Brothers Swine Farm has violated Section 12(a) of the Act, 415 ILCS 5/12(a), 35 Ill. Admin. Code 302.212 and 35 Ill. Adm. Code 304.120;

B. Permanently enjoin the Defendant from further violations of the Act and associated regulations pursuant to Section 42(e) of the Act, 415 ILCS 5/42(e);

C. Pursuant to Section 42(a) of the Act, 415 ILCS 5/42(a), impose upon the Defendant a monetary penalty of not more than the statutory maximum;

D. Pursuant to Section 42(f) of the Act, 415 ILCS 5/42(f), award the Plaintiff its costs in this matter, including reasonable attorney's fees and costs; and

E. Grant such other and further relief as the Court deems appropriate.

COUNT II

WATER POLLUTION HAZARD

1-57. Plaintiff re-alleges and incorporates by reference herein paragraphs 1 through 57 of Count I as paragraphs 1 through 57 of this Count II.

58. Section 12 of the Act, 415 ILCS 5/12, provides, in pertinent part, as follows:

No person shall:

* * *

d. Deposit any contaminants upon the land in such place and manner so as to create a water pollution hazard;

59. The Defendant has caused or allowed contaminants to be deposited upon the land in such place and manner as to create a water pollution hazard through its proximity to Panther Creek.

60. By depositing contaminants upon the land in such a place and manner as to create a water pollution hazard, the Defendant has violated Section 12(d) of the Act, 415 ILCS 5/12(d).

PRAYER FOR RELIEF

WHEREFORE, the Plaintiff, PEOPLE OF THE STATE OF ILLINOIS, respectfully requests that this Court grant the following relief:

A. Find that the Defendant Kenneth W. Fehr, d/b/a Fehr Brothers Swine Farm has violated Section 12(d) of the Act, 415 ILCS 5/12(d);

B. Permanently enjoin the Defendant from further violations of the Act and associated regulations pursuant to Section 42(e) of the Act, 415 ILCS 5/42(e);

C. Pursuant to Section 42(a) of the Act, 415 ILCS 5/42(a), impose upon the

Defendant a monetary penalty of not more than the statutory maximum;

D. Pursuant to Section 42(f) of the Act, 415 ILCS 5/42(f), award the Plaintiff its costs in this matter, including reasonable attorney's fees and costs; and

E. Grant such other and further relief as the Court deems appropriate.

COUNT III

NPDES PERMIT VIOLATIONS

1-57. Plaintiff re-alleges and incorporates by reference herein paragraphs 1 through 57 of Count I as paragraphs 1 through 57 of this Count III.

58. The Defendant does not have National Pollution Elimination System Discharge ("NPDES") permit coverage for his facilities and the application sites, nor has he submitted an application for permit coverage.

59. Section 12(f) of the Act, 415 ILCS 5/12(f), provides, in pertinent part, as follows:

No person shall:

> f. Cause, threaten or allow the discharge of any contaminant into the waters of the State, as defined herein, including but not limited to, waters to any sewage works, or into any well or from any point source within the State, without an NPDES permit for point source discharges issued by the Agency under Section 39(b) of this Act, or in violation of any term or condition imposed by such permit, or in violation of any NPDES permit filing requirement established under Section 39(b), or in violation of any regulations adopted by the Board or of any order adopted by the Board with respect to the NPDES program.
>
> * * *
>
> No permit shall be required under this subsection and under Section 39(b) of this Act for any discharge for which a permit is not required under the Federal Water Pollution Control Act, as now or hereafter amended, and regulations pursuant thereto.

60. Section 309.102(a) of the Board's water pollution regulations, 35 Ill. Adm. Code 309.102(a), states, in pertinent part :

NPDES Permit Required

a. Except as in compliance with the provisions of the Act, Board regulations, and the CWA, and the provisions and conditions of the NPDES permit issued to the discharger, the discharge of any contaminant or pollutant by any person into the waters of the State from a point source or into a well shall be unlawful

61. Section 502.101 of the Board's Agriculture Related Pollution Regulations, 35 Ill. Adm. Code 502.101, provides:

> No person specified in Sections 502.102, 502.103 or 502.104 or required to have a permit under the conditions of Section 502.106 shall cause or allow the operation of any new livestock management facility or livestock waste-handling facility, or cause or allow the modification of any livestock management facility or livestock waste-handling facility, or cause or allow the operation of any existing livestock management facility of livestock waste-handling facility without a National Pollutant Discharge elimination System ("NPDES") permit. Facility expansions, production increases, and process modifications which significantly increase the amount of livestock waste over the level authorized by the NPDES permit must be reported by submission of a new NPDES application.

62. Section 502.103 of the Board's Agriculture Related Regulations, 35 Ill. Adm. Code 501.103, provides

> An NPDES permit is required if more than the numbers of animals specified in any of the following categories are confined:

* * *

2,500	Swine weighing over 55 pounds

* * *

1,000	Animal units

63. Section 502.104 of the Board's Agriculture Related Pollution Regulations, 35 Ill. Adm. Code 502.104, provides:

a) An NPDES permit is required if more than the following numbers and types of animals are confined and either condition (b) or (c) below is met:

<u>Number of Animals</u> <u>Kind of Animals</u>

* * *	* * *
750	Swine weighing over 55 pounds
* * *	* * *
300	Animal Units

b) Pollutants are discharged into navigable waters through a man-made ditch, flushing system or other similar man-made device; or

* * *

64. Section 502.106 of the Board's Agriculture Related Pollution Regulations, 35 Ill. Adm. Code 502.106, provides:

a) Notwithstanding any other provision of this Part, the Agency may require any animal feeding operation not falling within Sections 502.201, 502.103 or 502.104 to obtain a permit. In making such designation the Agency shall consider the following facts:

1) The size of the animal feeding operation and the amount of wastes reaching navigable waters;

2) The location of the animal feeding operation relatives to navigable waters;

3) The means of conveyance of animal wastes and process wastewaters into navigable waters;

4) The slope, vegetation, rainfall and other factors relative to the likelihood or frequency of discharge of animal wastes and process wastewaters into navigable waters; and

5) Other such factors bearing on the significance of the pollution problem sought to be regulated.

65. Section 122.23, 40 CFR 122.23, provides, in pertinent part, as follows

Concentrated animal feeding operations

(A) *Scope*. Concentrated animal feeding operations ("CAFOs"), as defined in paragraph (b) of this section or designated in accordance with paragraph (c) of this section, are point sources, subject to NPDES permitting requirements as provided in this section. Once an animal feeding operation is defined as a CAFO for at least one type of animal, the NPDES requirements for CAFOs apply with respect to all animals in confinement at the operation and all manure, litter, and process wastewater generated by those animals or the production of those animals, regardless of the type of animal.

66. Section 122.23 (b)(1), 40 CFR 122.23(b)(1), provides, in pertinent part:

(b) Definitions applicable to this section:

(1) *Animal feeding operation* ("AFO") means a lot or facility (other than an aquatic animal production facility) where the following conditions are met:

(I) Animals (other than aquatic animals) have been, are, or will be stabled or confined and fed or maintained for a total of 45 days or more in any 12-month period, and

(ii) Crops, vegetation, forage growth, or post-harvest residues are not sustained in the normal growing season over any portion of the lot or facility.

67. Section 122.23(b)(2), 40 CFR 122.23(b)(2), provided, in pertinent part:

(2) *Concentrated animal feeding operation* ("CAFO") means an AFO that is defined as a Large CAFO or as a Medium CAFO by the terms of this paragraph, or that is designated as a CAFO in accordance with paragraph (c) of this section. Two or more AFOs under common ownership are considered to be a single AFO for the purposes of determining the number of animals at an operation, if they adjoin each other or if they use a common area or system for the disposal of wastes.

68. Section 122.23 (b)(8), 40 CFR 122.23(b)(1), provides, in pertinent part:

(8) *Production area* means that part of an AFO that includes the animal confinement area, the manure storage area, the raw materials storage area, and the waste containment areas.

69. Section 122.23(d) (1), 40 CFR 122.23(d)(1), provides, in pertinent part:

(d) *Who must seek coverage under an NPDES permit?*

(1) *Permit requirement.* The owner or operator of a CAFO must seek coverage under an NPDES permit if the CAFO discharges . . . . Specifically, the CAFO owner or operator must either apply for an

individual NPDES permit or submit a notice of intent for coverage under an NPDES general permit. If the Director has not made a general permit available to the CAFO, the CAFO owner or operator must submit an application for an individual permit to the Director.

70. Section 122.23(e), 40 CFR 122.23(e), provides, in pertinent part:

e) Land application discharges from a CAFO are subject to NPDES requirements. The discharge of manure, litter or process wastewater to waters of the United States from a CAFO as a result of the application of that manure, litter or process wastewater by the CAFO to land areas under its control is a discharge from that CAFO subject to NPDES permit requirements, except where it is an agricultural storm water discharge as provided in 33 U.S.C.1362(14).

71. The Defendant has indicated that manure waste from any one of his facilities may be moved to other of his facilities, as the manure is needed to meet the Defendant's manure land application contracts. The Defendant thus practices a common system of disposal of wastes and all of his facilities are considered a single CAFO for the purpose of determining animal numbers.

72. The Fehr Brother's Swine Farm is made up of six facilities with a common system of disposal of wastes: Neisler Swine Facility, Jake's Swine Facility, the Red Finisher Swine Facility, Jared's Swine Facility, Toby's Swine Facility, and the Home Farm. The Neisler Swine Facility houses 1800 finishers, Toby's Place houses 1800 finishers and 500 nursery piglets and the Fehr Home Place houses 700 sows. These numbers alone, which do not account for three of the six facilities, represent 4,300 swine over 55 pounds. A facility that confines 2,500 swine over 55 pounds is defined as a Large CAFO.

73. As a Large CAFO, Defendant's swine operation must have coverage under an NPDES permit if it discharges. Defendant has failed to obtain permit coverage for his large CAFO. Defendant discharged from point sources associated with his large CAFO as follows: on February 15 and 16, 2011 from the application site; on February 24, 2011 from various

production area locations at the Neisler Swine Facility, and on November 1 and 3, 2011 during land application from Toby's Place.

74. By causing or allowing the discharge of a contaminant into waters of the State from a point source without an NPDES permit, the Defendant has violated Section 12(f) of the Act, 415 ILCS 5/12(f), and 35 Ill. Adm. Code 309.102(a).

PRAYER FOR RELIEF

WHEREFORE, the Plaintiff, PEOPLE OF THE STATE OF ILLINOIS, respectfully requests that this Court grant the following relief:

A. Find that the Defendant Kenneth W. Fehr, d/b/a Fehr Brothers Swine Farm has violated Section 12(f) of the Act, 415 ILCS 5/12(f), and 35 Ill. Adm. Code 309.102(a);

B. Permanently enjoin the Defendant from further violations of the Act and associated regulations pursuant to Section 42(e) of the Act, 415 ILCS 5/42(e);

C. Pursuant to Section 42(a) of the Act, 415 ILCS 5/42(a), impose upon the Defendant a monetary penalty of not more than the statutory maximum;

D. Pursuant to Section 42(f) of the Act, 415 ILCS 5/42(f), award the Plaintiff its costs in this matter, including reasonable attorney's fees and costs; and

E. Grant such other and further relief as the Court deems appropriate.

COUNT IV

OFFENSIVE CONDITIONS

1-57. Plaintiff re-alleges and incorporates by reference herein paragraphs 1 through 57 of Count I as paragraphs 1 through 57 of this Count IV.

58. Section 302.203 of the Board's Water Pollution Regulations, 35 Ill. Adm. Code 302.203, states, in pertinent part:

> Waters of the State shall be free from sludge or bottom deposits, floating debris, visible oil, odor, plant or algal growth, color or turbidity of other than natural origin.

59. The February 14, 15 and 16, 2011 and November 1 and 3, 2011 discharges from Defendant's manure land application operations caused waterways tributary to Panther Creek and Panther Creek to be turbid, contain foam, be of an unnatural dark color and to have a swine waste odor.

60. By applying swine manure in such a manner as to allow a discharge and cause turbidity, unnatural coloration, foam and swine waste odor in waters tributary to Panther Creek and Panther Creek itself, the Defendant has violated Section 12(a) of the Act, 415 ILCS 5/12(a), and Section 302.203 of the Board's Water Pollution Regulations, 35 Ill.Adm. Code 302.203.

PRAYER FOR RELIEF

WHEREFORE, the Plaintiff, PEOPLE OF THE STATE OF ILLINOIS, respectfully requests that this Court grant the following relief:

A. Find that the Defendant Kenneth W. Fehr, d/b/a Fehr Brothers Swine Farm has violated Section 12(a) of the Act, 415 ILCS 5/12(a), and 35 Ill. Adm. Code 302.203;

B. Permanently enjoin the Defendant from further violations of the Act and associated regulations pursuant to Section 42(e) of the Act, 415 ILCS 5/42(e);

C. Pursuant to Section 42(a) of the Act, 415 ILCS 5/42(a), impose upon the Defendant a monetary penalty of not more than the statutory maximum;

D. Pursuant to Section 42(f) of the Act, 415 ILCS 5/42(f), award the Plaintiff its costs in this matter, including reasonable attorney's fees and costs; and

E. Grant such other and further relief as the Court deems appropriate.

COUNT V

AGRICULTURE RELATED POLLUTION VIOLATIONS

1-57. Plaintiff re-alleges and incorporates by reference herein paragraphs 1 through 57 of Count I as paragraphs 1 through 57 of this Count V.

58. Section 501.405 of the Board's Agriculture Related Pollution Regulations, 35, Ill. Adm. Code 501.405, states, in pertinent part:

> a) The quantity of livestock waste applied on soils shall not exceed a practical limit as determined by soil type, especially its permeability, the condition (frozen or unfrozen) of the soil, the percent slope of the land, cover mulch, proximity to surface waters and likelihood of reaching groundwater, and other relevant considerations. . . .

59. Section 560.206 of the Board's Agriculture Related Pollution Regulations, 35 Ill. Adm. Code 560.206, state, in pertinent part:

> Waste application on frozen or snow-covered land should be avoided. . . .

60. Section 506.204(g)(4) of regulations promulgated under the Illinois Livestock Facilities Management Act, 35 Ill. Adm. Code 506.204 (g) (4), requires that two (2) foot of freeboard be maintained in livestock waste lagoons, as provided in pertinent part as follows:

> 4) In addition to the lagoon's total design volume, a freeboard shall be provided as follows:
>
> A) For lagoons service a livestock management facility with a maximum design capacity of less than 300 animal units and not collecting runoff from areas of than the exposed surface fo the lagoon (including associated interior berm slopes and flat berm top areas), the top of the settled embankment shall be not less than 1 foot above the fluid surface level of the lagoon total design volume, or
>
> B) For all other lagoons, the top of the settled embankment shall be not less than 2 feet above the fluid surface level of the lagoon total design volume.

61. Section 580.105(a) of the Board's Livestock Waste Reporting Regulations, 35 Ill.

Adm. Code 580.105(a), provides as follows:

> Method of Reporting a Release of Livestock Waste.
>
> a. An owner or operator of a livestock waste handling facility shall report any release of livestock waste from the livestock waste handling facility or from the transport of livestock waste by means of transportation equipment within 24 hours after the discovery of the release. Report of releases to surface waters, including to sinkholes, drain inlets, broken subsurface drains or other conduits to groundwater or surface waters, shall be made upon discovery of the release, except when such immediate notification will impede the owner's or operator's response to correct the cause of the release or to contain the livestock waste, in

62. The Defendant did not report the February 2011 land application runoff release to the Illinois Emergency Management Agency until told to do so by an Illinois EPA inspector. The Defendant did not report the November 1, 2011 waste release to the Illinois Emergency Management Agency. The Defendant left a message at a regional office after hours.

63. At the time of an inspection conducted on February 15, 2011, there was less than 2 feet of available freeboard in the Red Finisher Swine Facility livestock wastewater lagoon. At the time of an inspection conducted on February 24, 2011 at the Neisler Swine Farm, there was less than 2 feet of available freeboard in the facility's livestock waste lagoon.

64. By land applying waste on soil conditions, namely frozen, snow covered ground, and over applying waste that resulted in the runoff of waste to waters of the State, the Defendant has violated Section 12(a) and (d) of the Act, 415 ILCS 5/12(a), (d), and 35 Ill. Adm. Code 501.405.

PRAYER FOR RELIEF

WHEREFORE, the Plaintiff, PEOPLE OF THE STATE OF ILLINOIS, respectfully requests that this Court grant the following relief:

A. Find that the Defendant Kenneth W. Fehr, d/b/a Fehr Brothers Swine Farm has violated Section 12(a) of the Act, 415 ILCS 5/12(a) and 35 Ill. Adm. Code 501.405;

B. Permanently enjoin the Defendant from further violations of the Act and associated regulations pursuant to Section 42(e) of the Act, 415 ILCS 5/42(e);

C. Pursuant to Section 42(a) of the Act, 415 ILCS 5/42(a), impose upon the Defendant a monetary penalty of not more than the statutory maximum;

D. Pursuant to Section 42(f) of the Act, 415 ILCS 5/42(f), award the Plaintiff its costs in this matter, including reasonable attorney's fees and costs; and

E. Grant such other and further relief as the Court deems appropriate.

Respectfully submitted,

PEOPLE OF THE STATE OF ILLINOIS,
ex rel. LISA MADIGAN,
Attorney General of the
State of Illinois

MATTHEW J. DUNN, Chief
Environmental Enforcement/Asbestos
Litigation Division

BY:____________________________
THOMAS DAVIS, Chief
Environmental Bureau
Assistant Attorney General

OFCOUNSEL
JANE E. MCBRIDE
500 South Second Street
Springfield, Illinois 62706
217/782-9031
Dated: December 20, 2011

Attachment 17:

Concentrated Animal Feeding Operations, Row Crops, and Their Relationship to Nitrate in Eastern Iowa Rivers
(Weldon and Hornbuckle 2006)

Environ. Sci. Technol. **2006**, *40*, 3168−3173

Concentrated Animal Feeding Operations, Row Crops, and Their Relationship to Nitrate in Eastern Iowa Rivers

MARK B. WELDON AND
KERI C. HORNBUCKLE*
University of Iowa, Department of Civil and Environmental Engineering, SC 4105, Iowa City, Iowa 52240

Concentrated animal feeding operations (CAFO) and fertilizer application to row crops may contribute to poor water quality in surface waters. To test this hypothesis, we evaluated nutrient concentrations and fluxes in four Eastern Iowa watersheds sampled between 1996 and 2004. We found that these watersheds contribute nearly 10% of annual nitrate flux entering the Gulf of Mexico, while representing only 1.5% of the contributing drainage basin. Mass budget analysis shows streamflow to be a major loss of nitrogen (18% of total N output), second only to crop harvest (63%). The major watershed inputs of nitrogen include applied fertilizer for corn (54% of total N input) and nitrogen fixation by soybeans (26%). Despite the relatively small input from animal manure (~5%), the results of spatial analysis indicate that row crop and CAFO densities are significantly and independently correlated to higher nitrate concentration in streams. Pearson correlation coefficients of 0.59 and 0.89 were found between nitrate concentration and row crop and CAFO density, respectively. Multiple linear regression analysis produced a correlation for nitrate concentration with an R^2 value of 85%. High spatial density of row crops and CAFOs are linked to the highest river nitrate concentrations (up to 15 mg/L normalized over five years).

Introduction

Heavily agricultural regions in the central U.S. often suffer from high concentrations of nutrients in surface waters (*1*, *2*). Surface water impairments from high nutrient concentrations include human health risks for consumption, elevated costs for water treatment, anoxia, and reduced biological diversity (*3*). Iowa is a prime example of this situation because Iowa's rivers have among the highest nitrate and phosphorus concentrations in the central U.S. (see Supporting Information Figure S-1). Nitrate is not efficiently removed by conventional drinking water treatment, and as a result, the Des Moines Water Works activates a nitrate removal system during times of potentially high nitrate in their source water from the Des Moines and Racoon Rivers (*4*). Iowa waters discharge to the Mississippi River where elevated nutrients cause an extensive region of low dissolved oxygen concentrations in the northern Gulf of Mexico near the Louisiana coast (*5*). In the Gulf of Mexico, the hypoxic zone has devastated local shellfish populations and driven fish populations to other waters (*6*). Goolsby et al. estimated nitrogen flux to the Gulf of Mexico to be 1.6 million metric tons per year (61% nitrate, 37% organic nitrogen, 2% ammonium) and phosphorus flux to be 136 000 metric tons per year (69% particulate or organic, 31% dissolved orthophosphate) (*1*).

Riverine nitrate fluxes are closely linked to anthropogenic inputs of nutrients to the watershed, over both spatial and temporal scales. For the lower Mississippi River system, McIsaac et al. showed that almost all (up to 95%) of the temporal variability in nitrate flux between 1960 and 1998 can be explained by variations in the net anthropogenic nitrogen input (*7*, *8*). Libra et al. performed a similar accounting of nitrogen and phosphorus but for the smaller study area of Iowa watersheds (*9*). Total nitrogen input was most strongly correlated with stream nitrate concentration although the use of chemical fertilizer and the percentage of row crops in the watershed were significant. Arbuckle and Downing have shown that that nitrogen:phosphorus ratios in Iowa lakes are linked to the spatial distribution of row crop and pasture land use in watersheds (*10*). Schilling and Libra developed a model correlating stream nitrate concentration as a function of row crop land use percentage (*11*). McIsaac and Hu showed that the presence or absence of tile drainage can be associated with variations of nutrient fluxes in surface waters (*12*). It is clear that land use for row crops is widely associated with increased nutrient concentrations and fluxes.

Agriculture is more than just row crops. Pasture-based livestock has traditionally been a significant component of the agricultural landscape. Recently however, concentrated animal feeding operations (CAFOs) have begun to replace pasture-based livestock operations, beginning in the 1950s for cattle and poultry and in the 1970s for swine (*13*). Manure management from CAFOs includes application to nearby crop land and may be applied at a higher rate than can be assimilated by crop requirements (*13*, *14*). This may result in increased fluxes and concentrations of nutrients in surface waters. Manure spills from CAFO waste storage failures also lead to significant pollution events into natural water bodies (*15*). In North Carolina, hurricanes have caused catastrophic failures and tremendous nutrient releases from CAFO waste storage systems (*15*). We know of no studies, however, that examine the relationship between water quality and typical (long-term) operations of CAFOs within the agricultural landscape.

The purpose of this study is to determine if CAFOs have an impact on river nutrient content that can be isolated through a spatial analysis of land use and water quality data. Iowa is an ideal place to study the relationship between CAFOs and water quality. It is a heavily agricultural state and recently led the nation in agricultural production of corn, soybeans, hogs and eggs (*16*). There are more than 3800 CAFOs in Iowa, and these operations are becoming more concentrated spatially (*17*).

Experimental Section

Our study area, the Eastern Iowa Study Area (EISA), includes the Cedar, Iowa, Skunk, and Wapsipinicon river basins. It is essentially the same area used in U.S. Geological Survey (USGS) water quality assessments (*18*, *19*) and identified there as EIWA. The study area is large enough to include the human activities and ecological functions that are of interest in this research. It contains enough monitoring stations to allow comparisons both within and between watersheds. Figure 1

* Corresponding author phone: (319) 384-0789; fax: (319) 335-5660; e-mail: keri-hornbuckle@uiowa.edu.

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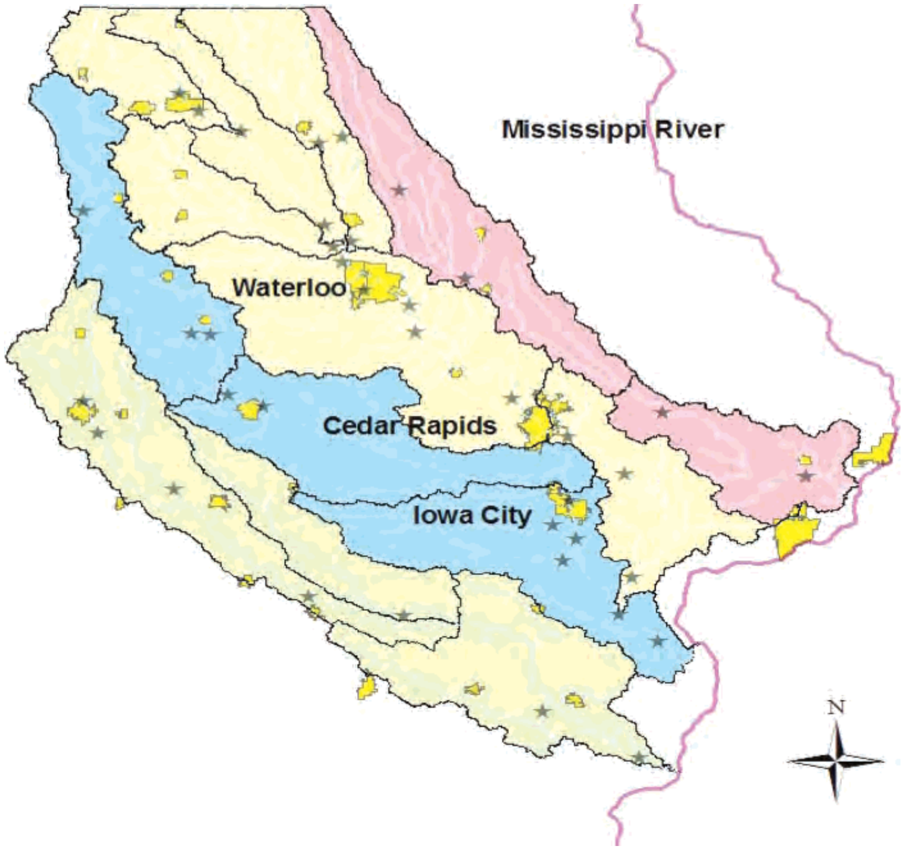


FIGURE 1. Eastern Iowa Study Area. The four major watersheds include the Wapsipinicon (pink), the Cedar (pale yellow), the Iowa (blue), and the Skunk (light green). The monitoring stations are shown by stars and urban areas are shown in bright yellow.

TABLE 1. Watershed Comparison of the Number of Monitoring Stations and the Average Number of Months of Data Represented

watershed	no. of monitoring stations	avg. months of data
Cedar	16	58.2
Iowa	11	61.9
Skunk	7	60.0
Wapsipinicon	4	62.8

shows the EISA including urban areas, monitoring stations, and rivers.

We identified 37 monitoring stations within the EISA that have a temporal data record of at least 40 months. We also include one station, the Skunk River at Augusta with 31 months of data, because of its location near the mouth of the Skunk River representing the entire Skunk River basin. This gives us a total of 38 monitoring stations and one station in each basin that is representative of the entire basin. Table 1 shows the number of monitoring stations in each of the four watersheds and their average temporal records. Water samples are typically taken once per month so we required a 40 month record to lessen the impacts of short-term weather variability. Since most sites had 5 years of data, we normalized mass fluxes on a 60 month basis to allow comparison between stations with differing temporal record lengths.

The 38 monitoring stations are distributed as a network over the landscape. We segregated the data from the 38 stations into independent and dependent stations. We categorized stations located on the upper reaches of the river systems, with no other stations above them, as independent. Stations downstream of other stations are dependent as water samples taken from these stations may be influenced by conditions at upstream stations. Seventeen of the stations in our study area are independent, and they are the focus of this study.

We hypothesized that CAFOs and nutrient concentrations and fluxes were positively correlated and the relationship could be determined independent of other major sources of nutrients. Our methodological approach was organized into five steps: Water quality data database development; nutrient flux analysis; creation of landscape variables; nitrogen mass budget; and statistical correlation and regression analysis.

River water quality data in the form of nutrient concentrations and flows were available from the Iowa Department of Natural Resources' (IDNR) STORET Ambient Water Quality Database and from USGS reports. From this raw data, we produced estimates of mass fluxes, concentrations, and mean streamflows. Our data cover the time period from 1996 to 2004 with the majority of the data collected in the 1999−2004 time period. Both nutrient concentrations and mass fluxes are of substantial interest. Nutrient concentrations are the primary factors when considering the quality of aquatic habitat or drinking water sources. Mass fluxes are the primary factors when considering issues such as nutrient transport to the Gulf of Mexico.

Samples for the STORET program are collected and analyzed by the University of Iowa Hygienic Laboratory (UHL). Samples are collected by an individual visiting each site and collecting a grab sample. Flow measurement at the time of sampling is made either by reference to a nearby gauging station or by manual measurement following established IDNR procedures. Quality assurance and quality control guidelines are established as a normal part of UHL operations (See Table S-1 in the Supporting Information for method references and uncertainties). Approximately 10−15% of the samples collected are blank or split samples for the purpose of monitoring measurement procedures and techniques.

TABLE 2. Nitrate and Total Phosphorus Flux and Concentration from the EISA

river	reference	mean flowrate (m^3/s)	NO_3 Flux (MT/yr)	NO_3 conc (mg/L)	total P Flux (MT/yr)	total P conc (mg/L)
Cedar R. at Waterloo	this study[a]	82.5	18 851	7.23	937	0.36
	Goolsby et al.[b]	122.3	18 014	4.67	887	0.23
Cedar R. near Conesville	this study[a]	140.2	30 932	7.00	2,250	0.51
	Libra et al.[c]		37 019	NR	1,461	NR
Cedar and Iowa combined at Wapello	this study[a]	281.5	59 856	6.74	4397	0.50
	Goolsby et al.[b]	288.9	44 573	4.99	2826	0.31
Iowa R. at Columbus Jct.	this study[a]	69.6	28 924	6.74	2147	0.50
	Libra et al.[c]		11 475		565	
Skunk R. at Augusta	this study[a]	107.1	22 123	6.55	3517	1.04
	Goolsby et al.[b]	92.6	17 280	4.23	1338	0.30
	Libra et al.[c]		10 664		540	
Wapsipinicon R. at De Witt	this study[a]	42.5	9534	7.11	481	0.36
	Libra et al.[c]		13 378		460	
EISA total	this study[a]		91 513		8395	
	Libra et al.[c]		75 236		3026	

[a] This study includes data from 1996 through 2004. [b] Goolsby et al. includes data from 1980 through 1996. They report results for the Cedar River at Waterloo, the Cedar and Iowa Rivers combined, and the Skunk River. They do not report results for the Wapsipinicon River. [c] Libra et al. include data from 1999 through 2001. They do not report nutrient concentrations.

To create landscape variables, we first delineated the drainage area associated with each monitoring station. This was done within a geographic information system (ESRI Arc Suite version 9.0) by overlaying the stream network and monitoring station locations on the level 12 HUC watershed map. The level 12 HUC maps represent a fine scale of resolution. Starting with the monitor furthest upstream on the stream network, level 12 basins were selected that drain to the selected station. This collection of areas was saved as a new file to be used later as an identification template. This process continued for each station downstream on the network, with previously selected areas excluded from the area for the station under consideration. This process delineated each watershed into areas uniquely associated with one monitoring station.

Landscape variables were created by identifying and counting land usage types, including agricultural activities, that occur within the boundaries of each monitoring station drainage area. Land use data is categorized into 17 different land use types including wetlands, forested areas, cropland, urban areas, etc. (see Table S-2 in the Supporting Information for a complete list). These data is available from the IDNR as a GIS grid file with 15 m $\times$ 15 m resolution and $\pm$30 m accuracy. It is based on satellite imagery from Landsat 5 and Landsat 7 taken from May 13, 2002 through May 27, 2003. Two images were acquired for each area, one from a spring time frame, the second from a summer date. Nearly cloud-free images were acquired in almost all cases. Using the GIS, the monitoring basins are overlaid on the land use grid such that the land use grid cells can be counted for each land use category within each monitoring station basin. The number of CAFOs are counted in a similar fashion, except the monitoring basin boundaries are overlaid on a different GIS file which contains CAFO location and size data. This file includes the number and type of animals raised at each CAFO. Landscape variables were divided by the total acreage for each station area to calculate density values for each landscape variable.

We created a mass budget for nitrogen in the EISA based on preliminary results indicating nitrate to be the most highly correlated water quality component in our dataset. Nitrogen input and output fluxes were estimated for each monitoring station drainage basin using landscape information gathered in the creation of the landscape variables. Nitrogen inputs included inorganic fertilizer, manure, nitrogen fixation, and deposition. Outputs included the nitrogen content of crops, nitrogen loss through volatilization mechanisms and nitrogen leached to streams. Our analysis centered on agricultural land and considered the main nitrogen fluxes that were applied to the land or evolved from the land. We did not include microbial nitrogen transformations that occur within soil due to the small net effect of these activities (*8*, *9*, *12*).

Finally, we linked the water quality data with the landscape variables. The data were checked for correlation between water quality parameters and landscape variables. Highly correlated parameters were further analyzed via multiple regression analysis to identify the strength of relationship between the landscape variable and water quality parameter.

Results and Discussion

Database Development. Our water quality database for the EISA contains streamflow and mass flux and concentration estimates for elemental phosphorus, orthophosphate, total suspended solids, total Kjeldahl nitrogen, nitrate plus nitrite, ammonia nitrogen, organic nitrogen, total nitrogen, and total phosphorus. Nutrient concentration is reported as mg/L of the nutrient (N or P). The database includes monthly sample information for 38 water monitoring stations with an average temporal record length of 60.1 months (standard deviation = 11.3 months). There are a few instances of multiple samples in a month. In these cases, the data are averaged to produce a single monthly mass flux estimate. Station mass flux estimates are summed over the length of the data record and then normalized to a 60-month basis by multiplying the summed flux by 60 and dividing by the number of months in the data record. This was done to facilitate comparisons between stations. Station concentration estimates are calculated by dividing the summed mass flux by the total streamflow.

Nutrient Flux Analysis. Our nutrient flux analysis shows that the EISA is a major contributor of nitrate to the Gulf of Mexico, exporting approximately 91 000 metric tons per year. Table 2 shows our estimates of average annual nitrate and total phosphorus flux and concentration from the four river basins of the EISA along with comparable estimates from Goolsby et al. (*1*) and Libra et al. (*9*). Estimates are reported at multiple locations for the Cedar and Iowa Rivers in order to offer direct comparison with estimates from Goolsby et al. and Libra et al.

The flux estimates show reasonable agreement, considering that they represent three different time periods. Our data

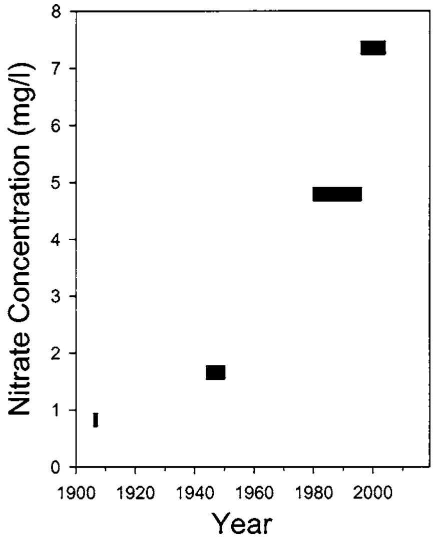


FIGURE 2. Average concentration of nitrate concentrations in the Cedar River. The samples collected between 1996 and 2004 are the data described in this study. All other data is from Goolsby et al., 1999 (1).

comes from the 1996−2004 time period, the data of Goolsby et al. come from the 1980−1996 time period, and the data for Libra et al. was based on data from 1999 to 2001. Our concentration data show a significant increase when compared to Goolsby et al. While our research is not specifically targeted toward temporal differences, the data suggest that nutrient concentrations in our study area have become greater over time, which is consistent with Goolsby et al.'s reported historical increase in nitrate concentration in the Cedar River in the 20th century (Figure 2).

The EISA total of 91 513 metric tons per year (MT/yr) of nitrate can be compared to Goolsby's estimate for nitrate flux for the entire Mississippi and Atchafalaya River basin (MARB) of 950 000 MT/yr (1.6 million MT/yr, with 61% being nitrate). Therefore, as much as 9.6% of the annual nitrate flux entering the Gulf from the Mississippi River can be attributed to the EISA, while the EISA represents only 1.5% of the land area of the MARB. Likewise, the EISA total phosphorus flux of 8395 MT/yr represents over 6% of Goolsby's estimate of 136 000 MT/yr for the MARB.

Nitrogen Budget. Our nitrogen budget is based on the land use within each sub-watershed. Knowing the number of acres planted to different crops, and knowing the location and size of CAFOs, permitted us to estimate nitrogen fluxes to and from each hectare of land within the sub-watershed. We used rate factors that were applicable to the EISA, as reviewed and reported by Libra et al. (*9*), Illinois data from McIsaac et al. (*8*), the United States Department of Agriculture (*20*), and the IDNR. Rate factors and literature source for each of the flux categories are listed in the Supporting Information (Tables S-3 and S-4) and briefly summarized here. Fertilizer nitrogen was based on application to all corn acres within each sub-watershed at a rate of 150 lbs. N per acre, as reported to the IDNR. We assumed no fertilizer was applied to soybeans. Manure nitrogen ranged from 0.003 lb N/d for chickens to 0.7 lbs/d for dairy cows (*9*). We estimated nitrogen flux from manure for each sub-watershed by multiplying these factors by the number of animals at each CAFO and then by suitable loss factors to account for nitrogen lost to the atmosphere from the CAFO buildings and from the application of manure. Nitrogen fixation was based on factors for soybeans (100 kg/ha), alfalfa (200 kg/ha), and hay (100 kg/ha) (*9*). Nitrogen deposition was estimated as the sum of wet and dry deposition mechanisms (*21*).

Nitrogen export in crops was based on the average crop yields from 1988 to 2004 (*20*) multiplied by the nitrogen content of each crop. Nitrogen loss through volatilization was based on crop senescence emissions, fertilizer application emissions, and manure application emissions of nitrogen (*8*, *9*). Nitrogen leaching to streams was calculated from our EISA water quality database.

Figure 3 shows the EISA nitrogen balance and fair agreement between inputs and outputs. The largest fluxes are fertilizer application and nitrogen fixation for the inputs and nitrogen export in crops for the outputs. Manure application represents a minor flux in this mass balance. Nitrogen lost to streamflow is significant at 18% of the total output. Total annual nitrogen inputs is expected to fall within the range 9.6−12.2 metric tons per square kilometer (MT/km^2), while total annual nitrogen outputs is expected to fall within the range 12.7−14.8 MT/km^2. The net mean imbalance is 136 000 MT/yr excess annual exports.

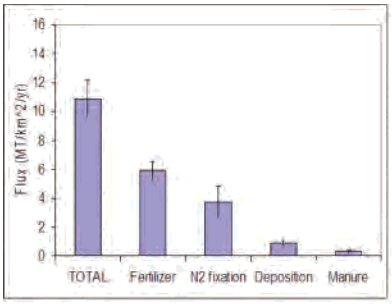
The uncertainty factors included within Figure 3 are intended to show the relative uncertainty for each flux category and represent the authors' best judgment. The observed imbalance between inputs and outputs (21% $\pm$ 12%) is large relative to that which might be expected to arise from the component errors. This suggests that there may be errors in the rate factors used or that there may be an unidentified input flux. We believe that most of the uncertainty lies in the fixation category among the inputs and the volatilization category among the outputs. It is also possible that local redeposition of atmospheric emissions from CAFO buildings and waste storage lagoons is a significant unidentified input flux to the budget.

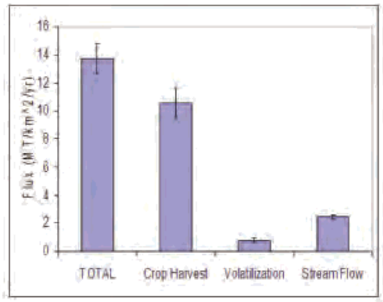
The nitrogen budget shows manure to be a small factor in the overall budget and far overshadowed by fertilizer applied to corn and nitrogen fixed by legumes. This is somewhat contradictory to the results of correlation and regression analyses which will show CAFOs and animal units to be important factors in explaining nitrogen concentration in rivers.

Correlation Analysis. Correlation analysis was employed to select landscape variables with strong Pearson Correlation Coefficients (PCC) corresponding to probability values (*P*-value) of 5% or less. Examination of parameter concentrations indicated a strong relationship between nitrate and the number of CAFOs (PCC = 0.627, *P*-value = 0.007), animal units (PCC = 0.756, *P*-value = 0.000), CAFO density (PCC = 0.885, *P*-value = 0.000), animal unit density (PCC = 0.891, *P*-value = 0.000)and row crop density (PCC = 0.585, *P*-value = 0.014). Total nitrogen is also correlated with these variables. Row crop density is negatively correlated with ammonia (PCC = −.559, *P*-value = 0.020), Elemental phosphorus (PCC = −.608, *P*-value = 0.010), Total phosphorus (PCC = −.583, *P*-value = 0.014) and Total suspended solids (PCC = −.663, *P*-value = 0.004).

Correlation analysis of parameter mass fluxes indicated a moderate relationship between nitrate and row crop acres (PCC = 0.537, *P*-value = 0.026). Once again row crop density is negatively correlated with elemental phosphorus (PCC = −.461, *P*-value = 0.010) and total suspended solids (PCC = −.598, *P*-value = 0.011). In general, correlation values are stronger for concentration data than for mass flux data (See Table S-5 and S-6 in the Supporting Information).

The high correlation between nitrate concentration and animal unit density suggest that CAFOs produce measurable impacts to water quality. Of all the water quality parameters examined, nitrate was found to be the most responsive to livestock and row crop agricultural activities at the watershed scale. This led us to choose nitrate as the dependent variable for multiple regression analysis. Correlation analysis also indicated that the agricultural variables of livestock production and row crop acreage are best represented as respective





Nitrogen Inputs (MT/km^2/yr) →

Fertilizer	Corn	5.92 +/- 0.59
	Soybeans	0.00 +/- 0.00
Fixation	Soybeans	2.82 +/- 0.85
	Alfalfa	0.50 +/- 0.15
	Hay	0.42 +/- 0.13
Deposition	Wet	0.52 +/- 0.10
	Dry	0.37 +/- 0.07
Manure	Cattle	0.04 +/- 0.01
	Swine	0.21 +/- 0.04
	Chickens	0.06 +/- 0.01
	Turkey	0.02 +/- 0.01

→ Nitrogen Outputs (MT/km^2/yr)

Crop Harvest	Corn	4.49 +/- 0.45
	Soybeans	4.74 +/- 0.47
	Alfalfa	0.50 +/- 0.05
Volatilization	Crops	1.87 +/- 0.37
	Fertilizer Application	0.59 +/- 0.12
	Manure Application	0.04 +/- 0.01
Stream Flow		2.43 +/- 0.24

FIGURE 3. EISA nitrogen budget results.

densities, and the regression analysis uses them in this form. While this transformation improves correlation moderately for livestock it improves correlation markedly for row crops. These improvements related to density suggest that nitrate fate, flux, and flow paths are influenced by local landscape characteristics and that it is possible to overwhelm the landscape's potential for assimilating nitrate before it reaches the river network.

Except for ammonia (present as dissolved ammonium ion), the water quality parameters that have negative correlations with row crop density are particle associated parameters. This suggests that soil conservation measures on intensively managed croplands may be having a beneficial impact. The ammonia results may also be related to soil conservation since oxidation of ammonia to nitrite and nitrate occurs in the soil.

Regression Analysis. Regression analysis was performed on nitrate concentration and CAFOs, animal unit, and row crop densities. Simple linear regression showed a strong relationship between nitrate concentration and animal unit density ($R^2 = 79.4\%$) and a moderate relationship for nitrate concentration and row crop density ($R^2 = 34.2\%$). We then employed multiple linear regression analysis to examine both animal unit density and row crop density together. This improved the strength of the relationship by accounting for approximately 5% more of the variability. The regression equation and R^2 value in this case are shown in eq 1:

$$\text{Nitrate concentration (mg/L)} = 2.39 + 7.65 \text{ animal unit density} + 7.01 \text{ row crop density } R^2 = 84.8\%$$

Equation 1 explicitly recognizes the modern duality of row crop agriculture and CAFOs, much like Arbuckle and Downing's (*10*) consideration of row crop and pasture lands. While the percentages of land in row crops and pasture are inversely related, modern livestock operations (CAFOs) typically vary directly with row crop density out of the practical concern of manure disposal. Equation 1 also complements and extends Schilling and Libra's (*11*) finding that river nitrate concentration can be approximated as 0.1 times the watershed's row crop percentage.

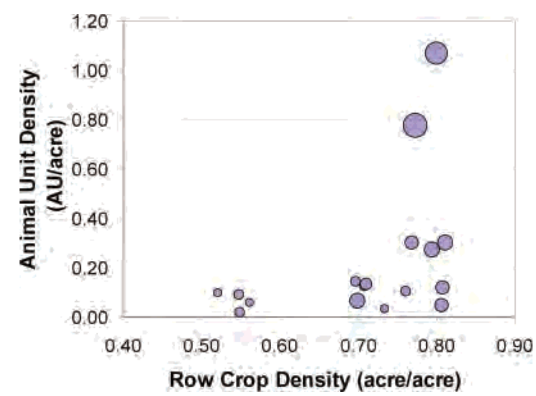


FIGURE 4. Nitrate concentration (mg/L) for 17 independent EISA monitoring stations. The smallest point represents 6.35 mg/L. The largest point represents 15.8 mg/L. See also Table S-7 (Supporting Information).

The relationship between nitrate, animal unit density, and row crops is displayed in Figure 4. The 5 year normalized nitrate concentration clearly increases with both animal unit and row crop densities, and exceeds the U.S. Environmental Protection Agency limit of 10 mg/L for drinking water (*22*) in some cases.The two sub-watersheds in the upper right of Figure 4 have nitrate concentrations greater than 15 mg/L. In our data set, animal unit density ranges from near zero to 1.07, and row crop density ranges from 0.47 to 0.81. Higher animal unit densities occur only at high row crop density, whereas high row crop density occurs at high or low animal unit density. The situation of high animal unit density at low row crop density does not occur in our data set.

Correlation and regression analyses point to animal unit density, and therefore, CAFO density as a prime indicator of nitrate concentration in streams. This stands in marked contrast to the nitrogen budget analysis which did not identify manure as a large factor in the total budget. One possible explanation to this apparent contradiction is that manure application practices permit excessive leaching of nitrogen to streams. Other possible explanations are that local atmospheric deposition of nitrogen from CAFO buildings is a significant nitrogen pathway or that plant uptake of nitrogen from manure is somehow inhibited. These issues of manure nitrogen management have been discussed by other researchers (*13*, *14*, *23*) as well and warrant further quantitative investigation.

The development of a water quality database linked to agricultural parameters by stream monitoring station allows us to analyze the impacts of local agricultural practices within the EISA. For example, drainage tiling has been shown to be an important factor in nitrogen flux to streams from some agricultural lands (*12*). We considered tiling as a potential landscape variable for this research but were unable to obtain a data set of sufficient accuracy at the sub-watershed scale to warrant its inclusion. With further refinement, however, an accurate dataset of tiled lands could be incorporated into the database.

Our research suggests that restricting nitrate to no more than 10 mg/L in eastern Iowa rivers may require a combined limitation of livestock and row crop agricultural densities. In our study area, the two instances of very high animal unit density were associated with nitrate concentrations above 15 mg/L. High row crop densities were associated with nitrate concentrations in the 9−10 mg/L range. At animal unit densities less than 0.2 and row crop densities less than 0.6, nitrate concentrations were in the 6−7 mg/L range.

Agricultural densities are not currently used as a decision making tool with regard to permitting agricultural activities under Iowa law. Rather, Iowa law focuses on local conditions for CAFO permitting, typically separation distances between CAFO sites and other land uses such as residences, public buildings, water or agricultural drainage wells, and streams (*24*). State requirements do include preparation of manure management plans indicating the availability of land for manure application. This local focus, however, does not fully account for spatial concentrations of agricultural activities that we found to be important. Consideration of the agricultural−environmental linkages at the watershed scale may be a beneficial addition to our current regulatory approach to CAFOs.

Acknowledgments

We thank the Iowa Department of Natural Resources, particularly the Iowa City Geologic Survey Office for providing basic water quality and GIS data sets. We are also grateful to the U.S. Geological Survey and the University of Iowa Hygienic Laboratory for water quality information. Technical assistance was provided by the University of Iowa Statistical Consulting Center. We appreciate the financial support of the University of Iowa Graduate College.

Supporting Information Available

One figure and seven tables are included as supporting information. Figure S-1 illustrates mean nitrate concentration for 24 rivers in the Mississippi-Atchafalaya River basin; Table S-1 lists water sample measurements, test methods and uncertainties for this study; Table S-2 lists the land cover categories used in the study; Table S-3 and S-4 show rate factors used in the nitrogen mass budget; Tables S-5 and S-6 show Pearson correlation coefficients; and Table S-7 provides mean nitrate concentrations for the 38 stations studied here. This material is available free of charge via the Internet at http://pubs.acs.org.

Literature Cited

(1) Goolsby, D. A.; Battaglin, W. A.; Lawrence, G. B.; Artz, R. S.; Aulenbach, B. T.; Hooper, R. P.; Keeney, D. R.; Stensland, G. J. Flux and Sources of Nutrients in the Mississippi-Atchafalaya River Basin (Report of Task Group 3 to the White House committee on Environment and Natural Resources, Hypoxia Work Group, *Fed. Regist.* **1999** 0097−6326.

(2) U.S. Department of the Interior. *The Quality of Our Nation's Waters;* U.S. Geological Survey, Circular 1225; U.S. Government Printing Office: Washington, DC, 1999.

(3) Carpenter, S. R.; Caraco, N. F.; Correll, D. L.; Howarth, R. W.; Sharpley, A. N.; Smith, V. H. Nonpoint pollution of surface waters with phosphorus and nitrogen. *Ecol. Appl.* **1998**, *8*, 559−568.

(4) Des Moines Water Works 2003 Consumer Confidence Report. www.dmww.com.

(5) Rabalais, N. N.; Wiseman, W. J.; Turner, R. E.; SenGupta, B. K.; Dortch, Q. Nutrient changes in the Mississippi River and system responses on the adjacent continental shelf. *Estuaries* **1996**, *19*, 386-407.

(6) Rabalais, N. N.; Turner, R. E.; Wiseman, W. J. Gulf of Mexico Hypoxia, A. K. A. The Dead Zone. *Annu. Rev. Ecol. Sys.* **2002**, *33*, 235−263.

(7) McIsaac, G. F.; David, M. B.; Gertner, G. Z.; Goolsby, D. A. Eutrophication−Nitrate flux in the Mississippi river. *Nature* **2001**, *414*, 166−167.

(8) McIsaac, G. F.; David, M. B.; Gertner, G. Z.; Goolsby, D. A. Relating net nitrogen input in the Mississippi River basin to nitrate flux in the lower Mississippi River: A comparison of approaches. *J. Environ. Qual.* **2002**, *31*, 1610−1622.

(9) Libra, R. D.; Wolter, M.; Langel, R. J. *Nitrogen and Phosphorous Budgets for Iowa and Iowa Watersheds;* Iowa Geological Survey Technical Information Series 47; Iowa Department of Natural Resources−Geological Survey: Iowa City, IA, December 2004. http://www.igsb.uiowa.edu/gsbpubs/pdf/TIS-47.pdf.

(10) Arbuckle, K. E.; Downing, J. A. The influence of watershed land use on lake N: P in a predominantly agricultural landscape. *Limnol. Oceanogr.* **2001**, *46*, 970−975.

(11) Schilling, K. E.; Libra, R. D. The relationship of nitrate concentrations in streams to row crop land use in Iowa. *J. Environ. Qual.* **2000**, *29*, 1846−1851.

(12) McIsaac, G. F.; Hu, X. T. Net N input and riverine N export from Illinois agricultural watersheds with and without extensive tile drainage. *Biogeochemistry* **2004**, *70*, 251−271.

(13) Mallin, M. A.; Cahoon, L. B. Industrialized animal production − A major source of nutrient and microbial pollution to aquatic ecosystems. *Popul. Environ.* **2003**, *24*, 369−385.

(14) Keeney, D. R.; Gilbert, E. M. Swine manure management plans in north-central Iowa: Nutrient loading and policy implications. *J. Soil Water Cons.* **2000**, *55*, 205−212.

(15) Mallin, M. A. Impacts of industrial animal production on rivers and estuaries. *American Scientist* **2000**, *88*, 26−37.

(16) U.S. Department of Agriculture National Agricultural Statistics Service, Bulletins 980a, 982, 986. http://www.nass.usda.gov/nass/pubs/histdata.htm.

(17) U.S. Department of Agriculture National Agricultural Statistics Service, Bulletin 1002. http://www.nass.usda.gov/nass/pubs/histdata.htm.

(18) Akers, K.; Schnoebelen, D.; Savoca, M.; Roberts, L.; Becher, K. *Water Quality Assessment of the Eastern Iowa Basins: Hydrologic and Biologic Data, September 1995 through September 1996,* United States Geological Survey, USGS Open-File Report 99-66; U.S. Government Printing Office: Washington DC, 1999.

(19) Akers, K.; Montgomery, D.; Christiansen, D.; Savoca, M.; Schnoebelen, D.; Becher, K.; Sadorf, E. *Water Quality Assessment of the Eastern Iowa Basins: Hydrologic and Biologic Data, October 1996 through September 1998;* United States Geological Survey, USGS Open-File Report 99-66; U.S. Government Printing Office: Washington DC, 2000.

(20) U.S. Department of Agriculture I*owa Agricultural Statistics;* National Agricultural Statistics Service. http://www.nass.usda.gov/ia/historics.

(21) National Atmospheric Deposition Program, http://nadp.sws.uiuc.edu/.

(22) U.S. Environmental Protection Agency. *List of Drinking Water Contaminants & MCL's;* EPA 816-F-02−013; 2002. http://www.epa.gov/safewater/mcl.html.

(23) Karr, J. D.; Showers, W. J.; Gilliam, J. W.; Andres, A. S. Tracing nitrate transport and environmental impact from intensive swine farming using delta nitrogen-15. *J. Environ. Qual.* **2001**, *30*, 1163−1175.

(24) Iowa State Legislature. *An Act Relating to Animal Agriculture, Providing for Fees, Providing for Penalties, and Including Retroactive Applicability and Effective Date Provisions;* Senate File 2293.

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Attachment 18:

Antibiotic Resistance, Gene Transfer, and Water Quality
Patterns Observed in Waterways near CAFO Farms
and Wastewater Treatment Facilities
(West et al. 2010)

Water Air Soil Pollut
DOI 10.1007/s11270-010-0602-y

Antibiotic Resistance, Gene Transfer, and Water Quality Patterns Observed in Waterways near CAFO Farms and Wastewater Treatment Facilities

Bridgett M. West · Peggy Liggit · Daniel L. Clemans · Steven N. Francoeur

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Abstract We examined water quality indicators (pH, temperature, turbidity, total phosphorus, and fecal coliform density) and bacterial antibiotic resistance (prevalence, conjugative transfer, and genetic linkage of resistance elements) at locations impacted by confined animal feeding operations (CAFOs) and compared them to nearby reference sites. Sites located upstream and downstream of two wastewater treatment facilities were also compared. Sites near CAFO farms had poor water quality (elevated total phosphorus and turbidity), while water quality remained relatively good downstream of wastewater treatment plants. High proportions of antibiotic-resistant bacteria were observed at all study sites, and frequent conjugative transfer of resistance was observed in laboratory assays. Out of a total of 830 environmental bacterial isolates, 77.1% were resistant to only ampicillin, while 21.2% were resistant to combinations of antibiotics including ampicillin (A), kanamycin (K), chlorotetracycline (C), oxytetracycline (O), and streptomycin (S). Multi-drug-resistant bacteria were significantly more common at sites impacted by CAFO farms. In conjugation assays, 83.3% of the environmental isolates transferred one or more antibiotic resistance genes to a laboratory strain of *Salmonella typhimurium*. A subset of multi-drug-resistant (A, C, and O) isolates was screened for specific tetracycline resistance genes and class I and II integrons. None of the screened isolates (n=22) were positive for integrons, while 13 isolates contained resistance genes for *tet* (B) and *tet* (C). Our results indicate that CAFO farms not only impair traditional measures of water quality but may also increase the prevalence of multi-drug-resistant bacteria in natural waters.

Keywords Antibiotic resistance · Conjugation · Confined animal feeding operations (CAFOs) · Fecal coliforms · Water pollution · Water quality

B. M. West · P. Liggit · D. L. Clemans · S. N. Francoeur (✉)
Center for Aquatic Microbial Ecology,
Biology Department, 316 Mark Jefferson,
Eastern Michigan University,
Ypsilanti, MI 48197, USA
e-mail: steve.francoeur@emich.edu

1 Introduction

Pollution by human and animal wastes is a common threat to water resources. Untreated human sewage frequently enters waterways as point source discharges (intentional or accidental) or via combined sewer overflows (CSO) from wastewater treatment plants (WWTPs; Stoner 2005; Whitlock et al. 2002; Wiggins et al. 1999). For example, from January 1, 2002, to December 31, 2005, WWTPs across Michigan reported having 2,542 CSO events, totaling 270 billion liters of discharged sewage (Michigan Department of

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Environmental Quality 2004a, b, 2005). Animal waste seeps into surface water as non-point source pollution as a result of housing large numbers of animals in confined areas, or as runoff from liquid manure applications onto croplands (a primary method of disposing of the waste generated by confined animal feeding operations (CAFOs); Carpenter et al. 1998; Esiobu et al. 2002).

Both human and animal wastes add nutrients (e.g., nitrogen, phosphorus; Carpenter et al. 1998; Tabbara 2003) as well as fecal coliform bacteria (Esiobu et al. 2002) to receiving waters. Excessive nutrients promote eutrophication, which decreases water transparency, creates foul odor and taste, depletes oxygen, causes fish kills, reduces biodiversity, and decreases esthetic, recreational, and property values along waterways (Boesch et al. 2001; Carpenter et al. 1998; Wetzel 2001). To reduce eutrophication, nutrient levels are often controlled via regulation and mitigation treatments (e.g., Carpenter et al. 1998; Cooke et al. 2005; Wetzel 2001). Human and animal wastes may also contaminate waterways with pathogenic bacteria (Burton and Engelkirk 2004; Guan and Holley 2003; Pell 1997). To avoid the difficulty and expense of direct testing for all possible types of pathogenic bacteria transmittable by feces, standard microbiological examination of water centers on determining the abundance of indicator bacteria, such as the fecal coliform bacteria (American Public Health Association 1998; Griffin et al. 2001). These bacteria are commonly found in the gut and feces of warm-blooded animals, and their presence in water environments can indicate contamination with human or animal feces (and by extension, contamination of water by pathogenic bacteria possibly present in feces; American Public Health Association 1998; Ash et al. 2002; Chao et al. 2003; Griffin et al. 2001). The greater the fecal coliform count, the greater the probability there is of contracting diseases from waterborne pathogenic bacteria (Mitchell and Stapp 1997). In Michigan, the Michigan Department of Natural Resources and Environment (MDNRE, formerly Michigan Department of Environmental Quality) monitors surface waters for a specific fecal coliform bacterium (*Escherichia coli*), rather than the entire fecal coliform group (Michigan Department of Environmental Quality 1999). In contrast, some other states in this region monitor fecal coliforms in surface waters. Both Ohio and Illinois require that surface waters used for extensive contact (e.g., swimming) have no more than 10% of samples exceeding a maximum fecal coliform count of 400 colony-forming units per 100 ml (Illinois Pollution Control Board 2002).

Antimicrobial agent resistance is an emerging global concern to both public and veterinary health. Antibiotics are heavily used to treat disease in both humans and animals, and there is a pattern of antibiotic resistance and transfer emerging among bacterial populations in proportion to the use of antibiotics, especially in agriculture (Levy 1997; Oppegaard et al. 2001). Antibiotics have been added to animal feed as growth promoters for some time, and the animal production industry has been identified as a potential reservoir for resistant *Enterobacteriaceae* (Witte 1997). The use of antibacterial drugs for prophylactic or therapeutic purposes in humans and for veterinary and agricultural purposes has provided selective pressure favoring the survival and spread of resistant organisms (Hooper 2002; Mascaretti 2003; Taber 2002). These resistant bacteria may transfer their resistance to previously non-resistant pathogenic bacteria or directly infect humans with bacterial diseases that cannot be treated by conventional antimicrobial therapies (Khachatourians 1998). The potential for antibiotic exposure and resistance development in human and animal gastrointestinal tracts, coupled with relatively great abundance in waters contaminated with human and animal waste, makes the fecal coliform bacteria a logical focal group for studies of antibiotic resistance and transfer in aquatic environments.

Despite active research investigating how antibiotic resistance enters and is maintained in the environment (D'Costa et al. 2006; Esiobu et al. 2002; Graves et al. 2002; Iwane et al. 2001; Rice et al. 1995; Sayah et al. 2005; Schwartz et al. 2003; Whitlock et al. 2002; Witte 1997), determining the mechanisms by which bacteria can transfer resistant genes within and across species (Arana et al. 1997, 2001; Bell et al. 1980; Oppegaard et al. 2001; Salyers et al. 2002), and identifying and characterizing new antibiotic resistance genes (Aminov et al. 2001, 2002; Chopra and Roberts 2001; Davies 1997; Gevers et al. 2003; Roberts 2005) and integrons (France et al. 2005; Hall 1997; Hall et al. 2003), the global ecological impact of antibiotic resistance and risks to human and veterinary health are yet to be determined. In the USA, there are no national or state water quality regulations requiring testing or reporting on antibiotic-resistant organisms (American Public Health Association 2005; Michigan Department of Environmental Quality 1999; US Environmental Protection Agency 2002).

In order to understand how CAFOs and WWTPs affect bacterial antibiotic resistance and transfer, we simultaneously conducted traditional water quality measurements (pH, temperature, turbidity, total phosphorus, and fecal coliform density) and microbiological and molecular techniques for determining patterns of antibiotic resistance and potential transfer of resistance genes at CAFO-impacted and unimpacted sites and at sites upstream and downstream of WWTPs. We also examined the relationships between traditional water quality parameters and conducted a preliminary analysis of the genetic location and mode of transfer of antibiotic resistance elements.

2 Materials and Methods

2.1 Study Design and Site Descriptions

Ten sites were selected for this study, based on the presence of previously documented point source and non-point source contamination (see following section) and proximity to public access (Table 1). Six sites were located in an agricultural region near Hudson, MI. Three of the agricultural sites were waterways approximately 1–2.5 km from different CAFO farms and were classified as agriculturally impaired (AI) based on the following criteria: (1) the waterways received direct runoff from fields sprayed with liquid manure from CAFO farms; (2) previous observations of fecal coliform densities greater than 1,000 colony-forming unit (CFU)/100 ml (Khachatourians 1998; J. Pernicano personal communication); (3) ratings of "poor" on biological (macroinvertebrate) assessments of these waterways (Michigan Department of Environmental Quality 2003a); and (4) the MDNRE listing these waterways on their non-attainment and total maximum daily load lists (Michigan Department of Environmental Quality 2004b). Three additional agricultural sites located in the same farming region were classified as agriculturally unimpaired (AUI) since they did not receive direct runoff from manure-sprayed fields and were not located near a CAFO farm. Two of these three sites (sites SJT8 and SJC7) have been classified by MDNRE as "least impacted" streams in this location and have been previously identified as reference streams (Michigan Department of Environmental Quality 2003a). Four additional sites were selected approximately 1–1.5 km both upstream and downstream of the Ann Arbor, MI, and Chelsea, MI, WWTPs in the Huron River watershed. Like all WWTPs, these facilities occasionally release partially treated and untreated sewage. The most recent such episode prior to this study was the release of ~50 million liters of sewage by the Ann Arbor WWTP in August 2003 (Michigan Department of Environmental Quality 2003b).

2.2 Sampling and Physical and Chemical Water Quality Procedures

The initial and final sampling dates (June 3, 2004, and August 9, 2004, respectively) were chosen during distinct dry periods (zero precipitation measured within the proceeding 72 h), and the middle three sampling periods (June 16, 2004, July 7, 2004, and July 18, 2004) occurred after rainstorms where >1 cm of precipitation was recorded within 24 h (Michigan State Climatology, http://climate.geo.msu.edu/Semcog/SEMdaily/2004/, SEMCOG Daily Precipitation Summary). Site VH25 was completely dry by the end of the study, so this site was sampled on only four out of five possible dates.

At each site, a handheld probe was used to measure pH and temperature (YSI 63, Yellow Springs Instrument Co., Yellow Springs, OH). Turbidity was measured in the field using a Hach 2100P portable turbidimeter. For total phosphorus measurements, 100 ml of water was collected into an acid-washed Nalgene bottle and stored at −20°C for later analysis using the protocol of Wetzel and Likens (2000). For fecal coliform isolation, 100 ml of water was collected into two sterile 50-ml Falcon conical tubes and kept on ice (<8 h) until it was processed in the laboratory (Harwood et al. 2000). All water samples were obtained by submerging the collection container approximately 1 cm below the water surface before opening the lid to collect the sample.

Fecal coliform density was determined as follows: water samples of various volumes (100, 1.0, 0.1, and 0.01 ml) were brought to a final volume of 100 ml with 0.01 M phosphate-buffered saline (0.138 M

Table 1 Site descriptions: designations, locations, and average discharge

Site name	Study site designation	Watershed	Latitude/ longitude	Road crossing	Average discharge[a] (cubic meters per second)
Vanderhoff-Haley (VH19) Rice Lake Drain	AI	River Raisin	41° 49′ 69″ N 084° 13′ 24″ W	Haley Rd.	ND
Vreba-Hoff I (VH21) Medina Drain	AI	Bean-Tiffin	41° 48′ 17″ N 084° 17′ 58″ W	Ingall Hwy	ND
Vreba-Hoff II (VH25) Lime Creek	AI	Bean-Tiffin	41° 47′ 37″ N 084° 22′ 39″ W	Lime Lake Rd.	ND
Hazen Creek (HC1)	AUI	River Raisin	41° 55′ 69″ N 084° 15′ 71″ W	Burton Rd.	ND
Unnamed Tributary to St. Joseph Creek (SJT8)	AUI	Bean-Tiffin	41° 52′ 29″ N 084° 25′ 20″ W	Waldron Rd.	ND
St. Joseph Creek (SJC7)	AUI	Bean-Tiffin	41° 52′ 70″ N 084° 25′ 20″ W	Waldron Rd.	ND
Mill Creek upstream from WWTP (MC1)	UPWWTP	Huron River	42° 19′ 54″ N 084° 1′ 06″ W	McKinley Rd.	2.2
Huron River upstream from WWTP (HR1)	UPWWTP	Huron River	42° 20′ 19″ N 083° 52′ 30″ W	Zeeb Rd.	19.2
Mill Creek downstream from WWTP (MC2)	DNWWTP	Huron River	42° 19′ 33″ N 083° 58′ 77″ W	Chelsea-Dexter Rd.	2.2
Huron River downstream from WWTP (HR2)	DNWWTP	Huron River	42° 15′ 23″ N 083° 37′ 30″ W	Huron Parkway	19.2

AI agriculturally impaired, *AUI* agriculturally unimpaired, *UPWWTP* upstream from wastewater treatment plant, *DNWWTP* downstream from wastewater treatment plant, *ND* no data for that body of water

[a] Source: USGS National Water Information System: Web Interface, http://nwis.waterdata.usgs.gov/mi/nwis/annual/ (Ann Arbor gauging station, site number 04174500) and Mill Creek near Dexter, MI (site number 04173500)

NaCl and 0.0027 M KCl, pH 7.4 at 25°C) and filtered onto a 0.45-µm filter (Pall Life Sciences, East Hills, NY) using a vacuum system as described by Mitchell and Stapp (1997) and the Standard Methods for the Examination of Water and Wastewater (American Public Health Association 1998). The filter membranes were transferred to Petri plates (BD Falcon, BD Biosciences, Bedford, MA) containing fecal coliform selective agar (modified fecal coliform, mFC; BBL, BD Biosciences, Franklin Lakes, NJ) without antibiotics and were incubated at 44.5°C in a humidified incubator for 24 h. For the first sampling date (June 3, 2004), 100-, 0.1-, and 0.01-ml volume water samples were used; filtration of 100 ml yielded colonies with confluent growth, while the low-volume samples (0.1 and 0.01 ml) had no growth. As a result, fecal coliform densities from June 3, 2004, were not considered in any further analyses, and volumes of 100 and 1 ml were analyzed for the remaining four sampling dates (June 17, 2004, July 7, 2004, July 18, 2004, and August 9, 2004). After incubation, bacterial density (CFU/per 100 ml) was determined for each site on each sampling using the colony counts from either the 100- or 1-ml filtered plate, depending on which plate produced countable isolated colonies.

2.3 Bacterial Isolation and Antibiotic Resistance Susceptibility

Individual colonies were picked from distinctly isolated typical colonies as recommended by the Standard Methods for the Examination of Water and Wastewater (American Public Health Association 1998). Approximately 30 isolates per site per sampling date were analyzed. To test for multiple antibiotic resistance, individual bacterial isolates were replica-plated onto tryptic soy agar (TSA; BD Biosciences, Franklin Lakes, NJ) supplemented with one of five antibiotics: ampicillin (A), kanamycin (K), streptomycin (S), chlorotetracycline (C), and oxytetracycline (O) (Sigma-Aldrich, St. Louis, MO). These antibiotics are commonly used in both human and veterinary medicine and demonstrate different mechanisms and pathways (inhibiting cell wall synthesis [ampicillin] vs. protein synthesis [streptomycin, kanamycin, and the tetracyclines]; Esiobu et al. 2002). The antibiotic concentration for all drugs was 20 μg/ml for samples processed from the initial measurement date (June 3, 2004). The antibiotic concentrations were increased to 30 μg/ml for the remainder of the sampling dates (June 17, 2004, July 7, 2004, July 18, 2004, and August 9, 2004) to ensure complete inhibition of the control bacterial strain in the conjugation assay. Some authors consider these to be high antibiotic concentrations for screening environmental bacteria (D'Costa et al. 2006), but they are similar to concentrations used in other screening studies (i.e., 5–80 μg/ml; Harwood et al. 2000; Wiggins et al. 1999). The plates were incubated at 37°C for 24 h. Bacterial isolates were scored as positive for resistance if there was visible, tangible growth. No growth or trace colony development was recorded as antibiotic susceptible. All resistant isolates were preserved in tryptic soy broth (TSB, BD Biosciences, Franklin Lakes, NJ) supplemented with the appropriate antibiotic and 10% glycerol and frozen at −80°C.

2.4 Conjugation Testing

An S-resistant *Salmonella typhimurium* strain, EM1000, was used as the recipient in all conjugation testing. EM1000 was originally obtained as SGSC452 from the *Salmonella* Genetic Stock Center (Calgary, Alberta, Canada; Bullas and Ryu 1983). All environmental isolates not resistant to S were tested as donor strains in individual conjugation assays. Isolates demonstrating multiple-drug resistance were processed and tested separately for the ability to transfer each type of drug resistance gene. For example, after the replica-plating assay, if a particular isolate grew on an A plate and also on a separate K plate, the isolate was subjected to two conjugation assays. In the first assay, the transconjugants would be selected on a dual antibiotic plate containing A and S, and in the second assay, the selective plate would include K and S.

An A- and K-resistant *E. coli* strain, SM10-Tn*pho*A, was used as a positive control donor for testing the efficiency of conjugation. SM10-Tn*pho*A was obtained originally from John Mekalanos (Taylor et al. 1989). Prior to conjugation, individual bacterial cultures were grown overnight at 37°C in TSB containing 30 μg/ml of the appropriate antibiotic (S for EM1000; A and K for SM10-Tn*pho*A; and either A, K, C, or O for the environmental isolates). The bacterial cells were pelleted by centrifugation and resuspended in fresh TSB lacking antibiotic. Conjugation experiments were conducted as described by Bell et al. (1983). Briefly, equal volumes (0.1 ml) of stationary-phase cells from the donor strain (an individual environmental isolate or the SM10-Tn*pho*A positive control donor) and recipient strain (EM1000) were mixed in a 1.5-ml tube with 0.8 ml of antibiotic-free TSB and incubated at 37°C for 2–4 h. After the incubation, mating mixtures were plated onto TSA containing dual antibiotics (S and either A, K, C, or O all at 30 μg/ml) and incubated for 24 h at 37°C to select for transconjugants. Transconjugants of interest were preserved in TSB supplemented with the appropriate antibiotic and 10% glycerol and frozen at −80°C.

Negative conjugation control experiments lacking either the donor or recipient bacteria were included in all conjugation experiments to check for mutation of either strain to antibiotic resistance. No mutation to specific antibiotic resistance was seen with any donor strains or the universal *S. typhimurium* EM1000 recipient strain.

2.5 PCR Screening for Tetracycline Resistance Genes

Environmental isolates positive for tetracycline resistance and their respective transconjugants were screened for specific tetracycline resistance genes using the polymerase chain reaction (PCR). The tetracycline resistance genes tested in this study included *tet* (B), *tet* (C), *tet* (E), *tet* (H), *tet* (Y), and *tet* (Z) based on primer sets from Aminov et al. (2002); *tet* (M) and *tet* (W) from Aminov et al. (2001); and *tet* (K) and *tet* (L) from Gevers et al. (2003). The presence of class I and II integrons was screened using primer sets from France et al. (2005). A typical PCR reaction was performed with a 50-μl mixture of the following reagents: 1-μl DNA template (50 ng) or bacterial cell suspension (one colony suspended in 10 μl H_2O), 0.2-μM primers, and 45-μl Platinum PCR Supermix (Invitrogen Corporation, Carlsbad, CA) supplemented with $MgCl_2$ to a final concentration of 2.0 mM. PCR amplification was performed as follows: 95°C for 5 min (one cycle), 94°C for 30 s, 50°C for 30 s, 72°C for 30 s (25 cycles), and 72°C for 7 min (one cycle) using an MJ Research PTC-200 thermocycler (Bio-Rad Laboratories, Hercules, CA). If amplification products were not detected in the first assay, the samples were subjected to another round of amplification using the same cycling protocol with the exception of reducing the annealing temperature to 45–48°C. PCR amplification products were analyzed by gel electrophoresis on a 2.5% (wt/vol) agarose gel (NuSieve; FMC Bioproducts, Rockland, MD) and stained with ethidium bromide. The Promega 1 KB Plus Ladder (Promega Co., Madison, WI) was used for verifying DNA fragment sizes. Amplification products were purified by the use of a Qiagen QIAquick PCR purification kit (Qiagen Inc., Valencia, CA) and sequenced at the University of Michigan DNA Sequencing Core (Ann Arbor, MI). The DNA sequences generated from environmental isolates were compared to known sequences using the Basic Local Alignment Search Tool family of programs from NCBI (http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=pubmed&list_uids=15215342&dopt=Citation; McGinnis and Madden 2004).

Plasmid DNA was purified from environmental isolates and their transconjugants using the Qiagen QIAprep plasmid purification system. The following modifications in the procedure were performed to optimize purification of low-copy plasmids and cosmids: (1) volumes of buffers P1, P2, and N3 were doubled, (2) the optional PB wash step was included, and (3) H_2O heated to 70°C was used to elute DNA from the QIAprep membrane.

2.6 Identification of Environmental Bacteria

Each bacterial isolate that screened positive for a particular tetracycline resistance gene was identified using the RapiD 20 E system (bioMerieux, Inc., Durham, NC). Individual isolates were inoculated on media containing trypticase soy agar with 5% sheep blood BBL (Becton Dickinson, Franklin Lakes, NJ) and incubated overnight at 37°C. A cell suspension was prepared using one to four well-isolated colonies diluted in 2 ml 0.85% NaCl to a turbidity equivalent to 0.5 McFarland. The cell suspension was immediately used to inoculate a RapiD 20 E test strip and incubated for 4 h at 37°C. The test results were entered into the RapiD 20 E Analytical Profile Index, and the species identification was provided at the bioMerieux *apiweb* site (https://apiweb.biomerieux.com/servlet/Authenticate?action=prepareLogin).

2.7 Statistical Analysis

Differences between the AI and AUI sites in (1) the proportion of samples which exceeded established water quality thresholds for total phosphorus (0.1 mg/l, Lind 1985), turbidity (40.1 NTU, Mitchell and Stapp 1997), and fecal coliform density (400 CFU/100 ml, Michigan Department of Environmental Quality 1999), (2) the proportions of single- vs multi-drug resistant bacterial isolates, and (3) the proportions of bacterial isolates able to transfer their resistance during conjugation assays were tested using Fisher's exact test (Zar 1999). Identical comparisons were examined for sites upstream and downstream of WWTPs. Spearman's rank correlation was used to examine the relationships among turbidity, temperature, total phosphorus, pH, and fecal coliform density across all samples and sites. For correlation analyses, fecal coliform densities plated as too numerous to count (TNTC) were coded as an arbitrarily large number greater than 7,900 CFU/100 ml (the largest recorded value). The SYSTAT computer package (v 10.2, SYSTAT Inc., Chicago, Illinois) was used to perform all statistical analyses for this study.

3 Results

3.1 Physical, Chemical, and Biological Water Quality

Temperature, pH, and turbidity were similar among the agricultural streams and within the WWTP sites (Table 2). Turbidity tended to be increased by rain events, especially in the agricultural streams. AI sites were one to eight times more phosphorus rich than all other sites. Fecal coliform densities ranged from 70 to >7,900 CFU/100 ml. For some measurements, particularly those taken during rain events and at AI sites, quantitative fecal coliform densities could not be determined as a result of confluent colony growth regardless of the water dilution (Table 3). The fecal coliform densities at agricultural sites ranged from 700 to TNTC, while fecal coliform densities in

Table 2 Water chemistry ranges across sampling dates for agriculturally impaired and agriculturally unimpaired sites

Sample site group	Sampling date	pH	Temperature (°C)	Turbidity (NTU)[a]	Total phosphorus (mg/l)[b]
AI	Initial (June 3, 2004)	7.19–7.53	18.6–23.4	9.09–15.80	0.12–0.25
	Rain event 1 (June 17, 2004)	6.89–7.66	19.5–20.5	114.00–846.00	0.50–0.65
	Rain event 2 (July 7, 2004)	6.52–7.14	21.5–22.6	9.30–56.60	0.10–0.34
	Rain event 3 (July 18, 2004)	7.06–7.56	18.4–18.9	5.36–72.90	0.13–0.32
	Final (August 9, 2004)	6.82–7.54	16.6–19.7	14.60–33.70	0.36–0.76
AUI	Initial (June 3, 2004)	7.44–7.98	20.7–21.5	2.22–3.36	0.02–0.06
	Rain event 1 (June 17, 2004)	7.23–7.57	19.3–19.7	21.40–96.70	0.10–0.15
	Rain event 2 (July 7, 2004)	7.64–8.18	19.0–21.5	13.20–28.90	0.07–0.12
	Rain event 3 (July 18, 2004)	7.71–8.28	16.9–17.7	4.92–28.80	0.02–0.07[d]
	Final (August 9, 2004)	7.37–8.09	14.0–16.8	2.65–11.60	0.00–0.05
UPWWTP	Initial (June 3, 2004)	8.18–8.19	15.3–17.8	3.04–4.26	0.02–0.03
	Rain event 1 (June 17, 2004)	7.99–8.11	20.5–22.5	6.89–7.47	0.02–0.03
	Rain event 2 (July 7, 2004)	7.95–8.20	22.5–24.4	5.72–17.50	0.02–0.05
	Rain event 3 (July 18, 2004)	7.84–8.13	19.5–23.8	5.26–10.50	0.02–0.03
	Final (August 9, 2004)	8.00–8.04	18.9–23.6	4.20–5.22	0.00–0.01
DNWWTP	Initial (June 3, 2004)	7.98–8.28	16.6–18.7	3.38–3.47	0.03–0.04
	Rain event 1 (June 17, 2004)	7.91–8.12	21.8–22.9	3.89–6.43	0.02–0.04
	Rain event 2 (July 7, 2004)	7.95–7.95	21.7–25.4	3.63–17.20	0.03–0.07
	Rain event 3 (July 18, 2004)	7.80–8.18	21.4–24.6	3.88–10.50	0.02–0.03
	Final (August 9, 2004)	8.09–8.28	21.6–23.9	3.16–5.37	0.01–0.03

AI agriculturally impaired, *AUI* agriculturally unimpaired, *UPWWTP* upstream from wastewater treatment plant, *DNWWTP* downstream from wastewater treatment plant, *NTU* nephelometric turbidity units

[b] Rating according to Mitchell and Stapp (1997): excellent=0–10, good=10.1–40, fair=40.1–150, poor=>150

[c] Phosphorus levels. Rating >0.1 mg/l is considered poor water quality according to Lind (1985)

waterways near WWTPs ranged from 70 to 2,300 CFU/100 ml. The highest fecal coliform densities were measured after rain events and occurred most frequently at the AI sites (Table 3). Turbidity, total phosphorus concentration, and fecal coliform density were highly correlated (turbidity–phosphorus $n=49$, $r_s=0.790$, $p<0.001$; turbidity–fecal coliform $n=39$, $r_s=0.688$, $p<0.001$; phosphorus–fecal coliform $n=39$, $r_s=0.684$, $p<0.001$), with AI sites tending to have the greatest values for these three parameters. Turbidity, total phosphorus concentration, and fecal coliform density were all negatively correlated with pH (turbidity–pH $n=49$, $r_s=-0.371$, $p<0.01$; phosphorus–pH $n=49$, $r_s=-0.606$, $p<0.001$; fecal coliform–pH $n=39$, $r_s=-0.471$, $p<0.01$). AI streams were significantly more likely than AUI streams to exceed a total phosphorus threshold of 0.1 mg/l ($p<0.001$) and marginally more likely to exceed a turbidity threshold of 40.1 NTU ($p=0.08$, Table 4). There was no difference in the total phosphorus or turbidity exceedance frequencies between sites upstream and downstream of WWTPs (p always >0.05). Although the AI sites tended to have greater fecal coliform densities than the AUI sites, there was no difference in the frequency of fecal coliform densities >400 CFU/100 ml ($p>0.05$), nor was there any difference observed in fecal coliform exceedance frequencies between sites upstream and downstream of WWTPs ($p>0.05$, Table 4).

3.2 Antibiotic Resistance Patterns

A total of 830 fecal coliform isolates were collected from mFC agar plates and replica-plated on TSA supplemented with one of five antibiotics. Overall, 98.3% of the fecal coliform isolates selected were resistant to at least one antibiotic (Table 5). Fourteen of the isolates (1.7%) did not grow on any of the

Table 3 Fecal coliform densities for each grouping of sites across sampling dates from filtered plates (CFU/100 ml)

Sites	Measurement dates[a]			
	Rain event #1 June 17, 2004	Rain event #2 July 7, 2004	Rain event #3 July 18, 2004	Final August 9, 2004
AI				
VH19	TNTC	TNTC [b]	900	2,800
VH21	TNTC	3,200	1,600	600
VH25	4,400	TNTC	4,900	No data[c]
AUI				
HC1	5,900	3,100	7,900	2,400
SJT8	2,900	2,300	2,400	700
SJC7	5,800	800	2,900	700
UPWWTP				
MC1	800	2,300	2,000	600
HR1	79	700	88	198
DNWWTP				
MC2	198	2,100	1,500	900
HR2	174	1,800	70	300

For specific site abbreviations, see Table 1.

AI agriculturally impaired, *AUI* agriculturally unimpaired, *UPWWTP* upstream from wastewater treatment plant, *DNWWTP* downstream from wastewater treatment plant, *TNTC* too numerous to count

[a] The Initial sample date (June 3, 2004) yielded zero colony growth for the 0.01- and 0.1-ml samples and TNTC=confluent growth for the filtered 100-ml water samples. The protocol was adjusted after June 3, 2004, to include a 0.1-, 1-, and 100-ml filtered sample

[b] Mann–Whitney *U* test shows AI sites significantly greater than AUI sites for rain event 2 (p=0.046); TNTC: confluent growth on filters growing on media plates (0.1, 1.0, and 100 ml)

[c] Dry creek, unable to sample water on this date

antibiotic plates, 640 isolates (77.1%) were resistant to only A, and the remaining 176 resistant isolates (21.2%) were resistant to two or more antibiotics. The only single-drug resistance pattern observed was to A, and all the multi-drug isolates included A resistance with the exception of one isolate that was KCO resistant (Table 5).

The AI sites had a significantly greater proportion of isolates that were resistant to multiple antibiotics (41.6% vs. 16.5%) and a lower proportion of isolates resistant to only ampicillin (58.4% vs. 83.5%) than the AUI sites (p<0.001, Tables 4 and 5). The sites upstream and downstream of WWTPs had similar proportions of isolates resistant to only ampicillin (81.0% vs. 89.1%) and multi-drug resistant (19.0% vs. 10.9%) (p>0.05, Tables 4 and 5).

3.3 Conjugal Transfer of Antibiotic Resistance Genes

Conjugation assays were conducted on 735 non-S-resistant isolates. Of the 640 single-drug resistant isolates (all were A-only resistant), 456 (71.3%) produced viable resistant transconjugants. For the multi-drug-resistant isolates (n=95), 27.4% were able to transfer all resistance determinants (AK, AO, and ACO), and 81.1% were able to transfer at least one multi-drug resistance pattern (AK, AO, AC, ACO, and AKCO) with A being the most prevalent resistance determinant transferred (Table 6). The proportion of isolates from each site category able to transfer resistance in laboratory conjugation assays ranged from 60.0% to 85.6% (Table 4) and did not differ significantly between either the AI and AUI

Table 4 Patterns of water quality and multi-drug resistance at study sites

Site groups[a]	Water quality			Multi-drug resistance and gene transfer	
	Turbidity rating[b] (%) 40.1 >150 NTU	Phosphate rating[c] (%) >0.1 (mg/l)	Fecal coliform densities[d] (%) >400 CFU/100 ml	Multi-drug resistance[e] (%)	Gene transfer of one or more resistance genes[f] (%)
AI	35.7[g]	100	100	41.6	80.0
AUI	6.7[g]	26.7[g]	100	16.5	86.5
UPWWTP	0	0	62.5[g]	19.0	76.0
DNWWTP	0	0	50[g]	10.9	60.0

NTU nephelometric turbidity units

[a] AUI, UPWWTP, and DNWWTP (15 water samples collected); AI (14 samples; VH25 site was dry at final collection)

[b] Rating according to Mitchell and Stapp (1997): excellent=0–10, good=10.1–40, fair=40.1–150, poor=>150

[c] Phosphorus levels. Rating >0.1 mg/l is considered poor water quality, according to Lind (1985)

[d] Fecal coliform density for second to fifth sampling dates, initial coliform density samples are not included in this comparison because of a dilution change in the protocol, see Section 2.

[e] AI (n=185), AUI (n=328), UPWWTP (n=147), DNWWTP (n=156)

[f] Ninety-five multi-drug resistant isolates (all non-streptomycin) were tested for gene transfer: the percent is the number of isolates showing positive for gene transfer of one or more resistance genes per number of isolates tested in each grouped site = AI (24/30), AUI (32/37), UPWWTP (14/18), DNWWTP (6/10)

[g] These water quality indicators reached their highest ratings during rain events

sites (p>0.05) or the sites upstream and downstream of WWTPs (p>0.05).

A subset of AO, AC, and ACO multi-drug-resistant isolates (n=22) and their transconjugants were further screened for the presence of specific tetracycline resistance genes and class I and II integrons. In all, 13 isolates were *tet* positive: six isolates were identified carrying the *tet* (B) gene, five with *tet* (C), and two with both *tet* (B) and *tet* (C). No class I or II integrons or *tet* (E, H, K, L, M, Y, W, or Z) genes were detected. The 13 *tet*-positive isolates were individually identified as *E. coli* by the RapiD 20 E system (Table 7). The identity of all *tet* (B) and *tet* (C) PCR products was confirmed by DNA sequence analysis.

To characterize the potential location and mode of horizontal transfer of *tet* (B) and *tet* (C) genes between the environmental isolates and the conjugative recipient, plasmid DNA was purified and PCR-amplified using primer sets for *tet* (B) and *tet* (C) (see Table 7). All eight isolates positive for the *tet* (B) gene showed the presence of *tet* (B) on plasmid DNA, as did their respective transconjugants, with the exception of two isolates (VH19-7R1 and VH25-16R1). For these two isolates, their transconjugants could not be subcultured and maintained on tetracycline-selective media (at 30 μg/ml C or O). Repeated conjugation experiments for isolates VH19-7R1 and VH25-16R1 failed to produce any viable colonies selected on tetracycline plates despite the evidence that the donor isolates contain plasmid DNA with the *tet* (B) gene. In this study, chromosomal DNA was not definitively screened in the absence of plasmid DNA.

For the *tet* (C) determinant, three different patterns of gene transfer were observed (Table 7): (1) In two out of eight isolates (SJ7-5-I and HC-1-R2), the *tet* (C) gene was identified with plasmid DNA in both the donor isolates and their transconjugants; (2) *tet* (C) was identified in total DNA from isolates VH19-8R3, VH19-11F, and MC2-9R1 and their transconjugants, but not associated with any plasmid DNA; and (3) for isolates MC1-22R4 and MC1-1F, the *tet* (C) gene was present in total DNA from the isolate but was unidentifiable in any plasmid DNA or total DNA from the transconjugant even though

Table 5 Percentage of antibiotic-resistant fecal coliform isolates from individual study sites

Antibiotic[a]	Individual study sites									
	VH19 (AI) n=58	VH21 (AI) n=57	VH25 (AI) n=70	HC1 (AUI) n=142[b]	SJT8 (AUI) n=93	SCJ7 (AUI) n=93	MC1 (UPWWTP) n=72	HR1 (UPWWTP) n=75	MC2 (DNWWTP) n=84	HR2 (DNWWTP) n=72
A	43.1%	54.4%	74.3%	88.7%	82.8%	76.3%	84.7%	77.3%	88.1%	90.3.4%
Total % by site for amp	AI=58.4%			AUI=83.5%			UPWWTP=81.0%		DNWWTP=89.1%	
AK	5.2%	5.3%	20.0%	4.2%	10.8%	10.8%	2.8%	9.3%	3.6%	4.2%
AO	0	0	0	2.8%	0	0	0	0	0	0
AC	0	0	0	0	0	1.1%	1.4%	0	0	0
AS	24.1%	7.0%	2.9%	3.5%	2.2%	5.4%	1.4%	4.0%	3.6%	2.8%
AKS	0	0	0	0	0	0	2.8%	2.7%	0	0
ACO	8.62%	3.5%	2.9%	0	3.2%	3.2%	6.9%	1.3%	2.4%	2.8%
KCO	0	0	0	0	0	0	0	1.3%	0	0
AOS	0	1.8%	0	0	0	0	0	0	0	0
COS	3.5%	0	0	0	0	0	0	0	1.2%	0
AKCO	0	1.8%	0	0	0	0	0	1.3%	0	0
AKOS	1.7%	0	0	0	0	0	0	0	0	0
ACOS	5.1%	0	0	0.7%	1.1%	3.2%	0	2.7%	1.2%	0
AKCOS	8.6%	26.3%	0	0	0	0	0	0	0	0
Total% multiple resistance	56.9%	45.6%	25.7%	11.2%	17.2%	23.7%	15.3%	22.7%	11.9%	9.7%
Total% by site for multi-drug	AI=41.6%			AUI=16.5%			UPWWTP=19.0%		DNWWTP=10.9%	

Summary: total N=830; 816/830=98.3% isolates resistant to at least one antibiotic; 640/830=77.1% resistant to amp only; 176/830=21.2% multi-drug resistant

A ampicillin, *K* kanamycin, *O* oxytetracycline, *C* chlorotetracycline, *S* streptomycin

[a] Values tabulated for a single antibiotic (e.g., A) indicate isolates resistant only to that individual antibiotic; values tabulated for a particular combination of antibiotics indicate isolates resistant to all the listed antibiotics and only the listed antibiotics (i.e., isolates tabulated as AKS resistant would not also be included in the AS category)

viable tetracycline-resistant colonies appeared after conjugation.

For the two isolates carrying both *tet* (B) and *tet* (C) genes (VH19-11F and MC2-9R1), both *tet* genes were identified in total DNA (in the isolates and transconjugants). The *tet* (B) gene was also amplified from plasmid DNA isolated from both isolates and their transconjugants, but this did not occur with the *tet* (C) gene (Table 7). Figure 1 shows gel PCR amplification products produced using *tet* (B) and *tet* (C) primer pairs with DNA from bacterial cells and isolated plasmid from isolate MC2-9R1 and its transconjugant, MC2-9R1T2-1.

4 Discussion

Chemical water quality at the WWTP sites was good; all WWTP samples had total phosphorus concentrations <0.1 mg/l, which is considered acceptable or unpolluted (Lind 1985; Mitchell and Stapp 1997), and turbidities of <40.1 NTU, which is considered excellent to good (Mitchell and Stapp 1997). Fecal coliform densities at the WWTP sites were marginal, as 56% of samples surpassed the 10% exceedance threshold (<400 CFU/100 ml). No statistically significant differences were detected in water chemistry measurements and fecal coliform densities between

Table 6 Resistance pattern observed after gene transfer for non-streptomycin isolates

Antibiotic resistance pattern	Number of isolates[a]	Resistance pattern genetically transferred via conjugation	Number of isolates transferring resistance[b]
Single-drug resistance			
A	640	A	456 (71.3%)
		No transfer	184 (28.8%)
Multi-drug resistance (*n* = 95)			
AK	61	A	25 (41.0%)
		K	2 (3.3%)
		AK	18 (30.0%)
		No transfer	16 (26.2%)
AO	4	A	1 (25.0%)
		O	2 (50.0%)
		AO	1 (25%)
AC	2	A	2 (100%)
ACO	25	A	5 (20.0%)
		AC	3 (12%)
		ACO	7 (28%)
		AO	2 (8%)
		CO	3 (12%)
		O	4 (16%)
		No transfer	1 (4%)
KCO	1	No transfer	1 (100%)
AKCO	2	A	1 (50%)
		No transfer	1 (50%)

A ampicillin, *K* kanamycin, *O* oxytetracycline, *C* chlorotetracycline, *S* streptomycin

[a] Number of isolates, lacking streptomycin resistance, that were tested for gene transfer, total 735

[b] Number of multi-drug isolates: (1) transferring one or more resistance genes 77/95=81.1%; (2) transferring all resistance genes 26/95=27.4%

the upstream and downstream sites. These results suggest that little, if any, WWTP point source pollution occurred during the study period. MDNRE (Michigan Department of Environmental Quality 2004a) confirmed that no untreated sewage was discharged from either the Chelsea or the Ann Arbor WWTPs during or immediately preceding the study period.

In contrast, the AI sites located near CAFO farms had indicators of poor water quality compared to reference sites in the same area. Total phosphorus concentrations were much more likely (100% vs. 26.7%) to exceed established thresholds in the AI sites than AUI sites, and turbidity was also somewhat more likely (35.0% vs. 6.7%) to exceed threshold values. Although the frequency of fecal coliform densities exceeding 400 CFU/100 ml did not differ between AI and AUI sites, only the AI sites had fecal coliform densities TNTC, indicating that maximal densities of fecal coliforms occurred at AI sites.

As expected, water quality decreased following heavy precipitation. At all study sites, turbidity levels were elevated after rain events compared to the initial and final measurements taken during dry periods. Increased phosphorus levels were also detected after precipitation in the agriculturally impacted areas, and fecal coliform densities were much higher after precipitation. The strong correlation of turbidity, total phosphorus, and fecal coliform densities suggests a common source for these parameters. Elevated total phosphorus, turbidity, and fecal coliform densities are presumed to be the direct result of runoff from nearby tiled fields sprayed with liquid manure as reported by MDNRE in numerous previous waste discharge infractions by the CAFO farms in close proximity to our AI sites (Michigan Department of Environmental Quality 2003a, 2004b). Our data are consistent with recent biological surveys of the same waterways, which classified the AI sites as poor habitat, supporting only macroinvertebrate and fish communities that

Table 7 Identification of tetracycline (tet) resistance genes in the environmental isolates and their transconjugants

Sample site groups	Environmental isolates (*Escherichia coli*)[a]				Transconjugants (*Salmonella typhimurium*, *strain* EM1000)			
	IDi[b]	Antibiotic resistance pattern on selective plates	Resistance gene identified by PCR (total DNA)	Resistance gene identified by PCR (plasmid DNA)	ID	Resistance pattern on selective plates after conjugation	Resistance gene identified by PCR (total DNA)	Resistance gene identified by PCR (plasmid DNA)
AI	VH19-7R1	ACO	*tet* (B)	*tet* (B)	VH19-7R1T2-1	AC	–	–
	VH25-16R1	ACO	*tet* (B)	*tet* (B)	VH25-16R1T2-1	AC	–	–
	VH19-8R3	ACO	*tet* (C)	–	VH19-8R3T3-4	ACO	*tet* (C)	–
	VH25-6R3	ACO	*tet* (B)	*tet* (B)	VH25-6R3T3-5	ACO	*tet* (B)	*tet* (B)
	VH19-3F	ACO	*tet* (B)	*tet* (B)	VH19-3FT2-1	ACO	*tet* (B)	*tet* (B)
	VH19-11F	ACO	*tet* (B) and *tet* (C)	*tet* (B)	VH19-11FT3-1	ACO	*tet* (B) and *tet* (C)	*tet* (B)
AUI	SJ7-5-I	ACO	*tet* (C)	*tet* (C)	SJ7-5-IT3-1	AO	*tet* (C)	*tet* (C)
	HC-1-R2	ACO	*tet* (C)	*tet* (C)	HC-1-R2T2-2	ACO	*tet* (C)	*tet* (C)
UPWWTP	MC1-17I	ACO	*tet* (B)	*tet* (B)	MC1-17IT2-1	ACO	*tet* (B)	*tet* (B)
	MC1-24I	ACO	*tet* (B)	*tet* (B)	MC1-24IT3-1	ACO	*tet* (B)	*tet* (B)
	MC1-22R4	ACO	*tet* (C)	–	MC1-22R4T1-1	AC	–	–
DNWWTP	MC1-1F	ACO	*tet* (C)	–	MC1-1FT2-1	AC	–	–
	MC2-9R1	ACO	*tet* (B) and *tet* (C)	*tet* (B)	MC2-9R1T2-1	AO	*tet* (B) and *tet* (C)	*tet* (B)

AUI ag. unimpaired, *AI* ag. impaired, *UPWWTP* upstream from wastewater treatment plant, *DNWWTP* downstream from wastewater treatment plant, *A* ampicillin, *O* oxytetracycline, *C* chlorotetracycline

[a] Environmental bacterial isolates were identified to species using the RapiD20 System (bioMerieux)

[b] Isolates are named after the site location, the day when the measurement was taken (*R* rain event, *I* initial, *F* final), and the colony number picked from the plate. Transconjugants are named after the isolate donor, along with T for transconjugant, and the number of colony picked

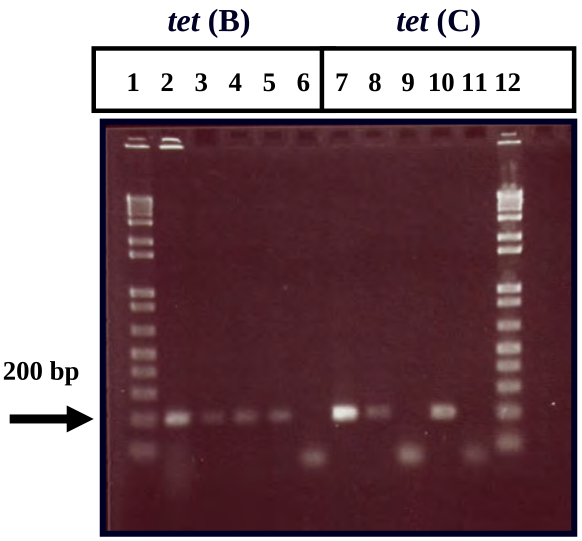

Fig. 1 PCR screening analysis of *tet* (B), *left-side* amplicons (206 bp), and *tet* (C), *right-side* amplicons (207 bp), from a representative tetracycline-resistant bacterial isolate MC2-9R1 and its transconjugant, MC2-9R1T2-1. *Lanes 1* and *12*, 1 KB Plus ladder (Promega); *lanes 2* and *8*, isolate MC2-9R1 cells; *lanes 3* and *9*, isolate MC2-9R1 plasmid DNA; *lanes 4* and *10*, transconjugant MC2-9R1T2-1 cells; *lane 5*, transconjugant MC2-9R1T2-1 plasmid DNA; *lanes 6* and *11*, *Salmonella* strain EM1000 cells (conjugal recipient, negative control); *lane 7*, pBR322 plasmid DNA (positive control for the *tet* (C) gene). The lower bands in *lanes 2*, *6*, *9*, and *11* are DNA primers

could survive in waters with low dissolved oxygen for sustained periods, whereas several of the AUI sites were listed as “reference” or “least impacted” systems in this region (Michigan Department of Environmental Quality 2003a).

Elevated nutrient levels can also improve the efficiency of horizontal gene transfer between species by the bacterial processes of transformation (Davison 1999; Quintiliani et al. 1999; Thomas and Nielsen 2005) and conjugation (Arana et al. 1997, 2001). High fecal coliform densities, as seen in this study, increase the likelihood of humans and animals coming in contact with pathogenic bacteria. Implementing a quantitative risk assessment analysis, as modeled by Hurd (2006), could help identify the “chain of causal events that connect on-farm antibiotic use to additional days of human illness caused by infections with resistant bacteria.”

Antibiotic-resistant fecal coliform bacteria were found in all water samples collected during the course of this study, with A resistance the most prevalent (77.1% overall, Table 4). This proportion of A resistance in fecal coliform bacteria parallels other river and waterway reports (Ash et al. 2002; McKeon et al. 1995; Ogan and Nwiika 1993). Some studies have reported lower frequencies of A resistance yet still found that resistance to A was the most common form of resistance (Iwane et al. 2001; Niemi et al. 1983). In contrast, other studies of soil, water, and manure samples from farm locations have found tetracycline and other drug resistance patterns in higher proportions than A resistance (Esiobu et al. 2002; Sayah et al. 2005). One plausible explanation for the differences seen in antibiotic resistance patterns is the source of the environmental sample, specifically human waste vs. animal waste. Harwood et al. (2000) determined the antibiotic resistance pattern from fecal coliforms isolated from domestic wastewater and various animal feces using four different concentrations of antibiotics; they showed a significantly greater percentage of A resistance in fecal coliform isolates from human sources (62% at 10 μg/ml A) than animal feces (15%) and a significantly greater percentage of C resistance in cattle feces (58% at 20 μg/ml C) compared to the human sources (35%). This could explain why we observed a significantly lower proportion of A resistance (58.4%) at the AI sites (sites near animal waste contamination) than at the AUI sites (83.5%) and sites upstream (81.0%) and downstream (89.1%) of the WWTPs.

The proportion of multi-drug resistance observed at the AI sites near CAFO farms (41.6%) was almost three times greater than at the AUI sites (16.5%). Certain multiple-resistance combinations were more common at some AI sites than others, perhaps reflecting site-specific antibiotic use patterns. In any case, the high proportion of multi-drug resistance at AI sites suggests that the fecal bacterial populations in these locations were subjected to conditions that fostered the acquisition of multiple-resistance determinants. In addition to likely increases in antibiotic-resistant bacteria from animals fed antibiotic-laden feed (Davies 1997; Lu et al. 2004; Wegener et al. 1999), soil-dwelling bacteria are thought to be a significant reservoir of resistance determinants (D’Costa et al. 2006), and studies on crop soils fertilized with animal manure show that horizontal transfer between fecal and soil bacteria is facilitated by the high nutrient availability of manure (Cooke 1976; Schmitt et al. 2006). We observed that fecal coliform levels increased after rain events at the AI sites, where periodic spraying of liquid manure was reported in nearby fields (Kauffman and Melmoth 2003; Michigan Department of Environmental Quality 2003a). Natural water environments may allow fecal coliforms a better selective advantage in becoming antibiotic resistant over other habitats such as soil, sand, or sewage effluent (Cooke 1976). Survival rates tend to be higher in bacteria containing plasmids (Arana et al. 1997, 2001; Ash et al. 2002; Schwartz et al. 2003), and the conjugative transfer of plasmids from one bacterium to another tends to occur more readily in aquatic habitats (Lebaron et al. 1993). The high rate of success for gene transfer as seen in our conjugation assays (83.3% overall) suggests that the environmental isolates carried conjugative plasmids or transposons. Of the multi-drug-resistant isolates, most exhibited resistance to combinations of antimicrobial drugs which included A. This is an indication that multiple-resistance genes may coexist on one plasmid (Davison 1999; Quintiliani et al. 1999; Sayah et al. 2005), a single conjugative transposon (Pembroke et al. 2002; Waters 1999), or an integron (Mazel 2004; White et al. 2001). This condition is particularly disconcerting given that exposure to one antibiotic agent may result in resistance to others without previous exposure (Sayah et al. 2005) or cost to bacterial fitness (Aminov et al. 2001).

The conjugation assay in combination with PCR was used to identify genetic patterns of transfer in bacterial populations. Using 10 primer pairs for the tetracycline resistance genes most commonly found in *E. coli* and other Gram-negative bacteria, we detected the presence of the *tet* (B) and *tet* (C) determinants in 13 of 22 ACO-resistant isolates. In strains isolated at all study sites, *tet* (B) was associated with plasmid DNA in isolates and their transconjugants, while *tet* (C) showed three different patterns of gene transfer. The first pattern of gene transfer showed that the *tet* (C) gene was associated with plasmid DNA in isolates and transconjugants from the AUI sites. The second pattern showed that the *tet* (C) gene from the UPWWTP isolates associated exclusively with total DNA in both the original isolates and the transconjugants and not with plasmid DNA; this suggests that the *tet* (C) gene was located on chromosomal DNA or on a large plasmid or cosmid that could not be purified by our methods. The final pattern of gene transfer found the *tet* (C) gene in total DNA in the isolate, but the *tet* (C) gene was undetectable in the resistant transconjugant. These results suggest that the transconjugant in the third pattern could have received a different tetracycline resistance gene from the donor isolate that was not detected by our PCR screening method (e.g., isolates MCI-22R4 and MCI-1F; Table 7). Two of 13 isolates (Table 7; isolates VH19-11F and MC2-9R1) showed the presence of both *tet* (B) and *tet* (C) genes, with *tet* (B), but not *tet* (C), associated with plasmid DNA, suggesting that the *tet* (B) and *tet* (C) genes are not genetically linked. Furthermore, both the differential distribution of the *tet* (B) and *tet* (C) genes in the VH19-11F and MC2-9R1 isolates (Table 7) between total and plasmid DNA suggested that the plasmid DNA preparations were not contaminated with chromosomal DNA. Additional studies with a larger population of tetracycline-resistant isolates would be needed to better characterize these genetic patterns.

Based on current chemical and biological water quality standards (turbidity, total phosphorus concentrations, and fecal coliform densities), study sites near the WWTPs were considered environmentally healthy, yet had high levels of single- and multi-drug-resistant fecal coliform bacteria (>81% for A alone and >10.9% for multi-drug resistance, Tables 4 and 5). Agricultural sites, especially the AI sites near CAFOs, had much lower measures of traditional water quality and also had high levels of single and multi-drug-resistant bacteria, with multi-drug resistance greatest (41.6%) at the AI sites near CAFOs. The risk to human and animal health posed by the high incidence of antibiotic resistance and gene transfer is unknown. Traditional measures of chemical and biological water quality do not appear to be direct surrogates for detection of the prevalence of antibiotic resistance. Those parameters most elevated in the AI sites (e.g., total phosphorus concentrations, turbidity), however, may have some predictive ability for the prevalence of multiple-drug resistance. We echo previous suggestions (Esiobu et al. 2002; Sayah et al. 2005) that testing for antibiotic resistance genes in bacterial strains become part of the standard methods for examining and regulating water quality and wastewater discharge in areas at high risk for pollution from human and animal waste.

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References

American Public Health Association. (1998). *Section 9000. Microbial examination. Standard methods for the examination of water and wastewater* (20th ed., pp. 56–74). Washington: APHA.

American Public Health Association. (2005). *Section 9260. Detection of pathogenic bacteria. Standard methods for the examination of water and wastewater* (21st ed.). Washington: APHA.

Aminov, R. I., Garrigues-JeanJean, N., & Mackie, R. I. (2001). Molecular ecology of tetracycline resistance: Development and validation of primers for detection of tetracycline resistance genes encoding ribosomal protection proteins. *Applied and Environmental Microbiology, 67*, 22–32.

Aminov, R. I., Chee-Sanford, J. C., Garrigues, N., Teferedegne, B., Krapac, I. J., White, B. A., et al. (2002). Development, validation, and application of PCR primers for detection of tetracycline efflux genes of gram-negative bacteria. *Applied and Environmental Microbiology, 68*, 1786–1793.

Arana, I., Pocino, M., Muela, A., Fernandez-Astorga, A., & Barcina, I. (1997). Detection and enumeration of viable but non-culturable transconjugants of *Escherichia coli* during the survival of recipient cells in river water. *Journal of Applied Microbiology, 83*, 340–346.

Arana, I., Justo, J. I., Muela, A., & Barcina, I. (2001). Survival and plasmid transfer ability of *Escherichia coli* in wastewater. *Water, Air, and Soil Pollution, 126*, 223–238.

Ash, R. J., Mauck, B., & Morgan, M. (2002). Antibiotic resistance of gram-negative bacteria in rivers, United States. *Emerging Infectious Diseases, 8*, 713–716.

Bell, J. B., Macrae, W. R., & Elliott, G. E. (1980). Incidence of R factors in coliform, fecal coliform and *Salmonella* populations of the Red River in Canada. *Applied and Environmental Microbiology, 40*, 486–491.

Bell, J. B., Elliott, G. E., & Smith, D. W. (1983). Influence of sewage treatment and urbanization on selection of multiple resistance in fecal coliform populations. *Applied and Environmental Microbiology, 46*, 227–232.

Boesch, D. F., Brinsfield, R. B., & Magnien, R. E. (2001). Chesapeake Bay eutrophication: Scientific understanding, ecosystem restoration and challenges for agriculture. *Journal of Environmental Quality, 30*, 303–320.

Bullas, L. R., & Ryu, J. I. (1983). *Salmonella typhimurium* LT2 strains which are r−m+ for all three chromosomally located systems of DNA restriction and modification. *Journal of Bacteriology, 156*, 471–474.

Burton, G. R. W., & Engelkirk, P. G. (2004). Epidemiology and public health. In J. Goucher, L. Horowitz, & P. C. Williams (Eds.), *Microbiology for the health sciences* (7th ed., pp. 271–307). Baltimore: Lippincott Williams & Wilkins.

Carpenter, S. R., Caraco, N. F., Correll, D. L., Howarth, R. W., Sharpley, A. N., & Smith, V. H. (1998). Nonpoint pollution of surface waters with phosphorus and nitrogen. *Ecological Applications, 8*, 559–568.

Chao, K.-K., Chao, C.-C., & Chao, W.-L. (2003). Suitability of the traditional microbial indicators and their enumerating methods in the assessment of fecal pollution of subtropical freshwater environments. *Journal of Microbiology, Immunology, and Infection, 36*, 288–293.

Chopra, I., & Roberts, M. (2001). Tetracycline antibiotics: Mode of action, applications, molecular biology, and epidemiology of bacterial resistance. *Microbiology and Molecular Biology Reviews, 65*, 232–260.

Cooke, M. D. (1976). Antibiotic resistance in coliform and fecal coliform bacteria from natural waters and effluents. *New Zealand Journal of Marine and Freshwater Research, 10*, 391–397.

Cooke, G. D., Welch, E. B., Peterson, S., & Nichols, S. A. (2005). *Restoration and management of lakes and reservoirs* (3rd ed.). Boca Raton: CRC.

D'Costa, V., McGrann, K. M., Hughes, D. W., & Wright, G. D. (2006). Sampling the antibiotic resistome. *Science, 311*, 374–377.

Davies, J. E. (1997). Origins, acquisition and dissemination of antibiotic resistance determinants. In D. Chadwick & J. Goode (Eds.), *Antibiotic resistance: Origins, evolution, selection and spread* (pp. 15–35). New York: Wiley.

Davison, J. (1999). Genetic exchange between bacteria and the environment. *Plasmid, 42*, 73–91.

Esiobu, N., Armenta, L., & Ike, J. (2002). Antibiotic resistance in soil and water environments. *International Journal of Environmental Health Research, 12*, 133–144.

France, A. M., Kugeler, K. M., Freeman, A., Zalewski, C. A., Blahna, M., Zhang, L., et al. (2005). Clonal groups and the spread of resistance to trimethoprim-sulfamethoxazole in uropathogenic *Escherichia coli*. *Clinical Infectious Diseases, 40*, 1101–1107.

Gevers, D., Danielsen, M., Huys, G., & Swings, J. (2003). Molecular characterization of *tet* (M) genes in *Lactobacillus* isolates from different types of fermented dry sausage. *Applied and Environmental Microbiology, 69*, 1270–1275.

Graves, A. K., Hagedorn, C., Teetor, A., Mahal, M., Booth, A. M., & Reneau, R. B., Jr. (2002). Antibiotic resistance profiles to determine sources of fecal contamination in a rural Virginia watershed. *Journal of Environmental Quality, 31*, 1300–1308.

Griffin, D. W., Lipp, E. K., McLaughlin, M. R., & Rose, J. B. (2001). Marine recreation and public health microbiology: Quest for the ideal indicator. *Bioscience, 51*, 817–825.

Guan, T. Y., & Holley, R. A. (2003). Pathogen survival in swine manure environments and transmission of human enteric illness—A review. *Journal of Environmental Quality, 32*, 383–392.

Hall, R. M. (1997). Mobile gene cassettes and integrons: Moving antibiotic resistance genes in gram-negative bacteria. In D. Chadwick & J. Goode (Eds.), *Antibiotic resistance: Origins, evolution, selection and spread* (pp. 192–205). New York: Wiley.

Hall, M. L. A., Blok, H. E. M., Donders, A. R. T., Paauw, A., Fluit, A. C., & Verhoef, J. (2003). Multidrug resistance among *Enterobacteriaceae* is strongly associated with the presence of integrons and is independent of species or isolate origin. *The Journal of Infectious Diseases, 187*, 251–259.

Harwood, V. J., Whitlock, J., & Withington, V. (2000). Classification of antibiotic resistance patterns of indicator bacteria by discriminate analysis: Use in predicting the source of fecal contamination in subtropical waters. *Applied and Environmental Microbiology, 66*, 3698–3704.

Hooper, D. C. (2002). Target modification as a mechanism of antimicrobial resistance. In K. Lewis, A. A. Salyers, H. W. Taber, & R. G. Wax (Eds.), *Bacterial resistance to antimicrobials* (pp. 161–192). New York: Marcel Dekker.

Hurd, H. S. (2006). Assessing risks to human health from antibiotic use in food animals. *Microbe, 1*, 115–119.

Illinois Pollution Control Board. (2002). *Illinois Administrative Code, Title 35, Subtitle C: Water Pollution, Chapter 1, Part 302: Water Quality Standards, Section 302.209: Fecal Coliform (As amended at 12 Ill. Reg. 12082, effective July 11, 1988)*. Illinois Pollution Control Board, Chicago, Illinois. http://www.ipcb.state.il.us/SLR/IPCBandIEPAEnvironmentalRegulations-Title35.asp. Accessed 21 February 2008.

Iwane, T., Urase, T., & Yamamoto, K. (2001). Possible impact of treated wastewater discharge on incidence of antibiotic resistant bacteria in river water. *Water Science and Technology, 43*, 91–99.

Kauffman, J., & Melmoth, K. (2003). *Final report, ECCSCM water monitoring project 2001–2003*. Environmentally Concerned Citizens of South Central Michigan, Hudson, MI http://www.nocafos.org/finalreport.htm. Accessed 13 June 2005.

Khachatourians, G. G. (1998). Agricultural use of antibiotics and the evolution and transfer of antibiotic-resistant bacteria. *Canadian Medical Association Journal, 159*, 1129–1136.

Lebaron, P., Roux, V., Lett, M. C., & Baleux, B. (1993). Effects of pili rigidity and energy availability on conjugative plasmid transfer in aquatic environments. *Microbial Releases, 2*, 127–133.

Levy, S. B. (1997). Antibiotic resistance: An ecological imbalance. In D. Chadwick & J. Goode (Eds.), *Antibiotic resistance: Origins, evolution, selection and spread* (pp. 1–14). New York: Wiley.

Lind, O. T. (1985). *Handbook of common methods in limnology* (2nd ed.). Dubuque: Hunt.

Lu, K., Asano, R., & Davies, J. (2004). Antimicrobial resistance gene delivery in animal feeds. *Emerging Infectious Diseases, 10*, 679–683.

Mascaretti, O. A. (2003). *Bacteria versus antibacterial agents: an integrated approach*. Washington: ASM.

Mazel, D. (2004). Integrons and the origin of antibiotic resistance gene cassettes. *ASM News, 70*, 520–525.

McGinnis, S., & Madden, T. L. (2004). BLAST: At the core of a powerful and diverse set of sequence analysis tools. *Nucleic Acids Research, 1*(32), W20–W25. http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=pubmed&list_uids=15215342&dopt=Citation. Accessed 22 October 2009 (web server issue).

McKeon, D. M., Calabrese, J. P., & Bissonnette, G. K. (1995). Antibiotic resistant gram-negative bacteria in rural groundwater supplies. *Water Research, 29*, 1902–1908.

Michigan Department of Environmental Quality. (1999). *Water quality standards. p A-47 (R323.1062 Microorganisms)*. Surface Water Quality Division, Michigan Department of Environmental Quality, Lansing, MI. http://www.michigan.gov/deq/0,1607,7-135-3313_3686_3728-12711–,00.html and http://www.deq.state.mi.us/documents/deq-wb-intreport-appendixa.pdf. Accessed 5 January 2007.

Michigan Department of Environmental Quality. (2003a). *Biological surveys of the tributaries in the vicinity of two CAFOs in the St. Joseph and Bean/Tiffin Watersheds Hillsdale and Lenawee counties, Michigan June 10, 11, and 19, 2003*. Report #MI/DEQ/WD-03-092. Water Quality Division, Michigan Department of Environmental Quality, Lansing, MI. http://www.michigan.gov/deq/0,1607,7-135-3304-54941–,00.html Accessed 23 October 2006.

Michigan Department of Environmental Quality. (2003b). *Combined sewer overflow and sanitary sewer overflow 2002 & 2003 annual report (January 2002–December 2003)*. Surface Water Quality Division, Michigan Department of Environmental Quality, Lansing, MI. http://www.deq.state.mi.us/documents/deq-wd-csossoreport03.pdf Accessed 3 June 2005.

Michigan Department of Environmental Quality. (2004a). *Combined sewer overflow and sanitary sewer overflow 2004 annual report*. Surface Water Quality Division, Michigan Department of Environmental Quality, Lansing, MI. http://www.deq.state.mi.us/documents/deq-wb-csossoreport04.pdf. Accessed 8 June 2005.

Michigan Department of Environmental Quality. (2004b). *Water quality and pollution control in Michigan: 2004 Sections 303(d) and 305(b) integrated report*. Report # MI/DEQWD-04/029. Water Quality Division, Michigan Department of Environmental Quality, Lansing, MI. http://www.michigan.gov/deq/0,1607,7-135-3304-54941–,00.html. Accessed 5 June 2005.

Michigan Department of Environmental Quality. (2005). *Combined sewer overflow and sanitary sewer overflow 2005 annual report*. Surface Water Quality Division, Michigan Department of Environmental Quality, Lansing, MI. http://www.deq.state.mi.us/documents/deq-wb-csossoreport05.pdf. Accessed 27 December 2006.

Mitchell, M. K., & Stapp, W. B. (1997). *Field manual for water quality monitoring* (11th ed., pp. 25–39). Dubuque: Kendall/Hunt.

Niemi, M., Sibakov, M., & Niemela, S. (1983). Antibiotic resistance among different species of fecal coliforms isolated from water samples. *Applied and Environmental Microbiology, 45*, 79–83.

Ogan, M. T., & Nwiika, D. E. (1993). Studies on the ecology of aquatic bacteria of the lower Niger delta: Multiple antibiotic resistance among the standard plate count organisms. *The Journal of Applied Bacteriology, 74*, 595–602.

Oppegaard, H., Steinum, T. M., & Wasteson, Y. (2001). Horizontal transfer of a multi-drug resistance plasmid between coliform bacteria of human and bovine origin in a farm environment. *Applied and Environmental Microbiology, 67*, 3732–3734.

Pell, A. N. (1997). Manure and microbes: Public and animal health problem? *Journal of Dairy Science, 80*, 2673–2681.

Pembroke, J. T., MacMahon, C., & McGrath, B. (2002). The role of conjugative transposons in the *Enterobacteriaceae*. *Cellular and Molecular Life Sciences, 59*, 2055–2064.

Quintiliani, R., Jr., Sham, D. F., & Courvalin, P. (1999). Mechanisms of resistance to antimicrobial agents. In P. Murray, E. Baron, M. Pfaller, F. Tenover, & R. Yolken (Eds.), *Manual of clinical microbiology* (pp. 1505–1525). Washington: ASM.

Rice, E. W., Messer, J. W., Johnson, C. H., & Reasoner, D. J. (1995). Occurrence of high-level aminoglycoside resistance in environmental isolates of enterococci. *Applied and Environmental Microbiology, 61*, 374–376.

Roberts, M. C. (2005). Update on acquired tetracycline resistance genes. *FEMS Microbiology Letters, 245*, 195–203.

Salyers, A. A., Shoemaker, N. B., & Bonheyo, G. T. (2002). The ecology of antibiotic resistance genes. In K. Lewis, A. A. Salyers, H. W. Taber, & R. G. Wax (Eds.), *Bacterial resistance to antimicrobials* (pp. 1505–1525). New York: Marcel Dekker.

Sayah, R. S., Kaneene, J. B., Johnson, Y., & Miller, R. (2005). Patterns of antimicrobial resistance observed in *Escherichia coli* isolates obtained from domestic- and wild-animal fecal samples, human septage, and surface water. *Applied and Environmental Microbiology, 71*, 1394–1404.

Schmitt, H., Stoob, K., Hamscher, G., Smit, E., & Seinen, W. (2006). Tetracyclines and tetracycline resistance in agricultural soils: Microcosm and field studies. *Microbial Ecology, 51*, 267–276.

Schwartz, T., Kohnen, W., Jansen, B., & Obst, U. (2003). Detection of antibiotic-resistant bacteria and their resistance genes in wastewater, surface water and drinking water biofilms. *FEMS Microbiology Ecology, 43*, 325–335.

Stoner, N. (2005). *EPA's proposed policy on sewage dumping during wet weather conditions*. Presented testimony, House Committee on Transportation and Infrastructure's Water Resources and the Environment Subcommittee, April 13, 2005. National Resources Defense Council, New York, NY. http://www.nrdc.org/water/pollution/tns0405.asp. Accessed 21 November 2005.

Tabbara, H. (2003). Phosphorus loss to runoff water 24 h after application of liquid swine manure or fertilizer. *Journal of Environmental Quality, 32*, 1044–1052.

Taber, H. W. (2002). Antibiotic permeability. In K. Lewis, A. A. Salyers, H. W. Taber, & R. G. Wax (Eds.), *Bacterial resistance to antimicrobials* (pp. 193–208). New York: Marcel Dekker.

Taylor, R. K., Manoil, C., & Mekalanos, J. J. (1989). Broad-host-range vectors for the delivery of Tn*pho*A: Use in genetic analysis of secreted virulence determinants of *Vibrio cholerae*. *Journal of Bacteriology, 171*, 1870–1878.

Thomas, C. M., & Nielsen, K. M. (2005). Mechanisms of, and barriers to, horizontal gene transfer between bacteria. *Nature Reviews. Microbiology, 3*, 711–721.

US Environmental Protection Agency. (2002). *United States Code, Title 33, Chapter 26: Federal Water Pollution Control Act*. (As amended through P.L. 107-303, November 27, 2002.). US Environmental Protection Agency, Washington, DC. http://www.epa.gov/region5/water/cwa.htm. Accessed 27 December 2006.

Waters, V. L. (1999). Conjugative transfer in the dissemination of beta-lactam and aminoglycoside resistance. *Frontiers in Bioscience, 4*, 416–439.

Wegener, H. C., Aarestrup, F. M., Jensen, L. B., Hammerum, A. M., & Bager, F. (1999). Use of antimicrobial growth promoters in food animals and *Enterococcus faecium* resistance to therapeutic antimicrobial drugs in Europe. *Emerging Infectious Diseases, 5*, 329–335.

Wetzel, R. G. (2001). *Limnology: Lake and river ecosystems* (3rd ed.). New York: Academic.

Wetzel, R. G., & Likens, G. E. (2000). *Limnological analyses* (3rd ed.). New York: Springer.

White, P. A., McIver, C. J., & Rawlinson, W. D. (2001). Integrons and gene cassettes in the *Enterobacteriaceae*. *Antimicrobial Agents and Chemotherapy, 45*, 2658–2661.

Whitlock, J. E., Jones, D. T., & Harwood, V. J. (2002). Identification of the sources of fecal coliforms in an urban watershed using antibiotic resistance analysis. *Water Research, 36*, 4273–4282.

Wiggins, B. A., Andrews, R. W., Conway, R. A., Corr, C. L., Dobratz, E. J., Dougherty, D. P., et al. (1999). Use of antibiotic resistance analysis to identify nonpoint sources of fecal contamination. *Applied and Environmental Microbiology, 65*, 3483–3486.

Witte, W. (1997). Impact of antibiotic use in animal feeding on resistance of bacterial pathogens in humans. In D. Chadwick & J. Goode (Eds.), *Antibiotic resistance: Origins, evolution, selection and spread* (pp. 61–75). New York: Wiley.

Zar, J. H. (1999). *Biostatistical analysis* (4th ed.). Upper Saddle River: Prentice Hall.