IDENTIFICATION AND REMEDIATION OF MICROBIAL CONTAMINANTS IN THE HEADWATERS OF AN AGRICULTURAL WATERSHED

DISSERTATION

Presented in Partial Fulfillment of the Requirements for the Degree Doctor of Philosophy in the Graduate School of the Ohio State University

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ABSTRACT

Forty years after the passage of the Clean Water Act, water contaminated with microbial pathogens is still one of the most severe challenges in the U.S. The inability to differentiate non-point sources of pathogens has severely limited the development of effective management strategies to reduce their presence. To reduce both point and non-point source pollution in U.S. waterways, USEPA established and implemented the limits of the total maximum daily load (TMDL) specific to each watershed since 1985. Meanwhile, USDA and other agencies try to diffuse conservation practices to improve streams. However, microbial contamination remains as one of the most severe problems in natural waterways particular in agricultural-dominant watersheds. To successfully address the problems, it is critical to understand sources of pollution, stream habitat characteristics, and attitudes of local people on their streams to develop effective remediation strategies from a holistic point of view.

Using the Upper Sugar Creek as a model watershed, we identified potential sources of microbial pathogens; correlated microbial diversity and stream habitats characteristics; and analyzed factors affecting local people’s willingness to adopt conservation practices to reduce the presence of microbial pathogens in the watershed. Study results show that the primary sources of microbial pollution in the Upper Sugar Creek were the human coliforms instead of animal coliforms. The increased pathogen
level resulted from failing septic systems and the lack of awareness and knowledge regarding microbial pollution and septic systems. Study results also demonstrate that microbial diversity largely depended on land use, precipitation, and temperature, and was positively associated with stream habitat characteristics. Habitat characteristics are determined by two positively correlated variables, the primary headwater habitat evaluation index (HHEI) and headwater macroinvertebrate field evaluation index (HMEFI). They can be used as a rapid tool to access headwaters in the field. Survey results indicated decisive factors affecting people’s adoption of conservation practices. They are farm size, income, farm succession, sense of place, and awareness and knowledge about stream pollution. Regression results of mean willingness to pay for farmers and non-farmers were $151.67 and $156.95 for scenario 2 that microbial pathogen level would be lower than EPA standards for 3 years, and $171.88 and $197.57 for scenario 3 that that microbial pathogen level would meet EPA standards for 5 years.
Dedicated to my parents
ACKNOWLEDGMENTS

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FIELDS OF STUDY

Major Field: Environmental Science
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CHAPTER 1

1.1 INTRODUCTION

This dissertation aims to: a) identify potential sources of microbial pathogens in the Upper Sugar Creek (Chapter 2); b) correlate microbial diversity and stream habitat characteristics (Chapter 3); and c) analyze factors affecting local people’s willingness to adopt conservation practices to reduce the transmission and presence of microbial pathogens in the watershed (Chapter 4). First, however, it is important to present some general concepts and background to water quality research on headwaters and then proceed to describe the Sugar Creek Watershed and why it is an ideal site for testing microbial contamination and microbial diversity.

The term “headwaters” generally refers to the aggregation of the small swales, creeks and streams that are located in the upper portion of a watershed (Schumacher, 2003 b; Nadeau and Rains, 2007). However, when it comes to defining a specific headwater stream, consensus is lacking. One proposed definition focuses on stream channel or definable stream banks. For example, Dietrich and Dunne (1993) defined headwater streams as having a channel head namely “the upstream boundary of
concentrated water flow and sediment transport between definable banks.” Richardson and Danehy (2006) described headwater streams as “the channels that occur at the fringe of any fluvial network.” Another definition is based on stream order (Nadeau and Rains, 2007, Meyer and Wallace, 2001). According to the stream order classification, headwater streams are considered to be first- and second- order streams on a map scale of 1:24000 or with catchment areas < 100ha (Fritz, et al., 2006; Clarke et al., 2008). Both definitions focus on ecological structure but tend to neglect ecological function. So the overemphasis on bank or stream order may underemphasize the importance of draining zero-order basins such as vernal pools or ephemeral streams, which may be dry for long periods of a year. For example, low flow streams that are hidden beneath forest canopy or streams in the prairie regions may appear as low grassy fields or woodlands with no clearly defined channel. These areas are able to transport water, nutrients and sediments to the larger downstream rivers during storm events. Equally, in many places in the world with rice paddy agriculture, standing water in the paddies is transferred underground as one of the salient environmental benefits thus preventing erosion and nutrient loss.

For the purposes of this study, I use the OEPA’s definition that a headwater stream is a stream with a watershed less than or equal to twenty square miles, namely streams that are zero-, first- and second-order streams. In fact, most streams of the Upper Sugar Creek, have a drainage area less than one square mile and maximum pool depth less than 40 centimeters. They are further classified as primary headwater streams by Ohio EPA.

These small headwater streams including those that are dry for long periods of the year play a significant role in the aquatic system. They constitute over 60% of total
stream length in fresh watersheds, contribute 70% of water volume to downstream, and directly connect the surrounding landscape to the watershed ecosystem (Sidle et al. 2000; Meyer and Wallance, 2001; Alexander et al., 2007; Freeman et al., 2007). Furthermore, they benefit surrounding areas and nourish downstream reaches in a number of ways such as maintaining water quality, recycling of nutrients, attenuating floods, trapping sediment and pollution, and providing diverse habitats for flora and fauna (Sidle et al., 2000; Schumacher, 2003a & b; Meyer et al., 2007; Nadeau and Rains, 2007). In short, headwater streams have a profound influence on shaping downstream water quality and quantity. Therefore, it is especially important to protect headwater streams to keep the entire stream systems healthy.

However, despite their critical roles in maintaining stream ecology, headwater streams are some of the most understudied and degraded water resources (Belsky, 1999; Meyer et al., 2007). They have been largely overlooked by most lotic ecologists, and have been lost from the landscape at an alarming rate. According to USEPA (2011a), of 971,156 (27.5%) miles of assessed streams in the United States, 53% of them are impaired, and 1% of them are threatened. Of 52,483 (90.1%) assessed streams in Ohio, impaired streams accounts for 97% with agriculture, hydromodification, sewage and municipal discharge listed as three primary pollution sources. Our target study watershed, Sugar Creek, was labeled by Ohio EPA as the second most impaired watershed in 1998 due to high level of nutrient loading, sedimentation, bacteria, and habitat loss (OEPA, 2002). Studies pertaining to nutrient loading and sedimentation in headwater streams have been conducted, but owing to the complexity and expense involved, studies on bacteria, heavy metals, and habitat are sparse.
Problem Statement

Forty years after the passage of the Clean Water Act of 1972, water contaminated with microbial pathogens remains one of the most severe challenges in the United States. Transport of pathogens into natural waters can result in serious risk to human health. A well-known example is the massive outbreak of *Cryptosporidium* in Milwaukee in 1993. It was estimated that the outbreak killed 104 and sickened over 403,000 people, which is the largest waterborne disease outbreak in documented U.S. history (MacKenzie et al., 1994). The cause of the tragedy was suspected to be from water contaminated by animal excrement from nearby factory farms. Another typical example is the consistently high number of beach closures over the past decade. According to USEPA (2011a & b; Table 1.1), 459 (24%) beaches were closed or advised in 1999, the number reached 1,184 (43%) in 2009, and 1,362 (37%) in 2010 with more than 60% attributed to fecal pollution. In Ohio, 942 (26%) beaches were closed in 2004. As shown in Table 1.1 the number jumped to 89% in 2009, and 97% in 2010. Most (73%) of the closures were due to high concentration levels of bacteria from non-point sources (USEPA 2011 b; Dorfman and Rosselot, 2011).

Microorganisms associated with human and animal fecal wastes can be transported to water through a number of different ways, such as manure field application, sewage overflow, failing septic systems, runoff from animal farms, pasture, and crop fields. However, it is difficult to track the true sources of microbial pathogens since most are from non-point sources. The inability to differentiate non-point sources of microbial contamination has severely limited the development of effective remediation strategies to reduce their presence. As a result, microbial contamination remains as one of the most
severe problems in natural waterways particular in agricultural-dominant watershed. Although it will not be explored in this dissertation, a rising concern in this watershed is the possible cross-contamination of microbes in hydro-fracturing. The brine trucks which deliver brine water to injection wells also sometimes pump water from Sugar Creek (Source: local citizen and Wayne County Soil & Water Conservation Districts).

To reduce both point and non-point source pollution in U.S. waterways, the USEPA has established and implemented the limits of the total maximum daily load (TMDL) specific to each watershed since 1985. The TMDL program, recently revised in 2003, requires state-level EPAs to prioritize local watersheds, develop remediation plans, and improve pollution to acceptable standards (Moore et al., 2008). The approval of a TMDL program for each specific watershed involves at least three steps. First, EPAs assesses the mainstem of watersheds that have been marked as having a high level of impairment in each state. A 303 (d) list, which is list of prioritized waters with effluent quality or nutrient levels exceeding EPA standards, is then submitted to the regional EPA associated with assessment results. In the reports both point sources and non-point sources are listed. For the point sources load allocations are established and usually nutrient reduction load goals for each subwatershed are set. A final report compiled with major problems of a watershed, remediation plans, technical support document, and GIS modeling is submitted to regional EPA for approval. After approval, the state has five years to improve pollution to the limitation level. Our target study watershed, the Upper Sugar Creek was the second watershed in Ohio to create an aquatic life use TMDL program (2002) and the first to have a bacteria TMDL program (2007).
Although TMDL programs have been implemented across the country for over ten years, the microbial pollution level in some natural waterways has not been reduced significantly. This may be due to the limited budget of EPA, limited staff, and a lack of scientific support to local agencies. It was estimated that assessed streams and rivers, and lakes respectively accounted for only 23%, and 42% of the total in 2000 (Moore, et al., 2008). Moreover, a criticism is that most of EPA’s effort has been directed toward more easily identified point sources such as effluent pipes from industrial plants. This makes sense from a historical point of view and, in fact, the NPDES permit program has actually been very effective in reducing point source pollution nationally and in Ohio. One problem in improving non-point pollution is that many small streams are scattered over a watershed, and pollution sources are hard to identify in agriculture-dominant watersheds. EPA has been sued a lot for both non enforcement of the Clean Water Act as well as for over-steeping its authority (current Farm Bureau case over TMDLs pending). Our study watershed has at least three problems associated with the EPA bacterial TMDL program.

First, OEPA employed a load duration model to develop its bacteria-impairment TMDL for the Sugar Creek watershed. This method provides a rapid and visual assessment of water quality, which helps to quantify and identify water quality problems surrounding the impairment. It also makes the comparison between pre- and post-TMDL possible in order to evaluate the effectiveness of best management practices (Benham et al., 2006; Kim et al., 2007). However, the model is weak in differentiating point- and non-point source transition. The load-duration curves present the integrated response from the entire watershed above the monitoring sites, but offer no information on loading and sources within the watershed. As a result, it is somewhat arbitrary when they derive
waste load allocations. Another problem of the model is that it lumped pasture and hay together. In fact, these two types of land use are very different. Pasture contributes more to bacteria loading in streams than hay does.

Second, the data used to develop the bacteria TMDL came from mostly from OEPA’s own intensive sampling conducted in the summer of 2005. The intensive data collected in a single year from multiple sources and simply combined casts some doubt on the reliability of the report, although it was a pioneer study. Furthermore, the water sampling was done in summer, so other seasons were entirely missed. Spring and autumn seasons, however, tend to have intensive rainfall, and manure and human wastes are more likely to be washed off into streams during these seasons.

The third problem associated with the Upper Sugar Creek bacteria TMDL is non-representative sampling sites. OEPA selected water sampling sites based on physical geographic setting (geometric and sentinel). They evenly distributed sampling sites over the entire watershed to make sure adequate spatial coverage. However, previous study in the same sub-watershed showed that the number of E. coli on three headwater streams varied from site to site even on the same stream. Equally, there were different strains of E. coli present on different days in the same stream at the same sampling site (Moore et al., 2009). The results suggest that the magnitude and types of E. coli are closely associated with complex landscape. Thus selection of sampling sites based on a geographic setting is problematic and results generated from may skew the real situation.

To successfully address the pollution problems in agriculture-dominant watersheds such as Sugar Creek, it is critical to understand the ecosystem processes operating in the watershed and to develop effective remediation strategies from a holistic
point of view (Park, et al., 2009). Source identification and enumeration of microbial contaminants provides specific information about land management practices. Assessment of stream habitat offers additional useful information for decision makers as well as local residents. Delivering study results back to local communities increases local people’s awareness on the stream degradation, and enhances communications between neighbors, the research center, and government agencies. Effective management of complex watershed requires the interconnectedness of social-ecological systems. After all, people living in the watershed are the key component to protect and remediate impaired headwater streams.

1.2 SIGNIFICANCE OF THIS STUDY

Interest in understanding functions of headwater streams is longstanding. Over the past two decades, studies of nutrients recycling, crop field runoff, and chemical pollution have attracted the attention of soil scientists and environmental agencies. However, relatively less research has been done on pathogen loadings due to difficulties of source tracking. Our study is critical to understand the fate and transport of microbial pathogens in agriculture-dominant watershed, which can be replicated in other similar watersheds in the Midwest and worldwide.

Moreover, although water pollution has been studied for last 50 years and much knowledge related to water systems is available (Hack and Goodlett 1960, Hewlett and
Hibbert 1967, Linkens et al. 1977), the dynamics of microbial diversity from upper to downstream in headwater systems are poorly understood. Furthermore, no method is universally accepted to assess headwater streams, and no research has addressed the relationship between microbial diversity and habitat characteristics. This study is one of the first to apply a local-agency-developed method to evaluate headwaters, and to examine the possibility of the habitat index as an indicator of microbial diversity.

Finally, this study will produce needed empirical data about headwaters degradation in USA. It will identify local people’s willingness to pay (WTP) to adopt best management practices (BMPs). It will also identify obstacles and opportunities of BMPs diffusion. Thus, it will contribute information potentially useful to policy-makers as they struggle to develop policies and improve TMDL programs, to the researchers in the field of community development, and potentially to the public at large.

1.3 OBJECTIVES AND HYPOTHESES

The central goal of the study is to discover practical intervention strategies to interrupt transmission of the disease problems associated with contaminated water. The study, based on the Upper Sugar Creek Watershed in Ohio, is designed to determine the original sources of microbial contamination, microbial diversity, stream habitat characteristics, as well as local people’s adoption behavior of conservation practices. It
focuses on not only water quality but also in-stream habitat and factors constituting biologically healthy streams from a holistic point of view.

The objectives are:

- To identify potential sources of microbial pathogens in the Sugar Creek headwater streams;
- To correlate microbial diversity and habitats characteristics of streams;
- To analyze factors affecting local people’s willingness to adopt conservation practices to reduce the transmission and presence of microbial pathogens in the Sugar Creek headwater streams.

Hypotheses

In this study, I put forward three sets of hypotheses for each specific objective. They are discussed in detail in later chapters.

Hypotheses on microbial contamination (objective 1)

a) The primary source of microbial pollution in the Upper Sugar Creek is human coliform instead of animal coliform;

b) The dominant septic system is soil adsorption or leach fields in the watershed, and failing septic systems are the main cause of pathogen pollution;

c) Fifty percent of respondents lack awareness and knowledge regarding microbial pollution and septic systems

Hypotheses on microbial diversity and stream characteristics (objective 2)

a) Microbial diversity in the Upper Sugar Creek Watershed largely depends on land use and climate;
b) Primary headwater habitat evaluation index (HHEI) and headwater macroinvertebrate field evaluation index (HMEFI) are positively correlated, and can be used as a rapid tool to assess headwater stream in the field;

c) Microbial diversity is positively associated with HHEI and HMFEI, and can be used as an indicator of quality of water and stream ecosystem in agricultural watersheds.

_Hypotheses on local people’s adoption behavior (object 3)_

a) Farm size and income are positively correlated with the adoption of conservation practices;

b) Land tenure attributes (farm succession and sense of place) are closely correlated with people’s adoption behavior;

c) Lack of awareness and knowledge about stream pollution is a main barrier of conservation adoption.

### 1.4 DESCRIPTION OF STUDY AREA

#### 1.4.1 Geographic location

The Sugar Creek Watershed (HUC 05-040001-100) is located in north central Ohio (Figure 1.1). It covers 357 square miles and four counties including Holmes (26%), Wayne (28%), Stark (11%), and Tuscarawas (35%) (OEPA 2002). The main stem of Sugar Creek runs for 45 miles long, and flows from the north, Smithville in Wayne
County, to south, Tuscarawas River near Dover. It is the headwaters of the Muskingum Watershed (HUC 05-0400) where more than 70% of the basin land is devoted to agricultural uses (OEPA 2007). In 1998, the Ohio Environmental Protection Agency labeled the Sugar Creek Watershed as the second most impaired in Ohio with excessive nutrient loading, loss of habitat, high water temperature, low dissolved oxygen, and high level of bacteria. The watershed consists of eight sub-watersheds: Upper Sugar Creek, Little Sugar Creek, North Fork, Middle Fork, South Fork, Indian Trail, Walnut Creek, and East Bran of the South Fork. Our study site, the Upper Sugar Creek watershed is located in Wayne County, one of the largest dairy counties in Ohio. It drains 28 square miles, consisting of thirty-six primary headwater streams, ranging from Wooster to Orrville with Smithville in its heart.

1.4.2 Soil characteristics

The Sugar Creek watershed lies in two ecoregions. The northern half, which is our study site located, is in the glaciated Erie and Ontario Drift and Lake Plain (EOLP), while the southern half is in the non-glaciated Western Allegheny Plateau (WAP). According to Wayne and Holmes County soil maps (1988), there are more than 8 distinct types of soil in the watershed, but no single type dominants. In the Wooster area, Miami clay loam (40%) is the dominant soil type with an average depth of 10 inches. In Smithville and Orrville, Rittman-Wadsworth association, Melvin-Euclid-Orrville, and Fitchville-Glenford associations are dominant. The latter two are nearly level to moderately steep, somewhat poorly drained, deep soils that formed in silt and loamy alluvium.
1.4.3 Land use and population

The Upper Sugar Creek (Fig1.2) has different land uses such as crop fields (49%), forest (28%), pasture and hay (18%), residential (3.29%), wetlands (0.5%), commercial or industrial (0.8%), and others. Agriculture accounts for 67% of the total watershed area. Primary agriculture activities include row crops, dairy farms, beef and poultry confined feeding operations, forage production, and fruit production. Among the four counties where Sugar Creek flows through, Wayne County has the highest density in Ohio for all categories of livestock, with cattle constituting the majority of livestock numbers.

It was estimated that the population in Wayne County was 114,611 in 2010 with an increase of 2.6% from 111,564 in 2000 (U.S. consensus 2010). Approximately 457 households with 1,200 people reside in the Upper Sugar Creek Watershed area. More than 96% of them are white, while Black, American Indian, German and other races accounts for only 4%. While the Sugar Creek Watershed is home to one of the nation’s largest Amish population, the Upper Sugar Creek has no Amish (Parker, 2006). Female and male population is approximately equal. Forty-three percent of the population received high school education. The media age is 37.8 years. The average household income was $48,375.

In summary, the unique characteristics of the Upper Sugar Creek Watershed make it an ideal location for microbial contamination and microbial diversity study. First, the mix-use of streams representing various land uses allows us to examine associations among land use, microbial contamination, and microbial diversity. By testing the magnitude of microbial pathogens, identifying pollution sources, and differentiating
microbial communities, we are able to quantify the problems of the pollution and develop effective remediation strategies. Second, the relative small size of the watershed makes the Sugar Creek headwater streams an ideal location to detect the dynamic of microbial diversity along streams. It allows us to detect new microorganisms flowing from surrounding landscape before they die or consumed by predators in larger streams. Finally, over twelve years of research experiences in the watershed offers a solid background to conduct the research. Positive cooperation with local residents, government, and other research agencies makes the completion of this research possible including water sampling, conducting HHEI and distributing a social survey.
1.5 REFERENCES


28. Schumacher, B. 2003b. Fact Sheet: The Importance and Benefits of Primary Headwater Streams. State of Ohio Environmental Protection Agency. Columbus,


Figure 1.1 Sugar Creek Watershed
Data source: 1990 US Census TIGER/Line Files, USGS 8 and 14-digit HUC Coverage
Figure 1.2 Land use of the Upper Sugar Creek Watershed
Red dots with numbers represent the 21 water sampling sites for microbial the contamination test.
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*Incomplete data from 11 states
**Incomplete data from four territories

Table 1.1 Summary of beach report from 1999 to 2010
Data source: U.S. EPA Annual beach notification summary
CHAPTER 2

MICROBIAL CONTAMINATION IN THE UPPER SUGAR CREEK WATERSHED

2.1 ABSTRACT

Water contaminated with microbial pathogens poses a significant threat to the environment and human health worldwide. Clearly the tremendous growth of animal agriculture over the past few decades has contributed to stream degradation in the U.S. However, in some cases, although often being blamed, livestock is not always the source of microbial contamination.

In this chapter, using the Upper Sugar Creek Watershed as a model, we identified that the primary source of microbial contamination was human fecal coliforms (76.2%). The majority (63%) of septic systems are traditional soil adsorption/leach fields instead of mound or sand bioreactor systems that are considered suitable in the watershed area. The main causes of microbial pollution include straight pipes (25%) and failing septic systems (53.8%). Local people lack
awareness and knowledge of microbial pollution (over 50%) and septic systems (97%), which are barriers to conservation adoption.

2.2 INTRODUCTION

Water contaminated with microbial pathogens poses a significant threat to human health and the environment worldwide. According to World Health Organization (WHO, 2000), globally 3.2% of human deaths are attributable to unsafe water, and 2.2 million deaths are associated with diarrhea each year. It is most commonly caused by gastrointestinal infections of which a good percentage is due to microbial contamination of water (WHO, 2003; WHO, 2004). Unfortunately, even in the United States today, 40 years after the Clean Water Act, microbiological contamination of water is still one of the most important and difficult challenges.

The 2000 National Water Quality Inventory of United States Environmental Protection Agency (USEPA, 2002) reported that, of the impaired surface waters, 13% were due to the presence of pathogen indicator bacteria (e.g. fecal coliform, Escherichia coli) primarily from non-point sources including domesticated animals, wildlife and septic systems. The Centers for Disease Control and Prevention (CDC, 2004) recorded 65, the largest number since 1978, outbreaks of waterborne disease associated with recreational waters in 2001-2002, half of which were outbreaks of diarrheal illness resulting from microbial contamination. Furthermore, during the
swimming season in 2010, 1,362 (37%) beaches were closed or advised with more than 60% due to fecal pollution (USEPA 2011). In 2008, beach closures and advisories reached 20,341 days with 73% due to detected bacterial levels exceeding standards, and 62% due to unknown sources of pollution (Dorfman and Rosselot, 2004). Although a great proportion of pollution sources remain unidentified, in many cases, livestock is often the prime suspect.

Clearly the tremendous growth of animal agriculture over the past few decades has contributed to stream degradation and water pollution such as nutrient loading, chemical contamination, introduction of pathogens to surface and ground waters. However, in some cases, livestock is not always the source of microbial contamination. For example, in 2011, a massive outbreak of \textit{E coli} sickened 3,000 people across 14 countries and killed over 30 people in Europe (McAllister et al., 2012; Rasko, et al. 2011). A rare enterohemorrhagic strain of bacterium \textit{E coli} O104:H4 was identified as the source of infection. The source of the outbreak was initially assumed to be sprouts irrigated by water contaminated with livestock manure. However, Scheutz et al. (2011) found that \textit{E coli} O104:H4 had not been isolated from livestock, and was most often associated with humans. Understanding the true sources of microbial pollution is vital in predicting health risk and developing effective management strategies to reduce the presence of microbial pathogens.

In this chapter, I a) review variables affecting survival and transport of microbial pathogens in environment focusing on soil and water, the status of septic systems in U.S., possible reasons of septic systems failure, and related sanitary policy
in Ohio; b) identify the primary pollution sources of the watershed by using Quanti- 
Tray/2000™, molecular source tracking, and survey methods; c) summarize the 
microbial contamination of the watershed, characteristics of septic systems, and local 
people’s attitudes toward microbiological pollution of their streams; and d) discuss 
local people's willingness to pay to adopt suitable septic system as a best management 
practice and other potential strategies to reduce microbial contaminants in the 
watershed.

2.3 LITERATURE REVIEW

2.3.1 Survival and transport of microbial pathogens in environment

Microbial pathogens can be transported to the environment through a number 
of different ways including manure field application, directly discharging from 
wildlife or domestic pets, runoff from pasture or crop fields, sewage overflow, leaky 
septic systems, and straight pipe from houses. A range of interacting environmental 
factors have been reported that affect the survival and transport of pathogens in soil 
and water including both extrinsic and intrinsic factors. They can be chemical, 
physical and biological factors of soil and water. The chemical factors include 
temperature, pH, dissolved oxygen, and soil cation exchange capacity. Physical 
factors are soil porosity, soil texture, water conductivity, solar radiation, and organic
matter content. The biological environment is affected by competition, predators, and growth rate. The primary driving forces of water are water flow rate, precipitation, and water temperature.

2.3.1.1 Survival and transport of microbial pathogens in soil

Three primary transport pathways of pathogens in the environment are movement downward with infiltrating water, movement with surface runoff water, and transport of sediment and waste particles. The survival and transport of pathogens in the soil are affected by sets of variables such as soil moisture, soil porosity, nutrient availability, and other management factors (Reddy et al. 1981; Gerba and Bitton 1984; Gerba et al. 1975).

Soil moisture

Among numerous variables affecting pathogen microbes’ survival, soil moisture seems to be the most important (Sjogren 1994; Crane and Moore 1986). Myriad studies have shown that the significant increase in pathogenic bacteria is associated with high soil moisture. Hagedorn et al. (1978) found that E. coli population increased dramatically as the water table rose following major rainfall events. Kibbey et al. (1978) discovered that Streptococcus faecalis died more rapidly under low soil moisture conditions. Mubiru et al. (2000) linked the correlation between lower die-off rates of E. coli and higher soil moisture. In general, soil moisture favors the survival of bacteria. High moisture is associated with long survival of bacteria, in contrast, reductions in bacteria are observed under dry soil
conditions. However, too much moisture can have a negative effect on bacteria survival due to a low availability of usable organic carbon in diluted water (Jamieson et al. 2002)

Water saturation state of soil and the flow of water are important variables in the movement of pathogenic microbes. Microbes move rapidly under saturated conditions, but only for a few centimeters (Santamaria and Toranzos, 2003), because of the adsorption of microbes onto the soil particles. Studies have shown that bacterial concentration is higher in the top 0 to 5 cm of soil than in deep soil, which indicates that absorption most likely occurs in top soil. However, microbes can also be transported long distances through soil when the flow of water increases (e.g., heavy rainfall, melt snow). Tyrrel and Quinton (2003) correlated the diffuse catchment sources of fecal contamination with high river flows resulting from storm events. This association with storm events suggests that rapidly responding hydrological pathways such as overland flow are likely to be important.

Soil texture and porosity

The ability of the soil to filter microbes depends largely on its texture and pore space (McMurry et al. 1998). Most studies indicate increased movement of bacteria in coarse-grained soil (e.g. sand) as opposed to fine-grained soils (e.g., clays and silts) (Santamaria and Toranzos 2003). This is because of large surface areas and the negative charge of clay and organic matter in soil, which affect adsorption of bacteria (Marshall 1971; Reddy et al. 1981). In addition, clays can be a barrier to protect bacterial cells against microbial predators and parasites.
Mawdsley et al. (1996) found that transport of *Cryptosporidium parvum* oocysts through soil and into leachate were greater in silts and clays than in a loamy sand soil. Hagedorn et al. (1978) found that fecal bacteria moved faster in coarse soil materials. Similarly Tan et al. (1991) also observed the rapid movement in coarse as opposed to fine soil fractions. Studies also show that, filtration, sedimentation, and pore size have a greater impact on the transport of larger microorganisms such as protozoa and bacteria rather than small microorganisms such as viruses (Mawdsley et al. 1996). Natsch et al. (1996) observed more macropores and a higher concentration of Pseudomonas fluorescens in untilled grass plots than in tilled wheat fields. One explanation is that the rapid flow of water through macropores largely bypasses the filtering and adsorptive effects of the soil, and therefore greatly increases the risk of pathogen transport to ground water and land drains.

**Cation exchange capacity**

Cation exchange capacity is the major characteristic of soils involved in the adsorption of bacteria (Santamaria and Toranzos 2003). The retention of bacteria increased with an increased in cation exchange capacity of the soil (Reddy et al. 1981). Soil with higher organic matter and clay contents has greater cation exchange capacity, and therefore has high capacity to hold bacteria. Jewett et al. (1995) showed that in laboratory columns packed with silica spheres, bacterial retention was directly related to the ionic strength of the carrying solution. Decreasing the ionic strength caused the collision efficiency. Similarly, Fontes et al. (1991) found that increasing
ionic strength enhances the ability of bacteria to adhere to the soil particles by increasing the availability of ions in solution.

**Nutrient availability**

The nutrient supply and organic matter contained in soil promote the survival of bacteria, and sometimes contribute to the regrowth of enteric bacteria (Gerba et al. 1975). Nutrient availability is a key determinant of microbes’ survival in soil. It has been identified that enteric bacterial die-off in soil was primarily due to the inability of bacteria to lower their metabolic requirements in the situation of low availability of usable organic carbon (Klein and Casida 1967), and low nutrient availability (Reddy et al. 1981).

Organic matter content increases the adsorption surface areas, moisture retention properties and organic carbon, and therefore prolongs the survival of bacteria. Many studies have shown increased adsorption in soils with high organic matter or clay contents (Guy and Visser 1979; Huysman and Verstraete 1993). Zimbilski and Weaver (1978) found the same effect on organic waste materials, which may explain the extended bacterial survival in concentrated waste storage.

Some inorganic nutrients such as \((\text{NH}_4\text{)}_2\text{SO}_4\) foster (Carlucci and Pramer 1959), but some restrain (Jenkins et al. 1998) the growth of bacteria. Jenkins et al. reported increasing the die-off rate of microorganism associated with increasing concentration of ammonia (Jenkins et al. 1998). They found that even at low concentrations of ammonia, the viability of *Cryptosporidium oocysts* in vitro
significantly decreased, while at high concentrations of ammonia, a wide range of microorganisms died off.

**Temperature**

Most studies demonstrate that higher temperatures decrease the survival times of fecal bacteria, while lower temperatures prolong bacteria survival (Kibbey et al. 1978; Zimbilski and Weaver, 1978; Reddy et al. 1981; Jamieson et al. 2002). In addition, the extreme high or low temperatures are the most disruptive to bacterial survival. Extreme high temperatures, especially combined with drying conditions, will effectively increase die-off rates. At the same time, freeze-thaw cycles of soil will also decrease bacterial populations.

Van Donsel et al. (1967) observed that 90% of coliform bacteria died within 3.3 days in the summer compared to 13.4 days in the winter. Santamaria and Toranzos (2003) found that the die-off rates increased significantly for poliovirus as the temperature increased from 15 °C to 40 °C. Reddy et al. (1981) reported that die-off rates of pathogens approximately doubled with a 10 °C rise in temperature (5-30 °C), and the rates increased with decrease in soil moisture. Kibbey et al. (1978) found that the survival of fecal streptococci (FS) was prolonged for at least 12 weeks under cool, but freezing and thawing of soils decreased the population up to 95%. Zibilske and Weaver (1978) conclude that the high temperatures and dry soil conditions are a set of conditions that consistently led to the death of *Salmonella* within 1 week. It is important to note that Howell et al. (1996) reported greater fecal coliforms (FC) survival and sometimes regrowth under warmer conditions.
**Competition**

The growth and survival of pathogenic bacteria in soils and water must compete for essential nutrient and resources including carbon sources, amino acids, nutrients, and moisture with the indigenous soil microbial community. Competing microorganisms limits pathogen survival in the soil (Reddy et al. 1981), because indigenous soil organisms are usually resistant to new microorganisms in their environment (Ellis and McCalla 1976). Gerba et al. (1975) and Tate (1978) both reported increased pathogen survival, and sometime regrowth, in sterile soils. It also has been found that certain bacteriophage and free-living soil organisms, such as Bdellovibrio, can parasitize E. coli cells, thus limiting their survival (Klein and Casida 1967).

**Application methods**

Agricultural application methods place an important role in pathogen transport. For example subsurface injection of liquid manure may reduce surface bacteria losses but will decrease the amount of contact between surface soils and the bacteria, thereby increasing transport to groundwater or tile systems. Disturbing the top layer of the soil profile in the column that had macropores appeared to substantially decrease bacterial transport. The application time and frequency also influence the bacteria concentration and therefore influence their survival and transport to waterways.
2.3.1.2 Survival and transport of microbial pathogens in water

Although microbial pathogens are not well studied in aquatic systems, existing literatures show that the variation of pathogens is extremely complex in water from different localities. Their survival potential in natural waters is a function of interacting biological and physical factors (Rhodes et al., 1998). In general, the survival and transport of most pathogens, once discharged into water, is highly variable depending upon the water quality of the receiving water. These factors include light, temperature, sediments, pH, organic matter content, predation, and competition (O’Brien and Newman, 1977; Melnick and Gerba, 1980; Davies-Colley et al., 1994; Moore et al., 1998). Among those variables, light and temperature are identified as the most influential factors affecting their survival and transport in natural waters.

Light and solar radiation

Light intensity has been identified as the most influential variable causing bacteria die-off in natural waters (Gameson and Gould, 1975; Fujioka et al. 1981; Davies and Evison, 1991; Garvey et al., 1998; Wcislo and Chrost, 2000). The incline of bacteria die-off is believed to be a result of reduced radiolabeled amino acids being transported in enteric bacteria, and the accumulation of peroxides or other toxic substances due to UV-B photooxidation of organic matter (Kapuscinski and Mithel, 1981).

Garvey et al. (1998) studied the impacts of various environmental factors on coliform decay, and noticed that the numbers of *E. coli* and *Enterococcus faecalis*
were significantly reduced when exposed to visible light in both freshwater and marine systems. Sinton et al. (1999) tested the contribution of sunlight on the inactivation of various bacteria and bacteriophages from raw sewage, and detected that the inactivation rate were 10 times higher than that in dark condition. Linden et al. (2001) observed high inactivation rate of Cryptosporidium oocyst when the wavelengths were at the range of 250 and 270 nm. He noted that the inactivation was more acute at shorter wavelengths, but the greatest inactivation occurred at full sunlight. Likewise, Nyeleti et al. (2004) examined the effect of sunlight on the survival of Salmonella on surfaces, and draw the same conclusion that light particular UV-B wavelength is one of the most important factors to inactivate bacteria.

The effect of ultraviolet radiation on bacterial mortality has long been known (Barcina et al., 1990). Since 1916, UV light has been widely used as an effective technique for the inactivation of microbial pathogens (Clancy et al., 2000). UV radiation represents approximately 3-4% of incoming solar (<2800 nm) radiation (Ziegler and Benner, 2000). It appears that most lethal wavelengths are within the range. Thus, it is highly likely that UV dosing in summer condition has the potential to kill most bacteria in natural waters particular in lakes and reservoirs (Brookes et al. 2004).

**Temperature**

Most literature has shown a negative correlation between temperature and pathogens mortality in natural waters (McFeters and Stuart, 1972; Rhodes and Kator, 1988; Garzio-Hadzick et al. 2010). The response of enteric bacteria in water to
temperature is similar to that in soil. Namely high temperature limits the multiplication of bacteria, while low temperature prolongs bacteria survival. Temperature plays a minor role in affecting the survival and transport of bacteria when it is above a certain level. However, it is important to note that extreme low temperature including freeze-thaw cycles of water will also decrease bacterial populations.

McFeters and Stuart (1972) reported that the response of *E. coli* survival to temperature was inversely correlated between 5 and 15 °C. Temperature played a minor role in coliform survival above 15°C. Rhodes and Kator (1998) compared the survival of *E. coli* and *Salmonella* spp. in estuarine environments over a variety of seasonal temperatures, and found that the most pronounced reduction occurred during seasonally warm temperatures in the presence of the autochthonous microbiota. *Salmonella* spp. exhibited significantly less die-off and stress than did *E. coli* at temperatures of < 10 °C. Haley, et al. (2009) investigated *Salmonella* abundance from a rural watershed in different seasons, and observed that concentrations were significantly higher in the summer months compared to other seasons. They further concluded that bacteria density and diversity in water vary temporally and are strongly influenced by seasonal precipitation and water temperature.

**pH**

The adsorption characteristic of cells is affected by pH (Santamaria and Toranzos 2003). Extreme high and low pH values may decrease microbial viability, although this does not apply to viruses (Hekman et al., 1995). Bacteria especially
enteric bacteria have been shown to persist better in an alkaline environment, rather than in an acid condition (Gerba et al. 1975). Study results of McFeters and Stuart (1972) demonstrates that an optimum pH for coliform bacteria survival is between 5.5 and 7.5, with prompt drop both above and below these values. Similarly Reddy et al. (1981) found that pH of 6 to 7 is optimum for bacteria survival. However, Jewett et al. (1995) argued that pH had little effect on bacterial transport within the pH range 5.5 to 7, which is the pH range commonly encountered in soil and groundwater. It is believed that the pH range of a normal and healthy stream is close to 7 and would not affect pathogen survival. However, the pH of impaired streams under extreme condition (pH < 4.5 or >8.2) can accelerate pathogen die-off. The high pH of the water is a commonly reported problem in agricultural watershed area particular in the spring when intensive runoff from crop fields occurs.

**Sediment**

Numerous studies have investigated the density of pathogens in sediment systems. Significantly high levels of pathogens were observed in sediments than in overlying water, in upper layers of sediments than in bottom sediments (Grimes, 1975; Grimes, 1980; LaLiberte and Grimes, 1982; Garzio-Hadzick et al. 2010’). Adsorption, sedimentation, and extended survival are identified as the most important contributors to increased levels of pathogens in various water sediments (LaBelle et al. 1980; Burton et al. 1987). Once discharged into water, microorganisms often adsorb to sediment particles. Adsorbed to sediments may protect pathogens from UV radiation, high salinity, heavy metal toxicity, and attract by predators such as protozoa.
(Hood et al. 1982; Davies et al. 1995; Jamieson et al. 2005a,b). In addition, the environment of sediments provides relatively low temperature and rich nutrients associated with particles, which prolong their survival. Therefore, it is widely believed that stream sediment serves as a reservoir and potential source of pathogens for stream water (Moore et al. 2003; Garzio-Hadzick et al. 2010).

**Organic matter**

Microbial activity in natural waters is greatly encouraged by the presence of organic matter (Millis, 1989; Ferguson, 1994). As noted before, microorganisms are usually adsorbed by sediment particles in water. Organic matter increases the adsorption surface areas, organic carbon, and therefore prolongs the survival of bacteria. Many studies have shown increased adsorption in sediment particles with high organic matter or clay contents (Grimes et al. 1980; Garzio-Hadzick et al. 2010). Meanwhile, slower inactivation rate of pathogens was observed to associate with higher organic matter content. In addition, shortage of carbon and sometimes of nitrogen and phosphorus may limit the growth of bacteria population in lake water (Scheuerman et al. 1988). Moreover, the increase in the content of fine particles and organic carbon also led to lower sensitivity of the inactivation to temperature (Garzio-Hadzick et al. 2010). However, Banning et al. (2003) argued that an increase in organic matter may have very little effect on *E. coli* survival due to competition for nutrients by autochthonous microbiota.
**Predation**

Studies have shown the net decrease of bacteria in the presence of predators in natural waters (Davies et al. 1995; Scheuerman et al. 1988; Wcislo and Chrost, 2000). Protozoa and eucaryotes are identified as two major constrains on the growth of bacteria in waters receive large inputs of organic carbon (Findlay et al. 1986; Scheuerman et al. 1988). These grazers may have a dramatic impact on their prey. Protozoa are likely to live at sewage outfalls, or under environment where rich organic matter continuously flows in. These locations are also where enteric bacteria are introduced to receiving water. Therefore, the abundance of bacteria is a balance between predators and indigenous microorganisms that compete nutrients with introduced bacteria. Moreover, the grazing of bacteria by aquatic invertebrates affects their transport and dispersion in water (Brookes et al. 2004). Pathogens may be absorbed by microinvertebrates or be ingested by protozoa and microflagellates and subsequently excrete as a faecal pellet. These activities will affect the settling of the pathogens.

In summary, many factors influence the survival and transport of pathogen in soil and water. Soil moisture, temperature and texture are the most important variables affecting pathogens’ survival and transport in soil. Physical filtration is believed to be the primary process that limits pathogens mobility in soil. Removal of bacteria occurs largely at the soil surface by straining, sedimentation and adsorption. Temperature and solar radiation are the most influential factors affecting survival of pathogens in water.
Besides variables described above, earthworm burrows have been cited as potential macropores causing preferential flow in soils (Shipitalo et al. 2004). Variations in the salinity of surface water runoff affect pathogen mobilization. Pathogens live longer in high salinity water than in fresh water (Bradford and Schijven 2002). Moreover, the settling characteristics, the aggregation of particles on organic matter, and re-suspension of water are more likely to redistribute bacteria (Brookes et al. 2004).

2.3.2 Septic systems as a strategy to reduce pathogens

Soil provides a natural filtering action and adsorption site for the removal of pathogenic microbes, nutrient, chemical, and suspended solids (Reddy et al. 1981; Gerba and Bitton 1984, Qasim 1999). As early as 1870s, soil adsorption drain field and the septic tanks were reportedly first used by the French (Sease 2011). In 1884, the United States began to use the systems to dispose of domestic sewage from homes that were not connected to municipal wastewater treatment systems (Cech 2010). Since that, the septic systems are widely used as the most common type of on-site wastewater treatment system where sanitary sewer services are unavailable (Reed and Crites 1988). As of 2007, nearly 20% of the U.S. households (approximately 26.1 million) use home septic systems for wastewater disposal (USEPA 2008).

2.3.2.1 Function of a septic system

A typical septic system (Figure 2.1) consists of two parts: a septic tank that is buried underground to capture sewage directly from a house, and leach fields where
wastewater is being distributed and filtered. Wastewater first flow into a septic tank where the solid materials (sludge) settle out and are broken down by bacteria, and oils and grease float to the surface as scum (Figure 2.2). T-shaped outlet and screens keep the sludge and scum from leaving the tank and traveling to the drain field area. The layer of water between scum and sludge is distributed to leach fields through perforated plastic piping systems. The wastewater treatment efficiency largely depends on the physical properties of soil at the location of leach fields.

Among various soil-related variables affecting wastewater treatment, soil depth and soil permeability are the most important factors. Studies (McCoy et al. 1979; Karathanasis et al. 2006) have shown that fine-textured soils remove bacteria more efficient than coarse-textured soils do particularly at deep layer of soil. A consistent improvement in wastewater treatment was also observed to associate with soil depth. In addition, significant large populations of fecal bacteria were detected in saturated soil in comparison with unsaturated soil.

Many states have adopted the minimum soil depth standards, three vertical feet of unsaturated soil or fill material between the bottom of leach trench and bedrock or highest level of water table, to install a septic system (Baker, 1978). However, Karathanasis et al. (2006) argued that a minimum distance of 30-cm between limiting interface and the bottom of the leach field would not be sufficient to remove fecal bacteria, particularly in coarse-textured soils. Moreover, McCoy et al. (1979) observed similar bacteria concentrations recovered downslope in one soil series from 12-cm to 60-cm depth, which indicate that large zone of unsaturated soil
is needed to prevent the release and rapid transport of large populations of fecal microorganisms. The U.S. has no unified standards for the design and construction of septic systems. Local health departments may adopt more stringent standards, while the State Board of Health adopted minimum standards in 1977.

2.3.2.2 Failing septic systems in U.S.

A properly design, constructed, and maintained home septic system can perform reliably over a long period of time with little attention due to the large natural assimilation capacity of the soil, and discharge very little fecal bacteria loading (Qasim 1999; Young and Thackston 1999). However, if a system is failing, sewage will be pushed back into the house, or be running into the yard where surviving bacteria can be transported either to groundwater or to surface water during a runoff-producing rainfall event. Even worse is that a fraction of older homes with failed septic systems are more likely to discharge sewage directly to the stream through a straight pipe (Taylor et al. 2003). Bacteria from human wastes that reach the stream in this manner do not have the opportunity to die-off on the land surface, or to be removed by soil. As a result, failing septic systems with straight pipes can be a significant source of bacteria and pose a significant threat to human health (Borchardt el al. 2003).

The percentage of failed septic systems in U.S varied widely. It was reported that 1% of septic systems failed in 1981 (De Walle 1981). The number jumped to 35% in 2000 (Schueler 2000). According to EPA, the average failure rate is in the range of
10 to 15% (USEPA 2002). Scalf and others (1977) suggests that one half of U.S. soils are unsuited for conventional septic systems. Septic systems fail for one of three reasons: unsuitable soils; b) lack of a scientific basis for the design, construction, and management of systems; and c) population density.

2.3.3 Policy and regulations

The Ohio Department of Health (ODH) developed rules for siting, design and construction of on-site septic systems in 1977 (Ohio Administrative Code Chapter (OAC) 3701-29). According to the regulation, a minimum distance of 66 inches to limiting condition and 3-60 min/inch percolation rate are required to build a soil absorption system or leach field system. Twenty four and 12 inches of vertical distance to limiting conditions are required to build a mound and sand bioreactor system respectively on locations where soils are shallow or slowly permeable. Moreover, it requires General Household National Pollutant Discharge Elimination System (NPDES) permit for new and replacement discharging systems.

OAC-3701-29 had been remained in effect until January 1, 2012 when OAC-3718 was adopted. According to the new rules, a minimum vertical separation distance of 18 inches is required for low and moderate risk conditions. The vertical separation distance shall be set at a depth from 24 to 36 inches for high risk conditions, and shall not be lowered unless a reduction of vertical separation is granted in accordance with rules adopted under divisions of this section, such as pretreatment of sewage, or soil elevation (OAC-3718-02). Older sewage treatment
systems are not required to be replaced under this rule if it does not cause a public health nuisance, but repairs are needed if they do. The new regulation does not require the permit for new or replacement discharging systems, but for a system that an NPDES permit has been issued, the effluent discharge limitations is specified in the permit.

In the Upper Sugar Creek, Wayne County Health Department (WCHD) has adopted OAC-3701-29 since 1977 and the Wayne County Sanitary Code HD Regulation # 136R since 1995. WCHD adopt the new rules OAC-3718 in January, 2012, but the rules and regulations on the website has not been updated yet. In both new and old regulations, leach fields or soil adsorption system and other three types of home sewage disposal systems are listed along with specific requirements. However, mound and sand bioreactor systems that are considered suitable for the Upper Sugar Creek soils were not included. Additionally, it is important to note that OEPA has completed a bacterial total maximal daily load (BTMDL) program for the Sugar Creek Watershed in 2007. The BTMDL has outlined the land use, tested and modeled maximum bacterial concentration for recreational use of the Upper Sugar Creek.
2.4 HYPOTHESES

Based on the soil data of the Upper Sugar Creek, OEPA BTMDL report, and the policy of WHCD, I put forward three hypotheses:

a) The primary source of microbial pollution in the Upper Sugar Creek is human coliform instead of animal coliform;

b) The dominant septic system is soil adsorption or leach fields in the watershed, and failing septic systems are the main cause of pathogen pollution;

c) Local people lack awareness and knowledge regarding microbial pollution and septic systems

2.5 MATERIALS AND METHODS

After discussion with local farmers, our team established 21 sampling sites in the Upper Sugar Creek Watershed for microbial contamination study based on land use, ease of access, and representing a diversity of management practices and potential host sources of bacterial pollution (Figure 1.2). We also surveyed the local people concerning their septic systems, awareness of the microbial contamination in their neighborhood streams, and their willingness to pay (WTP) to maintain their septic systems.
2.5.1 Description of the study site

Our study watershed, the Upper Sugar Creek is located in Wayne County, one of the largest dairy counties in Ohio. It has different land uses with potential contaminant sources such as residential areas, crop fields, livestock operations, and natural forested areas (Fig1.2). This mixed-use watershed makes Upper Sugar Creek an ideal location for studying relationships between various land use and microbial water contamination.

Due to the special geographic location and topography, the glaciated Erie and Ontario Drift and Lake Plain (EOLP), the Upper Sugar Creek is nearly flat with poorly drained clayey soils, which causes water to pond on the land surface unless agricultural tile drains are used. According to Wayne and Holmes County soil maps (1988), Wooster-Canfield association, Coshocton-Rigley association, Coshocton-Brownsville-Berks association, Canfield-Wooster-Riddles association, and Rittman-Wadsworth association, are dominant soil types in Sugar Creek Watershed. The slopes range from 2 to 70%. Permeability is moderate, moderately slow or slow. The complex soil type makes the watershed area limited to septic systems. For example, soil absorption system is suitable in some area of Smithville where Wooster soil dominants (upper stream), but may not in Holmes County (downstream).

According to the study of Mancl (2002), percentages of soils suited to traditional leach lines or mound systems in Wayne and Holmes County are 15-19.9%, and 10-14.9% respectively. Percentages of soils suited to mound systems only in Wayne and Holmes County are 50% or more and 40-49.9% respectively. Therefore,
in theory, mound systems and sand bioreactor are suitable and should be the preferred
method used in the Sugar Creek area. However, mound and sand bioreactor systems
were introduced to Ohio in 1980s as experimental systems, and have not been
included in the local sanitary agency’s regulations. Therefore, conventional soil
adsorption systems such as leach fields are presently dominant in Wayne County,
while mound and sand bioreactor systems are rare (Mancl, 1990; WCHD 2010).

For this study, after closely discussed with the Upper Sugar Creek farmers, 21
sites were established for nutrient loading as well as microbial contamination test
based on land use, ease of access, representing a diversity of management practices.
All people reside within the watershed are targeted audiences for our survey. More
information about survey is available in Chapter 4.

2.5.2 Microbial contamination test

2.5.2.1 Coliform and *E. coli* test

A total of 42 water samples (2000 mL each) were taken from 21 sampling
sites once a month from June to October, 2008 in the Sugar Creek headwater streams.
Water samples were kept in a cooler box, taken back to the laboratory and processed
within six hours. Colilert ® Method with Quanti-Tray/2000™ (IDEXX, ME) which is
based on the Standard Methods Most Probable Number (MPN) model was used to
examine the presence of coliform and *E. coli*.

A 100 ml water sample was poured into a sterile glass bottle, and 1 packet of
Colilert reagent was aseptically added. The mixture of water sample and reagent was
shaken well until the reagent is mostly dissolved, and poured to a quanti-tray. Small wells of the tray were tapped 2-3 times to release any air bubbles that maybe present before it was placed into a rubber insert. The tray with water sample was sealed in a Quanti-Tray® Sealer and incubated at 37 °C for 24 hours. All samples were yellow in color after incubation indicated the presence of coliforms. All samples were blue fluorescence in color under long wave ultraviolet light indicated the presence of E. coli. The positive number of large wells and small well were counted separately, and referred to a MPN table to determine the MPN of coliform and E. coli present.

2.5.2.2 Microbial source tracking

Sample preparation and DNA extraction

A sample of 100 ml water was taken from each sampling site with three replicates. In total, five sets of samples were collected from July 2008 to March 2009. We avoided wet weather in order to capture microbial community in water column at base flow condition for a better comparison of results from different samplings. Water samples were kept in a cooler box, taken back to the laboratory, and frozen at -20 °C until further processing. Water samples were thawed and filtered using disposable vacuum filtration apparatus (Pall Corporation, NY) fitted with a 25 mm diameter filter paper (Supor 200 Membrane Disc Filter, 0.2µm filter) to collect the bacterial cells. DNA was extracted directly from whole filters using the Power Soil DNA Kit (MoBio Laboratories, CA, USA) according to the manufacture’s’ instructions and stored at-20 °C. DNA samples were diluted to 1ng/µl after
measurement of DNA concentration using the NanoDrop ND-1000 UV Spectrophotometer (NanoDrop Technologies, Thermo Scientific Wilmington, DE). All extracted DNA samples were stored at -20 °C until further processing.

**General and host specific PCR**

The general *Bacteroidales* PCR was performed using an initial predenaturation step at 94 °C for 3 min, 30 cycles consisting of 94 °C for 1 min, 53 °C for 45 sec, and 72°C for 2 min, and followed by a final extension at 72 °C for 7 min. The primers, Bac32F (AAC GCT AGC TAC AGG CTT) and Bac708R (CAA TCG GAG TTC TTC GTG), were used to detect the presence of the general *Bacteroidales* signal. Amplification of products of the expected size (approximately 200 bp) was verified by electrophoresis using 1% w/v agarose gel stained with ethidium bromide stain and compared using a 100 bp DNA ladder (Promega, Madison, WI).

Human and ruminant specific PCR assays were performed using the same method described above with exception of 35 cycles, and annealing temperatures of 62 °C and 63 °C for ruminant and human marker sets respectively. Two sets of primers, CF193F (TAT GAA AGC TCC GGC G) & CF128F (CCA ACY TTC CCG WT ACT C), HF134F (GCC GTC TAC TCT TGG CC) & HF183F (ATC ATG AGT TCA CAT GTC CG), were paired with Bac708R to detect human and ruminant specific *Bacteroidales* respectively.

A set of primers, AlllBacF (GAG AGG AAG GTC CCC CAC) and AlllBacR (CGC TAC TTG GCT GGT TCA G), was used to examine the quantitative PCR
(qPCR) in the samples. Moreover, fecal samples (feces of livestock, wild animal, domestic animal, and human) and water samples (from wastewater treatment plant and septic effluent) were used as positive controls of human and ruminant contamination (see the dissertation of Merrick, N. 2010 for more details).

2.5.3 Survey method

Two sets of questions were asked to examine watershed residents’ awareness, attitudes and behavior toward microbial contaminations of their neighborhood streams. The first set of questions deals with how respondents value their neighborhood streams, rate water pollutants, prioritize their major concerns, and use the streams. The second set of questions was designed to understand how much knowledge that respondents have about their septic systems including types, function, maintenance activities and cost, and outflow discharge. Local people’s WTP to maintain their septic systems on regular basis was also investigated.

The study involved two steps. First, we interviewed 21 households in the summer of 2009 to test the survey instrument. Second, we randomly surveyed 300 households living in the watershed area in the summer of 2010. We returned three times to follow up on the completion of the survey. Those who completed the survey within the first week were marked as early respondents. Those who completed or mailed the survey after the sixth week were marked as late response. Late response was treated as non-response, and was not included in the data analysis.
The survey instrument was based on a questionnaire format using pick-up and drop-off method. Data were collected using a “drop-off, pick-up” survey method, interviews, participatory observation, and interaction at farmer meetings and community activities. The sample frame was limited to people living in the watershed area and age above 18. More information about our survey study is described in chapter 4.

2.6 RESULTS AND DISCUSSION

2.6.1 Microbial concentration results

2.6.1.1 *E. coli* test results

Bacteria concentration in water samples varies dramatically by seasons. Significantly higher levels of *E. coli* were observed in cold months compared to other seasons (Figure 2.3). Relatively lower concentrations were found to be associated with dry weather conditions. These results support the idea of most scientists that cold temperature prolongs pathogens survival, while dry condition limits their multiplication (McFeters and Stuart, 1972; Rhodes and Kator, 1988; Garzio-Hadzick et al. 2010). In addition, the relatively lower levels of *E. coli* in summer months may due to the high light density and solar radiation.
The geometric mean of *E. coli* concentration data (Figure. 2.4) show that 20 out of 21 sampling sites greatly exceed Ohio’s recreational water quality standards (235 count per 100ml). The exception is site 9 which is a natural spring used as a drinking water supply for the local community. Moreover, consistently high concentrations of *E. coli* were observed at sites 14 and 21 in 2008 and 2009 (P<.01). These results are in accordance with OEPA BTMDL report (2007) that the Upper Sugar Creek watershed was heavily contaminated with fecal coliform.

### 2.6.1.2 qPCR and host specific *Bacteroidales* test results

Host specific *Bacteroidales* test results (Figure 2.5) show that 76.2% of sampling sites were contaminated with human feces (P<.01), and only 19% of them were contaminated with ruminant feces. The results support my first hypothesis that the primary source of microbial contamination of the watershed is human coliform instead of animal coliform. Moreover, general qPCR test results show that consistent high level of *Bacteroidales* (Figure 2.6), and strong positive signals of human markers were found at site 14 and 21, which is in accordance with *E. coli* test results presented in Figure 2.4. According to land use data (Figure 1.2), residential area, grassland and pasture are main types of land use. However, by closely observing both sites, we found straight pipes from septic systems located near the streams. Therefore, it is encouraged to combine the use of host specific PCR results and land use data to identify potential sources of fecal contamination.
In summary, human fecal coliform (76.2%) was identified as the primary microbial pollution source in the Upper Sugar Creek watershed. Sites 14 and 21 were identified as hot spots. In addition, the qPCR and *E. coli* test results are positively correlated (P<.01), which indicate the validity of using *E. coli* as fecal coliform indicator. However, it is important to point out that the reliability of *E. coli* as a sole pathogen indicator of water quality has been questioned repeatedly in the past ten years (Lemarchand and Lebaron, 2003; Fielda and Samadpourb, 2007). As a solution, multiple water quality indicators including both *E. coli* and *Bacteroidales* (Dick et al. 2004) are encouraged.

2.6.2 Survey results

In total we delivered 262 questionnaires, and received 214 back (81.7% response rate). Among the 214 respondents, farmers and non-farmers account for 39% and 61% respectively. The percentage of male respondents (56%) is slightly higher that female (44%). The mean value of respondents’ age is 49.7 years with a standard deviation of 16.99. In average, respondents have lived or farmed in the watershed for 19 years.

2.6.2.1 Local people’s attitudes toward their headwater streams

The survey explored attitudinal differences between farmers and non-farmers regarding the water quality and the potential sources of pathogens. Over 60% of respondents (60% non-farmers, 43% farmers) rated their stream water quality as good (Figure 2.7). The majority of farmers prioritized bank stability (14.1%), drainage,
agricultural land flood (10.56%) as their major concerns, while non-farmers cared more about loss of biodiversity (9.31%), reduced water recreation (4.31%), and pasture or crop field runoff (10.86%). Both farmers (15.3%) and non-farmers (16.5%) indicated special concerns about well water pollution (Figure 2.8). This difference between farmers and non-farmers is consistent with the results of a survey of the Upper Sugar Creek by Moore et al. (2008). They found that non-farmers had a much higher concern about decreased biodiversity and agricultural pollution, while farmers were more concerned about drainage, erosion, and EPA regulation.

Eight percent farmers and ten percent non-farmers indicated concerns over sewage pollution (Figure 2.8). However, when we asked how much a problem the bacteria pollution was in their or their neighborhood’s streams, 4% of non-farmers marked bacteria as a severe problem, 18% of farmers thought it was not a problem at all, while 43% of farmers and 57% of non-farmers indicated they were unsure (Figure 2.9).

2.6.2.2 The characteristics of septic systems in the watershed area

Ninety-seven percent (N=210, P<.01) of respondents did not have idea about the soil types and soil depth where they built their septic systems. Only 6 of them stated that the soil depth was around 2 to 10 feet, but they did not know the soil features. In most cases, WHCD is in charge of soil investigation, septic system design and installation. It is most likely that onsite septic systems would be designed by the same person issuing the permit in the watershed area. However, the detailed
information regarding the methods assessing soil suitability, issuing permit, and installing processes were not available for our study and further digital organization of septic system data by the WCHD would greatly aid their effectiveness. More research needs to be done in future.

Our study results show that 63% (N= 210, P < .01) of respondents have used traditional soil adsorption/leach fields to treat their sewage wastewater, and 24% of them have used the city system. The percentage of mound system and sand bioreactor usage accounts for only 4% and 1% respectively (Figure 2.10). Sixty-four percent of respondents rated the function of their septic systems as very good (Figure 2.11). However, over half of them (53.8%) reported signs of septic system failure including sewage backs up into house (12.7%), slow running drains and toilets (34.5%), sewage in the yard (8.2%), standing water over the tank (10%), and others (Table 2.1).

Moreover, a quarter of the respondents did not know where their sewage outflow ends up, and another quarter of them honestly admitted that they discharged their sewage wastewater directly to nearby streams (Figure 2.12). Close to one-third of them discharged wastewater to farmers’ fields. These respondents often owned 5-acre parcels that qualify a waiver of NPDES. Forty percent of respondents have inspected their septic systems once a year (Table 2.2), 8.4% checked them less often than every 4 years, and 13.7% have never checked their systems. The main activities (Table 2.3) include maintaining or replacing pump (43.3%), pumping septic tank (19.5%), flushing lateral lines (2.9%), and switching to alternative leach field (5.7%).
The mean cost of the maintenance was $129 per year with a standard deviation of $88.7.

2.6.2.3 Willingness to pay for regular inspection of septic system

Among 216 respondents, 60% of them indicated willingness to pay a certain amount of money (75.2% for $50, 25% for $100, 0.8% for $150) to have their septic system being inspected on a regular basis (Table 2.4). Among those who did not want to pay (21.5%), nearly half of them indicated a great interest in garnering more information regarding bacteria pollution in their neighborhood streams, and around one-third of them indicated willingness to take personal or group actions to improve water quality of the Upper Sugar Creek watershed.

In general, above results support my second hypothesis that the primary septic system in the Upper Sugar Creek watershed is traditional soil adsorption/leach fields instead of mound or sand bioreactor. Study results also demonstrate failing septic systems are the most likely sources of high pathogen concentration. Moreover, results indicate that local people lack awareness and knowledge to their septic systems and pathogen pollution, which is an important factor affecting people’s willingness to pay to adopt conservation practices.

2.6.3 Future work

Since failing septic systems are the main causes of pollution, it is important to reassess their functions, fix failing ones, and rebuild suitable systems if necessary. To design and install a suitable system, detailed soil information is needed including soil
depth, soil types, and soil permeability. Scientific support and digital soil data will greatly aid the effectiveness of local sanitary agencies’ work. Overall, improving the water quality is possible by encouraging the cooperation of people within the watershed, increasing awareness of microbial pollution risks, and enhancing the communication between local sanitary agencies, the university, and watershed residents.

2.7 CONCLUSION

The objective of this study was to identify primary source of microbial pollution in the Upper Sugar Creek. By tracking the potential sources of Bacteroidales, an indicator of human and animal waste, we found that human fecal coliforms (76.2%) were the primary sources of pathogen contamination in the watershed. Although animal coliforms were often considered as the prime suspect, they were detected at only 4 out of 21 sampling sites. The results support my first hypothesis that the primary microbial contaminant is human waste instead of animal waste.

Another objective of this study was to examine the dominant septic system, main causes of pathogen pollution, and people’s awareness and knowledge regarding microbial pollution and septic systems. By surveying 262 households living in the watershed area, we found that 63% of septic systems are traditional soil
adsorption/leach fields, and mound and sand bioreactor systems account only 5%. The main causes of pathogen pollution were straight pipes (25%) and failing systems (53.8%). Over half of respondents indicated unsure about bacteria pollution in their streams. Most of them (97%) have little or no knowledge about their systems. They were not sure the types and depths of soil where their systems were built on. Some of them even did not know that leach fields need to be switched every half year. These results support my second and third hypothesis that the majority septic systems are traditional soil adsorption/leach fields, the main cause of pollution is failing septic systems, and local people lack awareness and knowledge about microbial pollution and their systems.

Even though study results are consistent with OEPA’s results that Sugar Creek had high level of bacteria, improvements to reduce microbial pathogens are possible. Our study results demonstrate that 60% of respondents indicated willingness to pay certain amount of money to have their systems being inspected on a regular basis. Among those who were not willing to pay, close to half of them showed great interest to either take individual or group actions to make improvement. Other remediation strategies are discussed in details in Chapter 4.
2.8 REFERENCES


60. Merrick, Natsuko N. 2010. Microbial source tracking: watershed scale study of pathogen origin, fate, and transport in the upper Sugar Creek watershed, Northeast Ohio. Columbus, Ohio: Ohio State University


Figure 2.1 A conventional soil adsorption septic system
Source: EPA 832-B-02-006

Figure 2.2 Structure of a conventional septic tank
Source: EPA 832-B-02-006
Figure 2.3 *E. coli* concentrations over different months
Data source (Merrick, N. dissertation, 2010)

Figure 2.4 Average *E. coli* concentrations of 21 sampling sites
Values are the geometric mean of five sets of samplings and the error bar indicates one standard error. Data source: Merrick, N. dissertation, 2010
Figure 2.5 Host specific Bacteroidales PCR results
Values are the geometric mean of five samplings. Purple and blue dots represent human and ruminant specific Bacteroidales markers respectively. Data source: Merrick, N. dissertation, 2010
Figure 2.6 qPCR results with general *Bacteroidales* 16S rRNA gene markers
Values are the geometric mean of five samplings and the error bar indicates one standard error. Data source: Merrick, N. dissertation, 2010

Figure 2.7 Water quality rating of farmers and non-farmers
Figure 2.8 Respondents’ major concerns about the Upper Sugar Creek

- **Bank Stability**: 14.14% (Farmer), 7.41% (Non-farmer)
- **Sewage Pollution**: 10.69% (Farmer), 8.37% (Non-farmer)
- **Industry Pollution**: 4.66% (Farmer), 1.59% (Non-farmer)
- **Loss of Biodiversity**: 9.31% (Farmer), 1.39% (Non-farmer)
- **Reduce Water Recreation**: 10.86% (Farmer), 0.80% (Non-farmer)
- **Reduced Beauty of Streams or Lakes**: 2.93% (Farmer), 0.40% (Non-farmer)
- **Children's Health**: 9.48% (Farmer), 8.37% (Non-farmer)
- **Pet's or Farm animals' Health**: 9.96% (Farmer), 8.28% (Non-farmer)
- **Pasture and Crop Field Runoff**: 10.86% (Farmer), 6.37% (Non-farmer)
- **High Drinking Water Treatment Cost**: 3.62% (Farmer), 2.39% (Non-farmer)
- **Well Water Pollution**: 16.38% (Farmer), 15.34% (Non-farmer)
- **Agricultural Lands Flooding**: 10.56% (Farmer), 2.76% (Non-farmer)
- **Drainage**: 14.54% (Farmer), 6.03% (Non-farmer)
- **Regulation Concern**: 5.78% (Farmer), 3.28% (Non-farmer)
Figure 2.9 Respondents’ attitudes towards bacteria pollution

Figure 2.10 Types of septic systems in the Upper Sugar Creek
Figure 2.11 Rating of septic systems’ function

Figure 2.12 Discharge locations of home septic systems
<table>
<thead>
<tr>
<th>Signs of failing septic systems</th>
<th>Frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sewage backs up into house</td>
<td>14</td>
</tr>
<tr>
<td>Slow running drains and toilets</td>
<td>28</td>
</tr>
<tr>
<td>Sewage in the yard</td>
<td>9</td>
</tr>
<tr>
<td>Standing water over the tank</td>
<td>11</td>
</tr>
<tr>
<td>Foul odors in the house or yard</td>
<td>15</td>
</tr>
<tr>
<td>Lush-plant growth that appears near the septic tank or drain field</td>
<td>4</td>
</tr>
<tr>
<td>Other signs</td>
<td>19</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>110</strong></td>
</tr>
</tbody>
</table>

Table 2.1 Signs of failing septic systems

<table>
<thead>
<tr>
<th>Maintenance frequency of septic systems</th>
<th>Frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>Never</td>
<td>26</td>
</tr>
<tr>
<td>Less often than every 4 years</td>
<td>16</td>
</tr>
<tr>
<td>Every 3-4 years</td>
<td>17</td>
</tr>
<tr>
<td>Every 2-3 years</td>
<td>40</td>
</tr>
<tr>
<td>Once a year</td>
<td>76</td>
</tr>
<tr>
<td>Twice a year</td>
<td>15</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>190</strong></td>
</tr>
</tbody>
</table>

Table 2.2 Maintenance frequency of septic systems
<table>
<thead>
<tr>
<th>Maintenance Activity</th>
<th>Frequency</th>
<th>Percent</th>
<th>Cumulative Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flush lateral lines</td>
<td>6</td>
<td>2.9</td>
<td>2.9</td>
</tr>
<tr>
<td>Pump septic tank</td>
<td>41</td>
<td>19.5</td>
<td>22.4</td>
</tr>
<tr>
<td>Switch to alternative leach field</td>
<td>12</td>
<td>5.7</td>
<td>28.1</td>
</tr>
<tr>
<td>Maintain or replace pump</td>
<td>91</td>
<td>43.3</td>
<td>71.4</td>
</tr>
<tr>
<td>Collect and analyze effluent</td>
<td>3</td>
<td>1.4</td>
<td>72.9</td>
</tr>
<tr>
<td>Haven’t done anything</td>
<td>55</td>
<td>36.2</td>
<td>99.0</td>
</tr>
<tr>
<td>Other maintenance activities</td>
<td>2</td>
<td>1.0</td>
<td>100.0</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>210</strong></td>
<td><strong>100.0</strong></td>
<td></td>
</tr>
</tbody>
</table>

Table 2.3 The maintenance activities of septic systems

<table>
<thead>
<tr>
<th>Amount</th>
<th>Frequency</th>
<th>Percent</th>
<th>Cumulative Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>$0</td>
<td>26</td>
<td>21.5</td>
<td>21.5</td>
</tr>
<tr>
<td>$50</td>
<td>91</td>
<td>75.2</td>
<td>96.7</td>
</tr>
<tr>
<td>$100</td>
<td>3</td>
<td>2.5</td>
<td>99.2</td>
</tr>
<tr>
<td>$150</td>
<td>1</td>
<td>0.8</td>
<td>100.0</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>121</strong></td>
<td><strong>100.0</strong></td>
<td></td>
</tr>
</tbody>
</table>

Table 2.4 Willingness to pay to inspect septic systems on a regular basis
CHAPTER 3

CORRELATIONS BETWEEN MICROBIAL DIVERSITY AND HABITAT CHARACTERISTICS IN THE UPPER SUGAR CREEK WATERSHED

3.1 ABSTRACT

We correlated microbial diversity and stream habitat characteristics of the Upper Sugar Creek Watershed. Microbial diversity was tested by using PCR-DGGE method. Stream habitat characteristics were assessed by using the primary headwater habitat evaluation index (HHEI) and headwater macroinvertebrate field evaluation index (HMEFI). Study results show that HHEI and HMEFI are positively correlated (P<.01), and are encouraged to be used in headwater streams assessment. Although microbial diversity values largely depend on land use and climate, they are positively correlated with HHEI (P<.01) and HMEFI (P<.01), which indicates the effectiveness of microbial diversity as a sensitive indicator of quality of water and stream
ecosystem in agricultural watersheds. Giving the expensive and time consuming method to test microbial diversity, HHEI method can be used as a rapid tool to assess not only the habitat characteristics, but also microbial diversity dynamics in the headwater streams.

3.2 INTRODUCTION

Microorganisms comprise the largest number of living organism on our planet. They are ubiquitous janitors of the Earth, and can be found in any climate zones including harsh environment such as Arctic, Antarctic, oceanic hot vents, and deep within rocks (Colwell, 1977). Although only small fraction of microbes have been discovered and studied, it has never been denied that microorganisms including both discovered and unknown are significant importance to the sustainability of life on our planet. They decompose dead organisms, recycle valuable nutrients, degrade waste and pollutants, and therefore play key roles in conserving higher organisms and restoring degraded ecosystems (Ogunseitan, 2007).

Since microorganisms respond sensitively to the environment in which they live, they have been widely used as indicators of environmental quality over the past decades (Colwell, 1977; Ogunseitan, 2007). Besides the population of an individual microorganism such as E.coli, the bacteria indicator of water quality, microbial diversity has also been viewed as sensitive measure of environmental quality at a
given site or ecosystem. This is due to the rapid reproductive capacities of microorganisms under distinct environmental settings, which alter the numbers and types of microbes. Since 1990s, more studies have been done on microbial diversity in different environmental settings such as soil (Kennedy and Smith, 1995; Ibekwe et al., 2002; Nanniepieri, et al., 2003; Bever, J.D. 2012). However, microbial diversity in aquatic system has received little attention because of technical limitations. Among the limited studies, majority of them have either worked on marine water (Urakawa, et al., 1999; Köpke et al., 2005; Pedrós-Alió, 2006), or focused on sediments in lakes or centered on techniques exploration (Kemp and Aller, 2003; Jiang et al., 2006). Microbial diversity in freshwater particularly in agricultural headwater ecosystem has been simply passed over. Meanwhile, the dynamics of microbial diversity from the upper to downstream in aquatic systems are poorly understood. As a result, the effectiveness of microbial diversity as water quality or stream health remains uncertain and unclear.

Besides the measurement of microbial diversity, the assessment of headwater streams is also a challenge. First of all, headwater streams have relatively small drainage areas with less recharge potential, which are more likely to dry up than downstream parts. They could be perennial, intermittent, or seasonally dry. The dynamic of hydrologic permanence largely determines biodiversity in watercourses. Second, the water quality, physical habitats, and biodiversity in headwater streams are heavily influenced by its surrounding areas. The complexity of headwater streams makes the assessment hard. Currently, no method is universally accepted to
comprehensively assess headwater streams in the field. Moreover, no studies have addressed the relationship between stream habitat characteristics and microbial diversity and. Our study is one of the first to assess headwater streams, and link it to microbial diversity.

The objectives for this chapter are to a) measure the microbial diversity from upper to downstream reach by using PCR-DGGE technique; b) assess stream habitat characteristics of the Upper Sugar Creek by using primary headwater habitat evaluation index (HHEI) and headwater macroinvertebrate field evaluation index (HMEFI); and c) examine the linkages between microbial diversity and habitat characteristics.

### 3.3 LITERATURE REVIEW

#### 3.3.1 Measurement of microbial diversity

Many molecular genetic techniques are used to measure microbial diversity such as Guanine plus cytosine (G+C), single strand conformation polymorphism (SSCP), and Restriction fragment length polymorphism (RFLP) (Kirk et al., 2004). Three methods are most commonly used: namely rRNA method, randomly-amplified polymorphic DNA (RAPD) method, and most recently used polymerase chain
reaction & denaturing and temperature gradient gel electrophoresis (PCR-DGGE) method (Colwell, R.R., 1997).

rRNA method focuses on the comparison of the small subunit ribosomal DNA sequences with known rRNA sequences (Barns et al., 1994). This method requires multiple steps including DNA extraction, PCR processing, and ribosomal DNA sequences test, which is time-consuming. Since the discovered rRNA sequences are limited, rRNA method is not suitable for large sample test. RAPD method generates DNA fingerprints characteristic of given microbial communities, which allows screening and comparison of large mixed genome samples collected from different ecosystems. However, RAPD fragments may be unclear and hard to score, which may due to non-specific priming (Hadyrs, et al., 1992; Zande and Bijlsma, 1995; Bardakci, 2001). PCR-DGGE method is a fingerprinting approach that can generate a pattern of genetic diversity in complex microbial ecosystems such as agriculture-dominant watersheds (Muyzer et al., 1993; Muyzer et al., 1999). This technique allows analysis of large number of samples. It is relatively reliable, reproducible and rapid (Duineveld, et al., 2001; Maarit-Niemi et al., 2001). Unlike RAPD, the fragments are clear and easy to identify. However, this technique only detects dominant species. Therefore, one band can represent more than on species (co-migration) (Torsvik, et al., 1998; Maarit-Niemi et al., 2001).

All three techniques do not allow for more specific identification of organisms. However, the latter two do allow us to screen different organisms from complex ecosystems. Moreover, all three techniques are PCR-based. They are sensitive to
reaction conditions. Slight changes such as recycling times, annealing temperature, and sample storage time may affect the reproducibility of amplifications products. Considering time-consuming (rRNA) and ambiguous results (RAPD), we use PCR-DGGE to detect the microbial diversity in our water samples. Continuing examination of microbial diversity at the Upper Sugar Creek may allow us to constitute an early warning system for environmental changes in the watershed.

3.3.2 Field-based methods of headwater streams assessment

The unique role of headwater streams has fostered the development of stream assessment protocols. Currently, three existing field-based tools can be used to rapidly assess headwaters: the North Carolina Department of Water Quality Stream Classification Method (NCSC), the United States Environmental Protection Agency Rapid Bioassessment Protocol Habitat Assessment (RBP), and the Ohio Environmental Protection Agency Headwater Habitat Evaluation Index (HHEI) and Headwater Macroinvertebrate Field Evaluation Index (HMFEI).

NCSC field identification protocol has been developed for field determinations between perennial and intermittent streams in Fair County NC, which is based on the combination of hydrological, physical and biological characteristics of the stream. The original RBP was developed in the late 1980s and 1990s (Bode 1988, Plafkin et al. 1989; Barbour et al. 1999; Merrit and Cummins, 2008). It was designed as cost-effective and scientifically valid tools that allow multiple biological surveys in a single fined season (Barbour et al. 1999). Because of a quick turn-around of results
for management decisions, RBP has been used extensively in state and federal monitoring programs as well as in studies on impacts such as acid mine drainage, chemical pollution of streams, and wastewater treatment facilities.

HHEI and HMFEI are developed specifically for headwater streams use. They have been developed to provide standardized assessment methodologies for conducting use attainability analyses of primary headwater habitat streams since 1999. HHEI has been developed to provide a rapid assessment to predict headwater stream classes. Class III streams indicate healthy streams and support a cool water biologic community; class II streams are warm water streams, and tend to have less diversity than class III streams; class I streams are generally dry and ephemeral. HMFEI is used to properly classify the actual and expected biological conditions in headwater streams. OEPA method includes three categories information, biology, water quality, and physical habitat features. Biological variables are species and population of fish, salamanders, macroinvertebrates and amphibians. Water parameters include dissolved oxygen, pH, temperature, turbidity and conductivity. Physical habitat refers to stream discharge, water depth, flow velocity, channel gradient, sinuosity, and substrate particle size classes. According to OEPA protocol, the biological data always supersede HHEI scores, which suggest that low HHEI streams with high HMFEI could be classified as higher stream classes (OEPA. 2012).

All three tools classify streams in certain order. They are regionally relevant. In this study, we use OEPA protocol to assess Sugar Creek headwater streams. The protocol defines primary headwater as a small stream with a watershed less than or
equal to 20 square miles. Most streams of our target watershed have a watershed of less than one square mile, which are referred as primary headwater streams. Since 1999, HHEI has been extensively used for aquatic systems in Ohio, but the literatures cited the methods and study results are rare or not readily available particularly for large-scale monitoring programs and studies. Our study is one of the first to apply the method to the entire upper streams of the watershed, and continuously record data in details.

3.4 HYPOTHESES

a) Microbial diversity in the Upper Sugar Creek watershed largely depends on land use and climate

b) HHEI and HMFEI are positively correlated, and can be used as a rapid tool to assess headwaters in the field;

c) Microbial diversity is positively associated with HHEI and HMFEI, and can be used as an indicator of quality of water and stream ecosystem in agricultural watersheds.
3.5 MATERIALS AND METHODS

3.5.1 Description of the study site

Our study watershed, the Upper Sugar Creek (Figure 1.1) is located in northern central Ohio, predominantly in Smithville and Orrville in Wayne County, the leading dairy county in Ohio (USDA 2002). It covers twenty-eight square miles, flows from northern Smithville to south, Tuscarawas River near Dover. The watershed consists of thirty-six primary headwater streams associated with various landscapes and diverse land management practices (Figure 1.2). This diverse land use makes the Upper Sugar Creek an ideal location for studying relationships between microbial diversity and streams habitat.

Stream twenty was selected as the experimental stream (Figure 3.1). Based on diverse land uses and ease of assess, ten sites were established as regular sampling sites for this study. The stream drains five square miles and runs twenty miles long associated with diverse land management practices. It derives from a woody wetland northern Smithville, flows through a pasture, residential areas, crop farms, open fields, Smithville downtown, natural forests, and finally merges into the main steam of Smithville Park. Considering relatively stability of the stream in short term, the physical and biological characteristics of the stream were examined only one time by using the OEPA HHEI and HMFEI. The microbial diversity of the stream was examined three times by using the PCR-DGGE technique in June, July, and August 2011.
3.5.2 Microbial diversity examination

3.5.2.1 Sample collection, filtration and DNA extraction

A sample of 100 ml water was taken from each sampling site with three replicates. Water samples were kept in a cooler box, taken back to the laboratory, and frozen at -20 ºC until further processing. Water samples were thawed and filtered using disposable vacuum filtration apparatus (Pall Corporation, NY) fitted with a 45 mm diameter filter paper (Supor 200 Membrane Disc Filter, 0.2µm filter) to collect the bacterial cells. DNA was extracted directly from whole filters using the Power Soil DNA Kit (MoBio Laboratories, CA, USA) according to the manufacture’s’ instructions and stored at-20 ºC. DNA was quantified using a NanoDrop ND-1000 UV Spectrophotometer (NanoDrop Technologies, Thermo Scientific Wilmington, DE) (Table 3.1).

3.5.2.2 PCR-DGGE

Original DNA samples were diluted with DDI water to 1 ng/µl for PCR-DGGE processing. Amplification of bacterial 16S sRNA genes was performed using an initial denaturation step at 94 ºC for 9 min, 30 cycles of denaturation at 94 ºC for 30s, annealing at 55 ºC for 30s, and extension at 72 ºC for 30s, followed by a final extension at 72 ºC for 7 min. The universal bacterial primers GC clamp, 338F (ACTCCTACGGGAGGCAGCAG) and 518R (ATTACC CGCGGCTGCTGG) were used. This primer set amplifies a 236 base-pair DNA segment. Amplification of
products of the expected size (approximately 200 bp) was verified by electrophoresis in 1% w/v agarose gel.

The PCR products were further separated by DEEG gels using a BioRad D-Code apparatus (BioRad, Hercules, CA). The PCR products were loaded onto 8% (w/v) polyacrylamide gradient gels with a denaturing gradient of 35% to 65% (100% denaturant contains 7mol/l urea and 40% deionized formamide). The gels were run for 6 h at 130 V and 60 ºC in 1.0×TAE buffer (20 mM tris-Cl, 10 mM acetate, 0.5 mM Na₂EDTA). After DEEG, the gels were stained with ethidium bromide for 30 minutes and photographed under ultraviolet (UV) illumination (Gel Logic Unit, Kodak, California, USA).

### 3.5.3 Stream assessment

Field measurements of HHEI and HMFEI were carried out from July 30th to August 1st 2011. Water parameters were collected three times as we collect water samples in June, July, and August, 2011. For each site, channel morphology, physical habitat, water quality, and biology within a 200-feet stream length were assessed. The primary parameters regarding channel morphology and habitat are substrate particle size classes, maximum pool depth, bank full width, riparian zone, canopy opening, flow regime, sinuosity and stream gradient. Recorded water parameters are water temperature, pH, conductivity, turbidity, and DO. Biological parameters include fish, salamanders, macroinvertebrates, and other amphibians. Floodplain quality,
precipitation, suspected straight pipes were also recorded to increase resolution at these sites.

The species and quantity of salamanders were identified and recorded at upper stream and downstream of each assessment zone. Three dip nets were used to obtain fish and macroinvertebrate samples from multiple habitats such as run, riffle, pool, rapid, step-rapid, and waterfall. Samples were transferred to white trays by washing rocks and disturbing substrate. The fish quantity and species were recorded in the field and later released back to the stream. Macroinvertebrate samples were preserved in 99% ethanol in the field, and later transferred into 70% ethanol after being separated from the substrate. The macroinvertebrates were then counted and identified to family level (Merrit and Cummins, 2008).

3.5.4 Statistical analysis

A diversity richness index calculated using the DGGE banding pattern was used to quantify different water samples numerically. The mean band number for each sample was used to calculate the richness index. For our analyses, bands that could be clearly discerned as being distinct and separated from other bands, even if faint, were marked. The existence of the bands was further confirmed by comparing the normal gel pictures with an inverted image. The phylotype richness ($S$, number of bands) was calculated for each sample. In the evaluation of richness, the higher the value, the more diverse in terms of the number of dominant species that were in water sample.
DGGE banding data were used to estimate diversity indices by treating each band as an individual operational taxonomic unit. Each band was presumed to represent the ability of that bacterial species to be amplified (Ibekwe and Grieve, 2002). The Shannon–Weaver index $H$ (Shannon and Weaver, 1963) and Simpson index of dominance $D$ (Simpson, 1949) were used to measure the richness and evenness of each subspecies within water microbial community. The parameters were estimated using the following equation:

$$H = - \sum_{i}^{s} (P_i \ln P_i) = - \sum_{i}^{s} (N_i/N)$$

$$D = \sum_{i}^{s} (P_i^2)$$

where $s$ is the number of species and $P_i$ is the measure of $i$th species proportional to the total measure of all species (Zak et al., 1994). In the case of DGGE dataset, $P_i$ is the percentage of the $i$th DGGE fragment gray degree to each DNA sample, $N_i$ is the net gray degree quantity (subtracted by the background gray degree quantity of a gel) of the $i$th DGGE fragment to each DNA sample. $N$ is the total net gray degree quantity of all DGGE fragments examined in each DNA sample, and $s$ is the number of DGGE fragments to each DNA sample.

DGGE data set was used to measure microbial diversity (richness, Shannon-Weaver, and Simpson index) of each water sample. Two-way analysis of variance (ANOVA) was used to determine the differences of microbial diversity between treatments and sampling time, and land use and climate. General lineal model was
used to measure the impacts of independent variables on microbial diversity. Independent variables include time, treatment, land use, precipitation, air temperature, water parameters, HHEI, HMFEI, and salamanders. Pearson correlation was used to determine the relationships between microbial diversity and habitat characteristics. The net gray degree quantity was used for cluster analysis. The results were expressed as dendrograms so that differences of water microbial communities could be more easily ascertained. Data analyses were performed using analysis tools with Minitab 16.0. All graphs were generated using Professional Microsoft Excel 2010.
3.6 RESULTS AND DISCUSSION

3.6.1 PCR-DGGE results

Our PCR (Figure 3.2) and DGGE (Figure 3.3) study results demonstrate two close associations: microbial diversity and land use, and microbial diversity and climate.

3.6.1.1 Association of microbial diversity and land use

The microbial diversity index varies from site to site, but it is closely related to the surrounding landscapes (P < .01). High microbial diversity is found at sites where streams are mostly covered by forests, woods, tree buffers, high grass or shrubs, while low diversity is observed at sites where agricultural activities are dominant such as crop fields, residential area, and pasture where riparian buffer zone lack. The trend is consistent in May, June, and July, 2011.

In May (Figure 3.4), low microbial richness was observed at site T where the stream originates from. The number increased as the stream flowing through a forest woods, and crop fields where the tree buffer was employed as a conservation practice to improve water quality. The number dramatically increased at site where another small stream joins in and is covered by high shrubs and grass. The microbial richness
decreased at open crop fields, and gradually increased as it flowed in forest woods of the Smithville Village Park.

In June (Figure 3.5), all three graphs demonstrate relatively high microbial richness at upper and downstream site with a drop in the middle. Unlike the data shown in May, high microbial diversity was observed at its original site, a small woody wetland, the number dramatically dropped at a residential area, increased after the stream flowed into forest woods, decreased when it flowed out of the woods, and gradually increased in downstream.

In July (Figure 3.6), microbial diversity index decreased in comparison with that in May and June. The index was very low at sites with land use of crop fields and residential areas. It sharply increased as water flowing through woods. The diversity indices were observed above mean value at sites with land use of forest, woods, wetlands, and tall grass and shrubs.

The DGGE clustering tree (Figure 3.7) demonstrates that sites that have similar land use tend to have similar microbial communities. For example, the microbes of site A, B and Q belong to one community. They all have 90% vegetation coverage, and no apparent discharge from septic systems, livestock operations, and pastures. The microbes of site RU tend to group with site S since they are right next to each other with similar land use. However the site RU and RD belong to completely different microbial communities even though they are in one parcel. Site RU is a forestry wood, while site RD is a row crop fields with a quarter mile tree buffer. The difference between RU and RD indicates that great amount of
microorganisms from row crop fields on site RD were transported into the stream, which further confirm the association between microbial diversity and land use.

3.6.1.2 Association of microbial diversity and climate

Microbial diversity indices were observed to associate with climate particularly precipitation (P < .01). For this study, Richness (S), Shannon-weaver index (H), and Simpson index (D) were used to calculate the microbial diversity of water samples. Strongly positive correlations among these three indices were observed (Table 3.5). All three calculation methods explored similar results in May, June, and July. However, the diversity index was generally high in May, but low in July, which may due to the climate.

High values of microbial diversity in May result from the dynamics of the stream power due to the precipitation. The high precipitation associated with high stream power would wash off microorganism from surrounding areas and deliver them to downstream quickly (Chang, 1979). As a result, generally high diversity indices were detected in the entire stream, but relatively less diversity were found in upper than downstream. In addition, increased sinuosity and wide bank full width help decrease stream power at certain sites such as Q and I, which explains the dynamics of richness between sites. Finally, the use of tree, shrubs, and lawn buffer along the stream contribute to the gradually increased microbial richness in downstream.
The low values in July may due to the low precipitation and dry weather in that month. According to the Wooster weather station, the average precipitation of May, June, and July 2011 was 0.23, 0.1, and 0.09 inches respectively (Table 3.2). The mean air temperature was 61.32, 69.01, and 75.72 Fahrenheit respectively (Table 3.3). Low precipitation (Figure 3.8) and high air temperature of July may explain the low values of microbial diversity. Furthermore, in our experimental stream area, no precipitation was recorded in two weeks before the sampling. The study results support the idea of the majority of scientists that high precipitation contributes to increased microbial concentration and species in aquatic systems (Melnick and Gerba, 1980; Rhodes et al., 1998), while dry weather limits microorganism survival (McFeters and Stuart, 1972; Rhodes and Kator, 1988; Garzio-Hadzick et al. 2010).

In summary, although the microbial diversity indices vary largely from site to site and month to month, the clear associations of microbial diversity indices - land uses, and indices - climate could be made. Moreover it is important to note that, despite the similar function of three diversity-calculation methods, Simpson index captured slightly different information in July when the average precipitation was low within the water sample collection week, which suggests the necessity of using three indices as complimentary tools to exam the microbial diversity of small headwater streams.
3.6.2 Headwater stream assessment results

Field-based assessment results show that HHEI score, quantity of salamanders, and HMFEI score vary from 36 to 78 with standard deviation of 14.5, from 0 to 4, and from 5 to 25 with standard deviation of 6.5 respectively (Table 3.4). The HHEI score is positively correlated with both HMFEI score at level of P < .01 (Figure 3.9), and numbers of salamanders at level of P < .01 (Figure 3.10).

Similar to microbial diversity test results, the HHEI and HMFEI varied largely with landscape changes. High scores of HHEI and HMFEI associated with landscapes such as forests (A & B), and high grass or shrubs (O), while relatively lower scores associated with residential area (S), crop fields (RD) and pasture (P). For instance, the site T was categorized as class I stream in wood wetland, but it was improved to class II stream in forestry, degraded to class I stream in crop fields, and finally improved to class III stream in downstream in forestry (Figure 3.11). Moreover, No salamanders were observed in class I sites, only two-line salamander adults were observed in class II sites (Site P & Q), both larval and adults of two-line salamander were observed in class III sites (Figure 3.12).

Statistical analysis results demonstrate the closely correlation between HHEI, HMFEI, and numbers of salamanders, which indicate the validity of two-line salamander as biological indicators of healthy headwater streams (Table 3.5). Overall, the stream improves from upper to downstream. Similar improvements are also observed in other tributaries of the Upper Sugar Creek Watershed (data not shown),
which suggests the positive influences of conservation practices and the potential possibilities to improve small headwater streams in agricultural ecosystems.

Water chemical variables, temperature, pH, turbidity, conductivity, and DO range from 16.4 to 22, 8.04 to 9.27, 33.4 to 64.4, 0.35 to 1.03, and 5.53 to 7.22 respectively (Table 3.4). The numbers of these parameters varied from month to month, but the correlation test results show that temperature (P < .05), pH (P < .01), and turbidity (P < .01) are negatively correlated with HMFEI. DO is positively correlated with HMFEI at level of P < .01 (Table 3.5). Test results also show that pH and turbidity is negatively correlated with HHEI at level of P < .05, but DO is positively correlated with HHEI at level of P < .01 (Table 3.5). The results support the perspective that neutral pH (McFeters and Stuart 1972), and low water temperature (McFeters and Stuart, 1972; Garzio-Hadzick et al. 2010) favor the survival of macroinvertebrates and microorganisms in fresh waterways.

3.6.3 The correlations between microbial diversity and habitat characteristics

Our study results demonstrate the positive correlations among three microbial diversity indices (S, H, D). Similar to the microbial diversity, variables of stream habitat characteristics, HHEI, HMFEI, and numbers of salamanders, are positively correlated. Statistical analysis results show that microbial diversity index (S, H, D) is positively correlated with HHEI (Figure 3.13), HMFEI (Figure 3.14), and the number of two-line salamanders at the level of P < .01 (Table 3.5).
Two-way ANOVA results (Table 3.6) show that the dynamics of microbial diversity is due to sampling time (P < .01), which indicates the impact of precipitation and air temperature. General regression analysis show that HHEI and HMFEI have statistically significant impacts on microbial diversity (P < .05). Turbidity, pH, and water temperature also contribute to richness increase. Precipitation, air temperature and water temperature place primary roles (P < .01), given microbial diversity as a function of precipitation, air temperature, HHEI, HMFEI, salamanders, water temperature, pH, conductivity, turbidity and DO (Table 3.7). Study results confirm the validity of using microbial diversity as an indicator of water quality and stream ecosystem. Although HHEI and HMFEI have less impact than climate on changes of microbial richness over time, the strong correlations between them indicate that habitat characteristics could be used as indicators of the microbial diversity in headwaters of agricultural watershed.

3.6.4 Future work

The polymerase chain reaction coupled with denaturing gradient gel electrophoresis (PCR-DGGE) has been used widely to determine species richness and structure of microbial communities in a variety of environments (Campbell et al. 2009). However, use of PCR-DGGE to analyze microbial communities in fresh headwater streams has been largely unexplored. Our study demonstrates that PCR-DGGE appears to be a viable option for addressing the dynamics of microbial diversity along small headwater streams. However, the specific species of microbial
community and its relationships with stream habitat characteristics are not clear. This needs to be examined more closely in future.

Moreover, more studies need to be done on associations of microbial diversity and climate changes. Giving the context of global warming, increased temperatures, precipitation, and other weather conditions may greatly impact the transport and survival of microbes in headwaters. It will be a new attempt to establish a surveillance system of climate change by continuously monitoring the dynamics of microbial diversity in the same watershed.

Finally, the HHEI method provides a quick and clear summary of stream’s biological and physical characteristics. Our results show that the same stream was categorized as a Class I stream in its headwaters has been improved to a Class III stream downstream, which suggests the influence of conservation land use. Even though, the habitat characteristics of one stream would be relatively stable during a season, they could shape the downstream dramatically, and indirectly influence the microbial community in a number of ways. Giving the expensive and time consuming method to test the microbial diversity, HHEI could be used as a rapid and inexpensive tool to predict the dynamics of microbial diversity and changes of stream ecosystems. Therefore, it is encouraged to assess headwater streams on a regular basis to establish a monitor system of climate change in future.
3.7 CONCLUSION

The objectives of this chapter are to measure microbial diversity, assess stream habitat characteristics, and correlate these variables. PCR-DGGE tests support my first hypothesis that microbial diversity largely depends on land use and climate (P < .01). High diversity indices were found to associate with land use of forestry, high grass, and shrubs. Low precipitation and dry weather were observed to correlate with low diversity indices.

Correlation analyses detected positive correlations between HHEI, HMFEI, and numbers of salamanders (P < .01). High habitat indices are closely associated with land use of forestry, tall grass, and shrubs, while low values are associated with residential area and pasture. The results support my second hypothesis that variables of habitat characteristics are positively correlated, and the method can be used as a rapid tool to assess small headwater streams in the field.

Correlation and regression analyses also detected positive association between microbial diversity and HHEI and HMFEI (P < .01), which support my third hypothesis. The results confirm the validity of microbial diversity as a sensitive indicator of water quality and stream ecosystem health. Given the expensive and time consuming method to test the microbial diversity of numerous headwater streams, HHEI method could be used as a rapid and inexpensive tool to summarize not only the habitat characteristics, but also the dynamics of microbial diversity of the streams.
3.8 REFERENCES


Figure 3.1 Stream twenty of the Upper Sugar Creek watershed
Red dots represent ten water sampling sites for microbial diversity and HHEI, HMFEI tests
Figure 3.2 Genomic DNA extracted from water samples
“M” denotes marker, “B” denotes blank
Figure 3.3 DGGE fingerprints of the PCR products from water samples
“M” denotes marker.
Figure 3.4 Microbial diversity of water samples in May 2011
Bar represent standard deviation with three replicates.

Figure 3.5 Microbial diversity of water samples in June 2011
Bar represent standard deviation with three replicates.
Figure 3.6 Microbial diversity of water samples in July 2011. Bar represent standard deviation with three replicates.
Figure 3.7 UPGMA dendrogram of 16S rRNA in DGGE profiles
Figure 3.8 The precipitation of the Wooster in May, June, and July, 2011.

Data source: OARDC weather station, Wooster, Ohio

Figure 3.9 The correlation between HHEI and HMFEI
Figure 3.10 The correlation between HHEI and quantity of salamanders

\[ y = 0.0875x - 3.8392 \]

\[ R^2 = 0.8155 \]

Figure 3.11 The HHEI classification of stream 20
Figure 3.12 The presence of two-line salamanders in stream 20

Figure 3.13 The correlation between microbial diversity index and HHEI

\[ y = 5.7276x - 9.5468 \]

\[ R^2 = 0.4971 \]
Figure 3.14 The correlation between microbial diversity and HMFEI

\[ y = 2.4884x - 17.069 \]

\[ R^2 = 0.4699 \]
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<th>Site number</th>
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<th>June</th>
<th>July</th>
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<td>S</td>
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<td>B</td>
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Table 3.1 The average DNA concentration of water samples

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<th>N*</th>
<th>Minimum</th>
<th>Maximum</th>
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<td>0</td>
<td>0.75</td>
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Table 3.2 Precipitation data of Wooster in May, June, July 2011. Data source: OARDC weather station, Wooster, Ohio
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<th>Average Air Temperature (Fahrenheit)</th>
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<th>Minimum</th>
<th>Maximum</th>
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Table 3.3 Average air temperature of Wooster in May, June, July 2011. Data source: OARDC weather station, Wooster, Ohio

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<td>0.67</td>
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</tr>
<tr>
<td>DO</td>
<td>5.53</td>
<td>7.22</td>
<td>6.74</td>
<td>0.612</td>
</tr>
</tbody>
</table>

Valid N = 90

Table 3.4 Descriptive statistics of habitat characteristics and water chemistry
<table>
<thead>
<tr>
<th></th>
<th>Richness</th>
<th>Shannon</th>
<th>Simpson</th>
<th>HHEI</th>
<th>HMEFI</th>
<th>Salamanders</th>
<th>Temperature</th>
<th>PH</th>
<th>Turbidity</th>
<th>Conductivity</th>
<th>DO</th>
</tr>
</thead>
<tbody>
<tr>
<td>Richness</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Shannon</td>
<td>0.938**</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Simpson</td>
<td>0.753**</td>
<td>0.704**</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HHEI</td>
<td>0.339**</td>
<td>0.456**</td>
<td>0.335**</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HMEFI</td>
<td>0.330**</td>
<td>0.410**</td>
<td>0.370**</td>
<td>0.857**</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Salamanders</td>
<td>0.288**</td>
<td>0.379**</td>
<td>0.323**</td>
<td>0.903**</td>
<td>0.872**</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Temperature</td>
<td>-0.047</td>
<td>0.003</td>
<td>0.137</td>
<td>0.021</td>
<td>-0.240*</td>
<td>-0.069</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PH</td>
<td>-0.167</td>
<td>-0.142</td>
<td>-0.113</td>
<td>-0.240*</td>
<td>-0.541**</td>
<td>-0.429**</td>
<td>0.778**</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Turbidity</td>
<td>-0.073</td>
<td>-0.045</td>
<td>0.104</td>
<td>-0.260*</td>
<td>-0.365**</td>
<td>-0.436**</td>
<td>0.824**</td>
<td>0.709**</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Conductivity</td>
<td>0.139</td>
<td>0.082</td>
<td>0.191</td>
<td>-0.179</td>
<td>-0.089</td>
<td>-0.263*</td>
<td>0.222*</td>
<td>0.168</td>
<td>0.480**</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>DO</td>
<td>0.133</td>
<td>0.129</td>
<td>-0.015</td>
<td>0.346**</td>
<td>0.420**</td>
<td>0.445**</td>
<td>-0.683**</td>
<td>-0.789**</td>
<td>-0.791**</td>
<td>-0.639**</td>
<td>1</td>
</tr>
<tr>
<td>N</td>
<td>90</td>
<td>90</td>
<td>90</td>
<td>90</td>
<td>90</td>
<td>90</td>
<td>90</td>
<td>90</td>
<td>90</td>
<td>90</td>
<td>90</td>
</tr>
</tbody>
</table>

**. Correlation is significant at the 0.01 level (2-tailed).
*. Correlation is significant at the 0.05 level (2-tailed).

Table 3.5 The correlations of microbial diversity and habitat characteristics
<table>
<thead>
<tr>
<th></th>
<th>DF</th>
<th>SS</th>
<th>MS</th>
<th>F</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Time</strong></td>
<td>2</td>
<td>20.94</td>
<td>10.47</td>
<td>24.43</td>
<td>0.00**</td>
</tr>
<tr>
<td><strong>Treatment</strong></td>
<td>9</td>
<td>9.59</td>
<td>1.07</td>
<td>2.49</td>
<td>0.48</td>
</tr>
</tbody>
</table>

S = 0.65  \[R\text{-Sq} = 79.83\%\]  \[R\text{-Sq (adj)} = 67.5\%\]

Table 3.6 Two-way ANOVA analysis of microbial diversity versus time and treatment

<table>
<thead>
<tr>
<th>Coefficient</th>
<th>Standard Error</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Dependent variable: Simpson Index</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Independent variable</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Constant</td>
<td>2.73</td>
<td>4.01</td>
</tr>
<tr>
<td>HHEI</td>
<td>0.11</td>
<td>0.03</td>
</tr>
<tr>
<td>HMFEI</td>
<td>0.25</td>
<td>0.16</td>
</tr>
<tr>
<td>Salamanders</td>
<td>0.54</td>
<td>0.32</td>
</tr>
<tr>
<td>Water Temp.</td>
<td>0.42</td>
<td>0.09</td>
</tr>
<tr>
<td>pH</td>
<td>-1.28</td>
<td>0.42</td>
</tr>
<tr>
<td>Turbidity</td>
<td>-0.01</td>
<td>0.02</td>
</tr>
<tr>
<td>Conductivity</td>
<td>1.39</td>
<td>0.63</td>
</tr>
<tr>
<td>Dissolved Oxygen</td>
<td>0.02</td>
<td>0.34</td>
</tr>
<tr>
<td>Precipitation</td>
<td>2.11</td>
<td>2.62</td>
</tr>
<tr>
<td>Air temp.</td>
<td>0.10</td>
<td>0.03</td>
</tr>
</tbody>
</table>

R-Sq=74.48%

**. Correlation is significant at the 0.01 level (2-tailed).
*. Correlation is significant at the 0.05 level (2-tailed).

Table 3.7 Regression analysis of microbial diversity versus habitat characteristics
CHAPTER 4
ADOPTION OF CONSERVATION PRACTICES TO IMPROVE
THE WATER QUALITY IN UPPER SUGAR CREEK
HEADWATERS

4.1 ABSTRACT

This chapter examines factors affecting people’s adoption of conservation practices to improve water quality in the Upper Sugar Creek Watershed. It also analyzes local people’s willingness to pay (WTP) to adopt them by using contingent valuation method (CVM). The study results demonstrate that besides socioeconomic variables, farm size, farm succession, sense of place, and local people’s awareness of stream pollution play important roles in determining their adoption (P < .01), and therefore should be included in the research of innovation diffusion in future. Study results also show the greatly potential possibilities to improve water quality. Over sixty percent of respondents indicated WTP to adopt conservation practices to improve water quality. On average, one-time payments in three and five years are
$151.67 and $171.88 for non-farmers, and $156.95 and $197.57 for farmers. Among those who are not willing to pay, over half of them indicated a great interest to take either personal or group actions to make improvement.

4.2 INTRODUCTION

Increasing public awareness and concerns about water contamination has spurred the development of conservation practices over the past decades. Typical practices advocated by U.S. government agencies include best management practices (BMPs) such as farm animal-exclusion fence, riparian buffer, cover crops, and waste utilization. In most cases, these practices are cost-shared with qualified farm owners, with most of the cost covered by the government. Meanwhile, new regulations in the form of total maximum daily load (TMDL) standards have intensified regulatory attention on agricultural activities. However, the adoption rate of BMPs remains low. In fact, in most agricultural areas of Ohio, people who sign up for the cost-share programs are not those who need them most (Moore et al., 2008; Parker et al., 2009). Understanding factors affecting people’s adoption behavior is critical for the diffusion of conservation practices and water quality improvement.

The primary objective of this chapter is to identify variables affecting local people’s adoption behavior, and to estimate their WTP to adopt conservation practices to improve water quality in the Upper Sugar Creek Watershed. The
secondary objective is to benchmark local people’s attitudes towards water pollution in their neighborhood’s streams, and to estimate the potential possibilities to cooperate with others living within the watershed area to improve it. The central goal of the study is to create a model by which the local community can find a way to implement and fund conservation practices appropriately to reduce the transmission of microbial contaminants into the watershed.

4.3 LITERATURE REVIEW

4.3.1 Innovation diffusion theory

Two models are widely used to explain adoption behavior of conservation practices: innovation diffusion and farm structure. The former model is based on two primary models, namely Roger and Brown. Roger’s model has been used to show that accessing information will result in adoption phases of the new technology in which the principle variables in farmer’s adoption are rational choice, self-interest, and personality type (Roger 1962). This model is adopter-centered and technology-neutral, which assumes that the adoptive behavior results from rational choice in response to expert-driven knowledge. Brown (1981) focused less on the adopter side and placed greater emphasis on provider side such as access to information, cost, geographic location, and infrastructural needs associated with innovations.
Farm structure model emphasizes farm size and farmers’ net income. These two variables are considered main indicators in determining impacts on conservation adoption. Both innovation diffusion and farm structure models are not very different in explaining farmers’ adoption behavior (Tucker and Napier 2002; Napier et al 1984). Based on these two models, most researchers study the conservation adoption from the approach that is similar to neoclassical economics (Granovetter 1985, Parker et al. 2007). It emphasizes the roles of factors such as economic profits, self-interest, and people’s rational choice, while social network, culture, sense of place, kinship obligations, and other variables are considered as externalities.

However, these “external factors” do account for socially embedded behaviors of certain conservation practices. For example, Bultena and Hoiberg (1983) observed that farmers’ adoption behavior was dependent upon their perception of neighbors’ attitudes toward conservation tillage. Soule et al. (2000) found that owner-operators were more likely than cash-renters to adopt practices such as conservation tillage, grassed waterways, strip cropping, and contour farming that provide benefits in long term. Erickson (2002) concluded that aesthetics was a determining factor based on the type of conservation being employed. Park et al. (2007) singled out the specific importance of land tenure attributes as a new set of variables in determining the adoption of conservation practices. Wauters et al. (2010) identified awareness of soil erosion as the most explaining factor of soil conservation practice adoption.
4.3.2 Factors affecting people’s adoption behavior

Innovation-diffusion theory provides well-developed concepts and a large body of empirical results applicable to the study of technology evaluation, adoption and implementation. It provides tools, both quantitative and qualitative, for assessing the likely rate of diffusion of a technology, and identifies numerous factors that facilitate or hinder technology adoption and implementation. These factors include characteristics of adopters, characteristics of innovation itself, and the means by which adopters learn about and are persuaded to adopt it (Roger 2001). However, as an important type of environmental innovations, conservation practices are different from traditional innovations in several ways such as relatively long cycle of return on investment, relatively low competition in market, benefiting both private adopters and public in long term. Therefore, it is critical and necessary to understand the characteristics of environmental innovations in the first place.

4.3.2.1 Characteristics of environmental innovations

An environmental innovation is an innovation that “serves to prevent or reduce burdens on the environment, clean up damage already caused, or diagnose and monitor environmental problems” (Hemmelskamp 1997). In contrast to commercial innovations, environmental innovations are characterized by both economic and environmental benefits due to the positive spill-overs of these innovations that are accompanied by the internalization of negative environmental effects (Horbach 2007). They may lead to a “win-win” situation so that pollution is reduced and profits are
increased. However, it is important to note that most environmental innovations do not generate direct profits in short term, and therefore are less competitive in market.

A conservation practice, as an important type of environmental innovations (also called eco-innovations) is the recent concept that is at the very core of public policies towards sustainable development. It differs from traditional and commercial innovations in several ways. First, it could be a practice instead of a specific technology, such as vegetation buffers used to filter out excessive nutrients and bacteria. Second, its relative advantages are hard to observe in short period of time (Kemp, et al., 2001). For example, the practice of livestock stream exclusion fences farm animals out of streams. It helps to reduce non-point source pollution by reducing stream bank erosion and lowering the bacteria associated with livestock waste. However it takes years to observe the recovery of vegetation on stream banks and reduction of soil erosion. It is even impossible to know the dynamics of bacteria concentration over different seasons or years unless we consistently monitor water samples. Third, it is difficult to quantify the economic profits of the environmental impacts, such as improved water quality for downstream, improved animal health, reduced risk of human health etc.

4.3.2.2 Characteristics of adopters

The literature identifies six factors to characterize adopters: societal entity of innovators, familiarity with the innovation, status characteristics, socioeconomic characteristics, relative position in social networks, and personal characteristics. For
this study, I focus on income, farm size, land tenure, and people’s awareness of water pollution.

**Income**

Much research has concluded that early adopters are wealthier than laggards or non-adopters. Since innovations may have a high degree of uncertainty, early adopters must take risks that can be avoided by later adopters. Thus, they must be wealthy enough to absorb the loss from occasional failures (Rogers 2001). Brown (2001), from the perspective of supply-side, put forward the same idea. He argued that diffusion agencies often create segment markets depending upon potential adopters’ economic, social and locational characteristics. People who are wealthier and live closer to market usually are more likely to benefit earlier and more than the poor. Likewise, Fligel (2001) pointed out that differences among farmers in farm income are related to adoption behavior. Relatively low income farmers tend to lag behind others.

It is clear that the innovation itself is, in many cases, costly and does not always lead to direct profits (Lawrence 1995). For example, some Sugar Creek farmers rejected having riparian buffer on their land because this practice was seen as removing land from production, and therefore has the potential to decrease profitability (Moore et al. 2009). With regard to my study, I also expect the similar correlation, namely richer people are more likely to adopt conservation practices since they must have enough money to undertake the cost of the practices and the loss of failure at the same time.
Farm size

Farm size is an important indicator of household decision-making of conservation adoption (Burton and Walford 2005), and also a major constraint on land use decision-making (Ilbery and Bowler 1998). This variable affects adoption for the similar reasons as economic well-being. Rogers (2001) noted that earlier adopters have larger-sized operations than later or non-adopters. Similarly, Fligel (2001) stated that farm size is directly related to adoption behavior. The relatively resource-poor farmers tend to lag behind in technology adoptions.

I hypothesize a positive correlation between farm size and conservation adoption, namely the larger the farm size, the more likely that conservation practices will be adopted (Potter et al. 1991). However, it is important to note that the direction of this relation may not be always true especially when farmers rent large proportion of land. Midwest farmers, particularly those who have exclusively row crops, have enlarged their operations by renting land to keep its competitiveness in the market of corn and soybean (Hart 1991; Moore et al., 2008). As a result, they may not want to implement practices on rented land.

Land tenure

Salamon et al. (1992) put forward the perceptive that land tenure attributers, such as leasing-out, farm succession, farmers’ attitudes towards land, neighbors’ behavior and environmental degradation, should be considered as important social and cultural variables in determining farmers’ adoption behavior. Previous studies suggest that the motivations to adopt conservation practices rest in secure access to land (Salamon et al., 1992; Soule et al., 2000; Parker et al., 2007). Sugar Creek
Watershed studies (Moore et al., 2008) also show the similar correlation that farmers are less likely to implement CRP buffers on leased land.

Farm succession is major contributor to sustainability of farm, both by reducing transitions from agricultural to non-agricultural land use and encouraging management styles that focus on long-term productivity over short-term gain (Bennett 1982; Salamon 1992). Relationships that result in reciprocal support, both within and between farm and neighbors, contribute to stable, sustainable, and equitable production (Lobao 1990; Salamon 1992).

My study focuses on two attributes of land tenure: farm succession and sense of place (years of farming/living at the current watershed area). I hypothesize that the adoption of conservation practices are positively correlated to the potential possibilities of continuing farming and sense of place such as years of farming or living at current watershed location.

**Knowledge and awareness of stream pollution**

Beedell and Rehman (1999) studied farmers’ conservation behavior towards hedge management in UK. They found that ‘conservation minded’ people are under greater social pressure to their hedge. They value hedge management higher than other people, and therefore are more likely to adopt the practice. Prager and Posthumus (2010) examined socio-economic factors influencing farms’ adoption of soil conservation practice in Europe, and conclude that knowledge and awareness of soil degradation problems is a decisive factor of adoption. Likewise, Wauters et al. (2010) draw similar conclusion that farmers’ adoption behavior was dependent upon their attitude towards soil conservation practice.
I expect the same correlation: namely the adoption is closely correlated to people’s knowledge and awareness of stream degradation. These ‘stream-concern’ people are also expected to have higher WTP than others.

4.3.2.3 Contingent valuation method

Since the last century, contingent valuation method has emerged as the most direct and straightforward technique to evaluate public opinion on adoption of environmental innovations (Mitchell et al., 1989; Jordan et al., 1993). As a method stemming from the field of natural resource economics, CVM has been extensively used as an effective tool to estimate people’s willingness to pay taxes for many public services such as drinking water safety and preserved park management (Blaine et al., 2005). One of the most frequently used and easiest to apply version of CVM is the payment card.

As an essential and indispensable natural resource, water particularly drinking water has gained considerable attention among public health, natural resource economics, governments, environmental agencies, and others. Over the past two decades, the use of CVM to estimate residents’ WTP for improved water quality has been extensively studied and much information is available (Jordan et al. 1993; Mitchell et al. 1986; Mitchell et al., 1989; Shultz et al. 1990). However, the headwater pollution in agricultural ecosystems has received little attention due to the small drainage area, mixed-use of the water, and unease access. Moreover, most studies concerning agricultural watersheds deal with issues such as soil erosion and nutrients loading. Relatively little research has focused on households' WTP for
adopting conservation practices to improve water quality. Our study will be among the first to investigate the detailed usage of headwater streams in agricultural watersheds, examine local people’s attitudes towards their neighborhoods’ streams, and link this information to their WTP.

4.4 HYPOTHESES

a) Farm size and income are positively correlated with the adoption of conservation practices;

b) Land tenure attributes (farm succession and sense of place) are closely correlated with people’s adoption behavior;

c) Lack of awareness and knowledge about stream pollution is a main barrier of conservation adoption.

4.5 MATERIALS AND METHODS

4.5.1 Description of study site

The Upper Sugar Creek is one of the six sub-watersheds of Sugar Creek Watershed in Ohio. It is located in north central Ohio in Wayne County, one of the
largest dairy counties in Ohio and covers 28 square miles (USDA 2002). It consists of thirty-six primary headwater streams, ranging from Wooster to Orrville with Smithville in its heart (Figure 1.1).

More than 80% of the watershed land is devoted to agricultural uses with potential microbial contamination sources including pasture, hay, livestock operation, and row crop (OEPA 2002). As the headwaters of Ohio’s largest watershed flowing to the Ohio River, the Upper Sugar Creek contributes to the hypoxia issue in the Gulf of Mexico. Besides excessive nutrient loading, failing septic systems was cited as one of the most important issues in the watershed (Parker et al. 2008). The watershed is therefore one of the first to implement TMDL and bacteria TMDL plan which aims to reduce both point and non-source pollution in the watershed (Moore, et al. 2008; OEPA 2007).

According to 2010 U.S. consensus, the population in Wayne County was 114,611 with an increase of 2.6% from 111,564 in 2000. Approximately 457 households 1,200 people reside in the Upper Sugar Creek watershed area, mostly Smithville and Orrville. More than 96% of them are white. Close to half of the population received high school education. The media age was 37.8 years. The mean household income was $48,375.

4.5.2 Survey design and instrument

4.5.2.1 Survey questionnaire

The survey questionnaire (Appendix A) was devised as a payment card CV instrument in which four sets of questions were asked to examine factors affecting
people’s adoption behavior and the amount of their WTP. The first set of questions deals with how respondents value their neighborhood streams, rate water pollutants, prioritize their major concerns, and use the streams. Questions related to microbial pollution and failing septic system were inserted in the middle of the lists in order to avoid misleading.

The second set of questions was designed to understand how much knowledge respondents have about their septic systems including types, function, maintenance activities, cost, and outflow discharge. According to the Wayne County Health Department (WCHD), the main type of septic system is a traditional leach field due to its low cost. No agencies assess the function and the outflow discharge of septic systems on a regular basis. WCHD goes out to check them only when called by neighbors complaining leakage, odor, or other issues.

The third set of questions is about WTP to adopt conservation practices to improve water quality. Respondents were first given six conservation practices to choose what they have used or would like to use in future. Data about information source, major concerns, and trust of agencies was also collected. Before the payment card survey, E. Coli concentration test results and streams’ habitat evaluate index were mapped out and shared with respondents. Each respondent was confronted with 3 scenarios with a series of monetary amounts, and asked to circle their maximum WTP for conservation practice implementation. The first scenario provides the option of paying zero to keep the current condition. This situation is that presently twenty out of twenty one sampling sites have bacteria over the OEPA standards of 235 / 100 ml. The second scenario offers a series of amounts ranging from $5 to $400 to
improve water quality to the level that the bacteria count will be less than OEPA danger levels in three years. The third scenario offers a range of $50 to $600 amounts for respondents to choose from. This effort will improve the water quality to meet the OEPA standards in five years, to make sure children and pets playing in neighborhood streams or village parks play without danger, and to make sure farm animals can drink in streams without danger. Instead of offering a single amount to simply vote ‘yes’ or ‘no’, by presenting each respondent with a range of possible payment levels, the researcher is allowing respondents to choose his or her own price. As a result, it should not be surprising that researchers often get relatively low estimates on WTP.

The last section of the survey is about demographic and socioeconomic variables. They were used to analyze to what extent those variables determine people’s adoption behavior. Land tenure variables of farmers such as farm size, years of farming, ownership as well as succession were closely examined.

4.5.2.2 Survey steps

The survey instrument was based on a questionnaire format using pick-up and drop-off method. Data were collected using a “drop-off, pick-up” survey method, interviews, participatory observation, and interaction at farmer meetings and community activities. The sample frame was limited to people living in the watershed area and age above 18.

The study involved two steps. First, a preliminary survey was conducted to test the survey instrument and econometric methods. Based on our previous
cooperation and recommendation from Sugar Creek Farmer Partners, a self-selected voluntary farmer group in this area who meet once a month in the non-growing season, 21 households participated in an informal interview. The preliminary survey covered questions regarding loss of biodiversity, agricultural pollution, children’s health, biological characteristics of streams, and other water quality issues. Survey was then modified to form a larger questionnaire based on comments and suggestions from the preliminary interview.

Second, 300 households were randomly selected from the list of the people living in the watershed area. Researchers and summer interns of Ohio agriculture and research development center (OARDC) delivered 262 survey questionnaires in the summer of 2010. We did not plan to do any recruitment activities. We simply knocked on their doors, briefly explained study purposes, answered questions they had, and left a copy of questionnaire to those who were willing to participate in the study. Participants could either ask us to pick it up or mail it to OARDC. We returned three times to follow up on the completion of the survey. Those who completed the survey within the first week were marked as early respondents. Those who completed or mailed the survey after the sixth week were marked as late response. Since late respondents tend to share similarities with non-respondents, they were not be involved in data analysis (Miller and Smith, 1983).

4.5.3 Statistical analysis

The raw data was coded, entered into Microsoft Excel, and converted into Minitab for statistical analysis. In analyzing the data, a number of statistical tools
were applied including descriptive statistics, Pearson correlation analyses, and multiple regression analyses. Data analyses were performed using analysis tools with Minitab 16.0. All graphs were generated using Professional Microsoft Excel 2010.

Descriptive statistics were used to describe demographic and socioeconomic characteristics of respondents. They were also used to describe the attitudinal difference between farmers and non-farmers towards the streams. Pearson correlation was used to measure the relationships between adoption and independent variables such as farm size, income, farm succession, and others. Logistic regression was used to test impacts of independent variables in predicting adoption. Adoption was coded as 0 = non-adoption, 1 = adoption. Farm succession was coded as 0= will not happen, 1= unlikely, 2= unsure, 3= likely, 4= will definitely happen. Probit regression was used to estimate the mean WTP of farmers and non-farmers.

4.6 RESULTS AND DISCUSSION

4.6.1 Descriptive results

In total we delivered 262 questionnaires, and received 214 back (81.7% response rate). The survey covered most areas of Smithville, part of Wooster and Orrville. The results of selected questions among economic and socio-demographic variables are presented in Table 4.1 and 4.2. The sample is comprised of 93.7% white and 1% black respondents, whereas the U.S. Census estimates for Wayne County’s
voting age population in 2010 were 95.7% white and 1.5% black. The average age of respondents is 48.5 years compared with the census estimate of 47 years for 18 years and above. The sample represents Wayne County’s population race and age quite well. Classifying by respondents’ self-recognition, the sample consists of 39.7% farmers, and 60.3% non-farmers. The mode of farmers’ income is $80,000 - $89,000 associated with an average of 304 acres farm land. The high land value ($10,000/acre including the new value of fracking) makes local farmer’s economic assets much higher than non-farmers. Therefore, it is not surprising that more farmers indicated a higher willingness to pay than non-farmers.

The survey results show that 48% of respondents rated their stream water quality as good, and only 3% of them rated it as poor (Figure 4.1). Close to half (43%) of respondents marked pesticides as a severe problem, 23% and 26% of them thought chemicals and sedimentation were severe problems, but less than 10% of them viewed bacteria as a severe problem (Figure 4.2). The survey also explored attitudinal differences between farmers and non-farmers regarding the potential sources of pollution, concerns, and others. Thirty-seven percent of non-farmers perceived chemical/agricultural pollution as severe (11%) and moderate problem (26%), but no farmers thought it was a severe problem, and only 59% of them rated it as a slight problem (Figure 4.3). More farmers (56%) than non-farmers (42%) rated sedimentation as a problem (Figure 4.4). Close to half of the farmers (43%) and more than half of non-farmers (57%) indicated that they were unsure about bacteria pollution in their streams (Figure 2.9). Meanwhile, a quarter of the respondents stated
that their sewage wastewater was directly discharged to nearby streams, and another quarter indicated unsure about the sewage outflow discharge (Figure 2.12).

Nearly half of the respondents indicated willingness to pay a certain amount of money to adopt conservation practices to reduce microbial contamination in Upper Sugar Creek Watershed (Figure 4.5). A slightly higher number of farmers (37%) than non-farmers (35%) indicated WTP for scenario 2, which is a one-time payment of $5 or more (up to $400) to improve the water quality to the level that bacteria count will be less than Ohio EPA standards in 3 years. A greater number of farmers (24%) than non-farmers (8%) indicated WTP for scenario 3, which is one-time payment of $50 or more (up to $600) to make sure that water quality will meet EPA standards in 5 years. Survey results show that 57% of non-farmers and 37.9% of farmers indicated unwillingness to adopt any conservation practices. Among those, 30% of them showed interest in knowing bacterial concentration levels in their neighborhood streams, and over 60% indicated willingness to either take personal or group action to make improvements (Figure 4.6).

The results of the payment card portion of the survey are presented in Table 4.2. It shows that 14.3% and 4.4% of the total respondents were willing to pay at least the minimal amount on the payment card for scenario 2 and scenario 3 respectively, while 17.5% and 17.4% were willing to pay the ‘break even’ amount of $200 and $300. However, our survey does not follow the traditional trend of the reverse relationship between offered amount and percent of responding yes. For example, more people indicated willingness of one-time payment of $500 (29.1%) than $400 (13%) for scenario 3 (Table 4.6).
4.6.2 Correlation analysis

Statistics results show that farm size and income are positively correlated with adoption at the level of $P < 0.01$ (Table 4.4). The results are in accordance with perspectives of Brown, Roger, and Fligel that rich farmers are more willing to take risks and to try new innovations. Study results also demonstrate that farm succession ($P < .01$) and years of farming ($P < .05$) are closely correlated with adoption. That is, people who are farming longer, and are going to continue farming for the next generation are more likely to adopt conservation practices. Education, gender and age are also correlated with adoption, but not statistically significant. The results support my first and second hypotheses.

Moreover, a positive correlation ($P < .01$) was observed between WTP and awareness of water pollution (Table 4.3). Respondents who rated the problems of bacteria and sedimentation higher are more likely to be willing to pay. On the contrary over 65% of respondents who indicated unsure about their sewage discharge (25%) and bacteria pollution (50.5%) also indicated unwillingness to pay. The results support my third hypothesis that lack of awareness and knowledge towards stream pollution is one of main barriers of conservation adoption. However, it is important to note that among them over half of indicated willingness to take either personal or group actions to improved water quality. Additionally, besides cost, other primary barriers of conservation adoption are too much paper work or restriction associated with government program, attitudes of friends and neighbors, effective land management, and availability of funds for cost-share (Figure 4.7).
4.6.3 Regression analysis and mean willingness to pay

4.6.3.1 Regression analysis

To further explore the impacts of land tenure attributes and socio-demographic factors on conservation adoption of respondents, logistic regression were employed. Although correlation analyses indicate that the relationship between adoption, gender, age, years of farming, and education are not statistically significant, they are included as control variables.

The results (Table 4.5) show that farm size, income, farm succession, and sense of place have significant impacts on adoption, while age, gender, and education play minor roles. The $R^2$ value indicates that the models account for 63.1% of the variation in adoption.

4.6.3.2 Mean willingness to pay of farmers and non-farmers

To identify the characteristics of those who are more or less willing to pay for the conservation practices, I ran regression equations to estimate attitudes towards payment as a function of a set of land tenure and socio-demographic variables. Mean willingness to pay of farmers and non-farmers were regressed separately. Non-farmers’ WTP was regressed on age, gender, education, income, and years of living at current watershed area. Farmers’ WTP was regressed on age, gender, education, income, years of farming at current location, farm size, and farm succession. A maximum likelihood (ordered probit) procedure (Cameron and Huppert, 1989) was used. The results are presented in Table 4.7 and 4.8.
The models show that income (P < .01) and years of living at current location (P < .05) have statistically significant influences on non-farmers’ willingness to pay for both scenario 2 and scenario 3. Income, farm size, farm succession, and years of farming play significant role in determining farmers’ willingness to pay (P < .01). Regression results support the perspective that people who have strong sense of place also have strong social responsibility, and therefore are more likely to be willing to make efforts for watershed improvement (Nazarea et al., 1998; Moore et al., 2009; Sullivan, et al., 2010).

To calculate a parametric measure of WTP, I use a weighted average of the parameter estimates provided in the ordered probit and logistic regressions in Table 4.7 and Table 4.8. The formula is as follow:

\[
WTP_{\text{Payment Card}} = (B_0 + \sum_{i} B_i X_i)
\]

where \(B_0\) is the intercept, \(B_i\) is the parameter estimate on the \(i\)th land tenure and socio-demographic variable whose mean is denoted \(X_i\), \(m\) is the number of variables in the regression equation.

Using the numbers from Table 4.7 and Table 4.8 we get a mean of WTP for farmers and non-farmers per household of one-time payment.

\textbf{WTP for non-farmers}

\[
WTP_{\text{scenario 2}} = -25.56 + 46.28(2.27) - 1.42(50.85) - 6.91(0.47) + 28.9(2.36) + 1.53(18.5) = \$151.67
\]

\[
WTP_{\text{scenario 3}} = 29.10 + 32.5(2.27) - 1.24(50.85) -13.8(0.47) + 22.5(2.36) + 0.55(18.5) = \$171.88
\]
**WTP for farmers**

\[
WTP_{\text{scenario 2}} = 11.26 + 1.05(19.4) + 5.82(2.38) - 0.05(304) - 0.42(44.86) - 2.39(0.47) + 20.82(2.39) + 17.83(3.8) = $156.95
\]

\[
WTP_{\text{scenario 3}} = 7.46 + 3.14(19.4) + 7.09(2.38) + 0.32(304) - 1.31(44.86) - 4.8(0.39) + 14.31(2.39) + 10.64(3.9) = $197.57
\]

In general, farmers’ willingness to pay is higher than that of non-farmers for both scenario 2 and scenario 3. Income, farm size, and sense of place (years of living at current watershed area) have strong impacts on respondents’ willingness to pay. The role of types of farm operation, social networks (participation in community activities), and other social or cultural variables are not examined in this dissertation, and need to be studied in future.

### 4.6.4 Future work

Both the diffusion of innovation model and the farm structure model emphasize impacts of variables such as economic profits, social well-being, self-interest, people’s rational choice, while most social and cultural variables are considered externalities. Our study results support perspectives of the general innovation-diffusion model. At the same time, our results also show that sense of place, land tenure attributes, and awareness of stream pollution are decisive variables in determining local people’s adoption behavior. These variables therefore should be involved more in the research of innovation diffusion. In addition, types of farm operations are not examined in this dissertation and need to be further studied.
Moreover, it is critical to enhance the communication between governments, universities, and local communities to develop appropriate and effective remediation strategies. Our study results show that the primary sources of microbial contaminants are human instead of animal fecal coliforms. Straight pipes and failing systems are the main cause of pollution. Therefore, the emphases should be placed on inspecting and maintaining septic systems in the watershed. Although other conservation practices greatly help reducing excessive nutrients and bacteria from animal farms, suitable on-site wastewater treatment systems should be the focus to improve water quality in Sugar Creek. However, it is also important to note that the cost to repair or replace a leach field system ranges from $5,000 to $10,000 in Ohio. The high price will be a main barrier to reduce human pathogens transported into the watershed especially for the non-farmer population which has a much lower average income and assets than do the farmers.

4.7 CONCLUSION

The primary objective of this chapter is to identify variables affecting local people’s adoption behavior, and to estimate their WTP to adopt conservation practices to improve water quality in the Upper Sugar Creek Watershed. Study results show that the most decisive factors are farm size, income, farm succession, and sense of place (P < .05). Results also identified one main barrier of adoption, which is lack
of awareness and knowledge towards stream pollution (P <.01). Analyses of mean willingness to pay demonstrate that farmers were willing to pay $156.95 and $197.57 for scenario 2 and scenario 3, and non-farmers’ WTP were $151.67 and $171.88 respectively.

The secondary objective is to benchmark local people’s attitudes towards water pollution in their neighborhood’s streams, and to estimate the potential possibilities to cooperate with others living within the watershed area to improve it. Study results show that more non-farmers (37%) than farmers (5%) perceived agricultural pollution as a problem. Farmer (56%) concerned more about sedimentation than non-farmers (42%). Both farmers (43%) and non-farmers (57%) did not know much about bacteria pollution in their streams (Figure 2.9). Study results also demonstrate that 60% of respondents were willing to pay a certain amount of money to adopt conservation practices. Among those who were not willing to pay, most of them indicated willingness to take either person (75%) or group actions (66%) to make improvement.
4.8 REFERENCES


Figure 4.1 Rating of water quality in the Upper Sugar Creek

Figure 4.2 Respondent’s attitudes on water pollutants in the Upper Sugar Creek
Figure 4.3 Respondent’s attitudes on chemical pollutant

Figure 4.4 Respondent’s attitudes on sedimentation
Figure 4.5 Respondent’s willingness to pay to adopt conservation practices

Figure 4.6 Attitudes of respondents who indicated unwillingness to pay
Figure 4.7 Reasons of unwillingness to adopt conservation practices
<table>
<thead>
<tr>
<th>Variable</th>
<th>Non-farmer</th>
<th>Farmer</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Survey respondents</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td>Mean=50.85 years, SD=16.05</td>
<td>Mean=44.86 years, SD=18.69</td>
<td>Mean=48.5 years, SD=17.4</td>
</tr>
<tr>
<td>Gender</td>
<td>46.5% female, 53.5% male</td>
<td>43.5% female, 61.8% male</td>
<td>38.82% female, 56.5% male</td>
</tr>
<tr>
<td>Family size</td>
<td>Q1=2, Median=2, Q3=4</td>
<td>Q1=2, Median=2, Q3=4</td>
<td>Q1=1, Median=2, Q3=4</td>
</tr>
<tr>
<td>Race</td>
<td>93.02% white</td>
<td>94.05% white</td>
<td>93.7% white, 2.1% German, 3.2% Swiss, 1% Black</td>
</tr>
<tr>
<td>Marriage status</td>
<td>68.2% married</td>
<td>89.41% married</td>
<td>77% married</td>
</tr>
<tr>
<td>Education</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Primary</td>
<td>2.3%</td>
<td>0%</td>
<td>1.4%</td>
</tr>
<tr>
<td>Middle school</td>
<td>5.4%</td>
<td>1.18%</td>
<td>3.7%</td>
</tr>
<tr>
<td>High school</td>
<td>54.3%</td>
<td>65.88%</td>
<td>58.9%</td>
</tr>
<tr>
<td>College</td>
<td>30.2%</td>
<td>30.59%</td>
<td>30.4%</td>
</tr>
<tr>
<td>Graduate</td>
<td>7.8%</td>
<td>2.35%</td>
<td>5.6%</td>
</tr>
<tr>
<td>Income</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Less than $20K</td>
<td>9.3%</td>
<td>1.23%</td>
<td>6%</td>
</tr>
<tr>
<td>$20K-$39K</td>
<td>22.9%</td>
<td>3.7%</td>
<td>15.1%</td>
</tr>
<tr>
<td>$40K-$59K</td>
<td>21.2%</td>
<td>17.28%</td>
<td>19.6%</td>
</tr>
<tr>
<td>$60K-$79K</td>
<td>31.4%</td>
<td>23.46%</td>
<td>28.1%</td>
</tr>
<tr>
<td>$80K-$89K</td>
<td>9.3%</td>
<td>27.16%</td>
<td>16.6%</td>
</tr>
<tr>
<td>$90K-$99K</td>
<td>3.4%</td>
<td>12.35%</td>
<td>7%</td>
</tr>
<tr>
<td>$100K or more</td>
<td>2.5%</td>
<td>14.81%</td>
<td>7.5%</td>
</tr>
<tr>
<td>Years of living/farming in Sugar Creek watershed area</td>
<td>Mean=18.5 years, SD=14.5 years</td>
<td>Mean=19.41 years, SD=16.49 years</td>
<td>Mean=18.9 years, SD=15.5 years</td>
</tr>
<tr>
<td><strong>N=214</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>81.7% response rate (98.6% early, 1.4% late)</td>
<td>3 9.7% farmers, 60.3% non-farmers</td>
<td></td>
</tr>
</tbody>
</table>

Table 4.1 Socio-demographic characteristics of respondents
<table>
<thead>
<tr>
<th>Variables</th>
<th>Survey respondents</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Years of farming</strong></td>
<td>Mean=19.41 years, SD= 16.49 years</td>
<td></td>
</tr>
<tr>
<td><strong>Farm size</strong></td>
<td>Min=45, Max=1250, Mean=304, SD=92</td>
<td></td>
</tr>
<tr>
<td>Will not happen</td>
<td></td>
<td>4.76%</td>
</tr>
<tr>
<td>Unlikely</td>
<td></td>
<td>15.48%</td>
</tr>
<tr>
<td>Likely</td>
<td></td>
<td>33.33%</td>
</tr>
<tr>
<td>Will definitely happen</td>
<td></td>
<td>26.19%</td>
</tr>
<tr>
<td>Unsure</td>
<td></td>
<td>20.24%</td>
</tr>
<tr>
<td><strong>Farm operator</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sole owner and operator</td>
<td></td>
<td>27%</td>
</tr>
<tr>
<td>Operator only</td>
<td></td>
<td>4%</td>
</tr>
<tr>
<td>Rent from others</td>
<td></td>
<td>21%</td>
</tr>
<tr>
<td>Rent to others</td>
<td></td>
<td>13%</td>
</tr>
<tr>
<td>Partnership with non-family</td>
<td></td>
<td>12%</td>
</tr>
<tr>
<td>Partnership with spouse and family members</td>
<td></td>
<td>21%</td>
</tr>
</tbody>
</table>

Table 4.2 Descriptive data of land tenure attributes of respondents

<table>
<thead>
<tr>
<th></th>
<th>WTP</th>
<th>Bacteria</th>
<th>Chemicals</th>
<th>Pesticides</th>
<th>Sedimentation</th>
<th>Awareness of stream pollution</th>
</tr>
</thead>
<tbody>
<tr>
<td>WTP</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bacteria</td>
<td>0.165*</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chemicals</td>
<td>0.130</td>
<td>0.464**</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pesticides</td>
<td>0.114</td>
<td>0.307**</td>
<td>0.403**</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sedimentation</td>
<td>0.155*</td>
<td>0.245**</td>
<td>0.326**</td>
<td>0.259**</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Awareness of stream pollution</td>
<td>0.054*</td>
<td>0.065</td>
<td>0.101</td>
<td>0.068</td>
<td>0.154*</td>
<td>1</td>
</tr>
</tbody>
</table>

**. Correlation is significant at the 0.01 level (2-tailed).
*. Correlation is significant at the 0.05 level (2-tailed).

Table 4.3 Correlations of respondents’ WTP and their attitudes on water pollutants
<table>
<thead>
<tr>
<th></th>
<th>Adoption</th>
<th>Farming years</th>
<th>Farm succession</th>
<th>Farm size</th>
<th>Income</th>
<th>Education</th>
<th>Gender</th>
<th>Age</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adoption</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Farming years</td>
<td>0.076*</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Farm succession</td>
<td>0.73**</td>
<td>0.14</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Farm size</td>
<td>0.607**</td>
<td>0.226</td>
<td>0.511</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Income</td>
<td>0.599**</td>
<td>0.096</td>
<td>0.571**</td>
<td>0.654**</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Education</td>
<td>0.197</td>
<td>0.100</td>
<td>0.250*</td>
<td>0.071</td>
<td>0.227*</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gender</td>
<td>-0.092</td>
<td>-0.162</td>
<td>0.072</td>
<td>0.071</td>
<td>0.069</td>
<td>0.045</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td>-0.206</td>
<td>0.516**</td>
<td>-0.267*</td>
<td>-0.056</td>
<td>-0.209</td>
<td>-0.110</td>
<td>-0.160</td>
<td>1</td>
</tr>
</tbody>
</table>

**. Correlation is significant at the 0.01 level (2-tailed).
*  . Correlation is significant at the 0.05 level (2-tailed).

Table 4.4 Correlations of adoption, land tenure, and socio-demographic variables
<table>
<thead>
<tr>
<th>Dependent variable : Adoption</th>
</tr>
</thead>
<tbody>
<tr>
<td>Independent variable</td>
</tr>
<tr>
<td>Constant</td>
</tr>
<tr>
<td>Farm size</td>
</tr>
<tr>
<td>Farm succession</td>
</tr>
<tr>
<td>Farming years</td>
</tr>
<tr>
<td>Income</td>
</tr>
<tr>
<td>Age</td>
</tr>
<tr>
<td>Gender</td>
</tr>
<tr>
<td>Education</td>
</tr>
</tbody>
</table>

**R-Sq=63.10%**

**. Correlation is significant at the 0.01 level (2-tailed).
*. Correlation is significant at the 0.05 level (2-tailed).

Table 4.5 Regression of adoption on land tenure and socio-demographic variables
<table>
<thead>
<tr>
<th>Scenario</th>
<th>Amount</th>
<th>Percentage of those responding ‘yes’</th>
</tr>
</thead>
<tbody>
<tr>
<td>Scenario 1</td>
<td>$0</td>
<td>45</td>
</tr>
<tr>
<td>(Keep current condition)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Scenario 2</td>
<td>$50</td>
<td>14.3%</td>
</tr>
<tr>
<td></td>
<td>$100</td>
<td>19.0%</td>
</tr>
<tr>
<td></td>
<td>$150</td>
<td>12.7%</td>
</tr>
<tr>
<td></td>
<td>$200</td>
<td>17.5%</td>
</tr>
<tr>
<td></td>
<td>$250</td>
<td>6.3%</td>
</tr>
<tr>
<td></td>
<td>$300</td>
<td>15.9%</td>
</tr>
<tr>
<td></td>
<td>$350</td>
<td>0.0%</td>
</tr>
<tr>
<td></td>
<td>$400</td>
<td>9.5%</td>
</tr>
<tr>
<td>(Make a one-time payment to meet EPA standards in 3 years)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Scenario 3</td>
<td>$100</td>
<td>4.4%</td>
</tr>
<tr>
<td></td>
<td>$200</td>
<td>21.8%</td>
</tr>
<tr>
<td></td>
<td>$300</td>
<td>27.4%</td>
</tr>
<tr>
<td></td>
<td>$400</td>
<td>13.0%</td>
</tr>
<tr>
<td></td>
<td>$500</td>
<td>29.1%</td>
</tr>
<tr>
<td></td>
<td>$600</td>
<td>4.4%</td>
</tr>
<tr>
<td>(Make a one-time payment to Meet EPA standards in 5 years)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 4.6 Payment-card method results (total N=214)
<table>
<thead>
<tr>
<th>Scenario 2</th>
<th>Scenario 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Make a one-time payment of $5 or more to meet EPA standards in 3 years</td>
<td>Make a one-time payment of $50 or more to meet EPA standards in 5 years</td>
</tr>
<tr>
<td>Parameter</td>
<td>Std. Error</td>
</tr>
<tr>
<td>Dependent variable (WTP for non-farmers)</td>
<td></td>
</tr>
<tr>
<td><strong>Independent variables</strong></td>
<td></td>
</tr>
<tr>
<td>Intercept (constant)</td>
<td>25.56</td>
</tr>
<tr>
<td>Income</td>
<td>46.28**</td>
</tr>
<tr>
<td>Age</td>
<td>-1.42</td>
</tr>
<tr>
<td>Gender</td>
<td>-6.91</td>
</tr>
<tr>
<td>Education</td>
<td>28.9</td>
</tr>
<tr>
<td>Years of living at current watershed</td>
<td>1.53*</td>
</tr>
</tbody>
</table>

R-Sq=12.90%
N=129

**. Correlation is significant at the 0.01 level (2-tailed).
*. Correlation is significant at the 0.05 level (2-tailed).

Table 4.7 Regression results of willingness to pay of non-farmers
<table>
<thead>
<tr>
<th>Scenario 2</th>
<th>Scenario 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Make a one-time payment of $5 or more to meet EPA standards in 3 years</td>
<td>Make a one-time payment of $50 or more to meet EPA standards in 5 years</td>
</tr>
<tr>
<td>Parameter</td>
<td>Std. Error</td>
</tr>
<tr>
<td>-----------</td>
<td>------------</td>
</tr>
<tr>
<td>Intercept (constant)</td>
<td>11.26</td>
</tr>
<tr>
<td>Years of farming</td>
<td>1.05*</td>
</tr>
<tr>
<td>Farm succession</td>
<td>5.82**</td>
</tr>
<tr>
<td>Farm size</td>
<td>0.05**</td>
</tr>
<tr>
<td>Age</td>
<td>-0.42</td>
</tr>
<tr>
<td>Gender</td>
<td>-2.39</td>
</tr>
<tr>
<td>Education</td>
<td>20.82</td>
</tr>
<tr>
<td>Income</td>
<td>17.83**</td>
</tr>
</tbody>
</table>

R-Sq=12.10%

N=86

**, Correlation is significant at the 0.01 level (2-tailed).

*, Correlation is significant at the 0.05 level (2-tailed).

Table 4.8 Regression results of mean willingness to pay of farmers
APPENDIX A: SURVEY QUESTIONNAIRE
Identification and Removal of Fecal Contaminants in Upper Sugar Creek Watershed

SURVEY QUESTIONNAIRE

Let’s keep improving Sugar Creek, so our kids can safely play in the streams

119 A Williams Hall
1680 Madison Ave. Wooster, OH 44691
http://sugarcreekmethod.osu.edu/
Dear Watershed Resident,

We would like to invite you to participate in a survey of your views on the Upper Sugar Creek water quality. The survey is about attitudes and awareness of bacteria in your neighborhood streams and conservation measures. It will take you a maximum of 30 minutes to fill out the questionnaire.

As you may know our project started in 2000 in the Upper Sugar Creek surrounding Smithville when our researchers at OARDC teamed up with the Smithville area farmers (Upper Sugar Creek Farmer Partners). Since 2000 we have sampled the Sugar Creek every other week at 21 sites focusing on Nitrates and Phosphorus. In 2008 we started to evaluate the bacteria concentration (see page 8). During the summer of 2009 using community youth from this area, we surveyed each small stream using the Headwater Habitat Evaluation Index to better learn how to improve water quality (see page 9). Now we would like to extend the research and to explore more about your ideas how to use conservation measures to reduce bacteria in streams.

Efforts by our community to reduce pollution have lowered our average nitrate level at the mouth of Upper Sugar Creek, located south of Orrville, from 9 mg/l to 6 mg/l--a substantial decrease. Thank you!! Our goal is to continue to improve the water quality and the quality of our lives since we all depend on water.

If you would like to participate in this study, please fill out the questionnaire (please note that you do not need to answer all of the questions). We will return to pick it up within 2 days. Thanks again for your support. If you have questions or need further information regarding this study, please do not hesitate to contact me at (330)202-3538.

Sincerely,

Richard Moore
Director, Environmental Science Graduate Program/OSU
Sugar Creek Research Project Team Leader
Tel: (330)202-3538
Email: moore.11@osu.edu
Section 1: Your Watershed Streams

1. Overall, how would you rate the water quality in your neighborhood streams?
   □ Poor  □ Fair  □ Average  □ Good  □ Excellent  □ I don’t know

2. Below is a list of different types of water pollutants that are generally present in water bodies to some extent. In your opinion, how much of a problem are the following pollutants in the Upper Sugar Creek area (upstream and downstream of Smithville)?

<table>
<thead>
<tr>
<th>Pesticides</th>
<th>Not a problem</th>
<th>Slight Problem</th>
<th>Moderate Problem</th>
<th>Severe Problem</th>
<th>Don’t Know</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>Chemicals (Nitrogen, Phosphorus, etc)</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>Bacteria</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>Very muddy water</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td></td>
</tr>
</tbody>
</table>

3. What are your major concerns right now about your neighborhood streams and the area surrounding them? Please check all that apply

   Legal concern of being regulated by EPA or other government agency
   □
   Maintaining adequate drainage
   □
   Stream flooding onto agricultural lands
   □
   Well water pollution and groundwater pollution
   □
   High drinking water treatment costs
   □
   Pasture and crop field runoff
   □
   Pets or farm animals’ health threatened by coming in contact with pollutants in streams
   □
   Children’s health threatened by coming in contact with pollutants in streams
   □
   Reduced beauty of lakes or streams
   □
   Reduced opportunities for water recreation
   □
   Loss of desirable fish species and habitats for them
   □
   Pollution from industry
   □
   Pollution from sewage systems
   □
   Stream bank stability
   □
   Other, please specify
   □
Section 2: Your Septic Systems

4. What sewage system do you use to treat wastewater discharged from your house?
   □ Traditional soil absorption
   □ Alternative leach field
   □ Mound system
   □ Sand bioreactor
   □ I don’t know
   □ Other, please specify

5. It requires at least 4 of soil depth to build a leach field or 2 feet of soil depth for a mound system. Do you know the soil depth where you built your septic system?
   □ Yes, __________ Feet
   □ No
   □ I don’t know

6. Is your sewage system required to be inspected regularly by Wayne County Health Department?
   □ Yes
   □ No
   □ I don’t know

7. Where does your septic system outflow end up?
   □ Stream
   □ Soil
   □ Rock bed
   □ Sand
   □ Farmer’s field
   □ I don’t know
   □ Other, please specify

8. How often do you inspect your septic system?
   □ Twice a year
   □ Once a year
   □ Every 2-3 years
   □ Every 3-4 years
   □ Less often than every 4 years
   □ Never
   □ I don’t know

9. Check all maintenances you have done to your septic system in past 2 years.
   □ Flush lateral lines
   □ Pump septic tank
   □ Switch to alternative leach field
   □ Maintain or replace pump
   □ Collect and analyze effluent
   □ Haven’t done anything
   □ Other, please specify

10. Who maintains your septic systems?
    Please check all that apply
    □ Skilled operators
    □ Community technicians
    □ Wayne County Health Department
    □ My family members
    □ No one
    □ Friends
    □ Other, please specify

11. How much does it cost you to maintain your septic system each year?
    $ __________ per year
    □ I don’t know

12. Have you seen any of following signs of your septic system in the past two years?
    □ Sewage backs up into your home
    □ Slow running drains and toilets
    □ Sewage in the yard
    □ Standing water over the tank
    □ Foul odors in your home or yard
    □ Lush plant growth that appears near the septic tank or drain field
    □ Other, please specify

13. How do you evaluate the function of your septic system?
    □ Very good
    □ Somewhat good
    □ Average
    □ Fair
    □ Probably failing
    □ I don’t know
### Section 3: Stream Use of the Upper Sugar Creek Watershed

14. Regarding the part of the Sugar Creek that goes through Smithville Park and your property, please check all of your main areas of stream use below.

<table>
<thead>
<tr>
<th>Activity</th>
<th>Never</th>
<th>Very Rarely</th>
<th>Rarely</th>
<th>Occasionally</th>
<th>Very Frequently</th>
</tr>
</thead>
<tbody>
<tr>
<td>Children playing in Smithville Park</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>Children playing in or along stream closest to your house</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>Children playing in or along neighborhood streams</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>Pets playing in or along the stream</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>Farm animals drinking from the stream</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>Use of water for agricultural purposes (e.g. irrigation)</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>Use of water for drinking (e.g. well)</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>Picnicking along the stream</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>Camping/campfires along the stream</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>Hiking or walking along the stream</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>Picking berries, collecting nuts or mushrooms</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>School, church, or other non-profit expeditions or outings</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>Fishing or catching minnows or frogs in the stream</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>Bird-watching along the stream</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>Hunting along the stream</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
</tr>
</tbody>
</table>

Other, please specify
**Section 4: Practices to Improve Water Quality**

Our preliminary survey shows the following six conservation practices that are most commonly used in the Upper Sugar Creek Watershed. They have the potential to reduce bacteria in the water in your area.

a) Buffers (riparian, lawn, forest, natural vegetation buffers)
b) Use of cover and green manure crops
c) Exclusion fencing (keeping livestock out of streams)
d) Grassed waterways
e) Animal waste utilization
   Biogas
f) Animal waste utilization
   Manure storage

15. Please circle the conservation practices you have used or will use. Check all that apply

<table>
<thead>
<tr>
<th>Practice</th>
<th>Doesn’t apply</th>
<th>Currently use it</th>
<th>Tried but no longer use it</th>
<th>Would you be willing to try or continue using this practice?</th>
</tr>
</thead>
<tbody>
<tr>
<td>a. Grass waterways</td>
<td></td>
<td></td>
<td></td>
<td>Yes</td>
</tr>
<tr>
<td>b. Buffers (e.g. riparian, lawn, forest, vegetation buffers)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>c. Use of cover and green manure crops</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>d. Exclusion fencing (keeping livestock out of streams)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>e. Manure storage</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>f. Biogas</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Bacteria Test Results of Upper Sugar Creek Watershed

20 out of 21 sites exceed Ohio recreational water quality standards

Not recommended for recreational use

You are welcome to keep this page
Primary Headwater Habitat Evaluation Index (HHEI) of the Upper Sugar Creek Watershed

Class III streams indicate healthy streams. They tend to contain species of animals that have adapted to the year around presence of cool water, such as two-line salamander or certain fish species. They are in the highest quality primary headwater habitat streams.

Class II streams are warm water streams, and tend to contain less diversity than class III streams.

Class I streams are often dry for long periods of time with no aquatic animal species present.

HHEI results show that most Class III streams center between Smithville and Orville where most two-line salamanders were found.

You are welcome to keep this page
16. How much do you trust each of the following agencies or organizations working in your community?

<table>
<thead>
<tr>
<th>Agency/Program</th>
<th>Do not trust</th>
<th>Somewhat distrust</th>
<th>Neutral</th>
<th>Somewhat trust</th>
<th>Trust very much</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ohio Environmental Protection Agency (OEPA)</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>Ohio Department of Agriculture</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>Ohio Department of Natural Resources (ODNR)</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>Ohio Farm Bureau</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>Wayne Soil and Water Conservation District</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>Wayne County Health Department</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>Ohio State University Extension</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>Ohio Agricultural Research Development Center (OARDC)</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>Sugar Creek Farmer Partners living near Smithville</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
</tr>
</tbody>
</table>

17. When you make decisions about new management practices, what are your major concerns? Please check all that apply

- [ ] Personal out-of-pocket expenses
- [ ] Availability of government funds for cost-share
- [ ] Amount of time required for implementation
- [ ] Cost to maintain
- [ ] Requirements or restriction of government program
- [ ] Complexity of the practices
- [ ] Concerns about reduced yields
- [ ] Whether I am familiar with the practice
- [ ] My own views about effective land management methods
- [ ] Whether or not my neighbors adopt
- [ ] Whether people I know are implementing the practice
- [ ] Whether it will interfere with my flexibility to change land use practices as needed
- [ ] Uncertainty about environmental benefits of the new practice
- [ ] Other, please specify
18. How much are you willing to pay to apply conservation practices along your neighborhood streams in order to improve the water quality? Please circle the highest amount you are willing to pay. Please check only one scenario

☐ Scenario 1

<table>
<thead>
<tr>
<th>I would like</th>
<th>keep Current Condition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Note: presently 20 out of 21 sampling sites have bacteria over the Ohio EPA standards of 235/100ml</td>
<td></td>
</tr>
</tbody>
</table>

☐ Scenario 2

| I would like to make one-time payment of |
|------|------|------|------|------|------|------|------|------|
| $5 | $50 | $100 | $150 | $200 | $250 | $300 | $400 | $400 |

To improve water quality to the level that the bacteria count will be less than Ohio EPA standards in 3 years.

To make sure children and pets playing in neighborhood streams or Smithville Park play without danger or to make sure animals drink in streams without danger.

☐ Scenario 3

| I would like to make one-time payment of |
|------|------|------|------|------|------|------|------|------|
| $50 | $100 | $150 | $200 | $250 | $300 | $400 | $500 | $600 | $600 |

To improve water quality to the level that the bacteria count will be less than Ohio EPA standards in 5 years.

To make sure children and pets playing in neighborhood streams or Smithville Park are without danger and to make sure pets and animals can drink the water in the stream without danger.
19. How much are you willing to pay to have your septic system be inspected each year in order to reduce bacteria concentration in your neighborhood streams? Please check only one box.

<table>
<thead>
<tr>
<th>I would like to pay</th>
<th>to keep my septic system in its current condition</th>
<th>Level of Management</th>
</tr>
</thead>
<tbody>
<tr>
<td>$0</td>
<td>to have my soil absorption or alternative leaching system be inspected each year</td>
<td>No more inspection</td>
</tr>
<tr>
<td>$30-50/year</td>
<td>to have my mound system be inspected each year</td>
<td>One inspection per year</td>
</tr>
<tr>
<td>$100-150/year</td>
<td>to have my sand bioreactor system be inspected each year</td>
<td>Two inspections per year</td>
</tr>
<tr>
<td>$250-300/year</td>
<td>Two inspections per year</td>
<td>Flush lateral lines</td>
</tr>
<tr>
<td></td>
<td>Pump septic tank as needed</td>
<td>Maintain &amp; replace pump</td>
</tr>
</tbody>
</table>

20. Potential possibility to collaborate to improve water quality of the Upper Sugar Creek

a. Are you aware that the OEPA has established a Bacterial Total Maximum Daily Load for the Upper Sugar Creek Watershed?
   - Yes
   - No

b. Have you ever attended conservation sessions offered by the OARDC, OSU Extension, or Wayne SWCD?
   - Yes
   - No

c. Are you interested in knowing the bacteria concentration levels of your own or your neighborhood streams on a regular basis?
   - Yes
   - No

d. Would you be interested in working on an individual basis to make environmental improvements with streams on your property?
   - Yes
   - No

e. Would you be interested in forming a small group with your immediate neighbors to improve the stream quality based on your own local priorities?
   - Yes
   - No

f. Are you a member of the Upper Sugar Creek Farmer Partners?
   - Yes
   - No
   - I don’t know

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Section 6: About Your Farm Land

Section 5 questions are for farm families, skip to section 6 if you are not farming family

21. Which of the following best describes your position as a farm operator?

☐ Sole owner and operator
☐ Operator only
☐ Partnership with spouse
☐ Partnership with family members
☐ Partnership with non-family members
☐ Rent farmland to others
☐ Rent farmland from others
☐ Farm manager
☐ Limited liability corporation
☐ Other

22. How many years have you been farming at your current location? ________

23. How likely is it that any family member may continue farming when you retire or quit farming?

☐ Will not happen
☐ Unlikely
☐ Likely
☐ Will definitely happen
☐ Unsure

24. Please indicate your farm type and size that you manage (include rented acreage).

Corn ________ acres
Soybeans ________ acres
Small grains ________ acres
Canning crops ________ acres
Clover ________ acres
Pasture ________ acres

Please list the average herd size, if you have any livestock.

☐ Dairy cattle
☐ Beef cattle
☐ Hogs
☐ Poultry
☐ Other livestock

25. Are your pets or farm animals kept from entering the drainage area?

☐ Yes
☐ No
☐ I don’t know

26. Do you spread manure on your soil?

☐ Yes
☐ No

27. What time of the year do you spread manure? Please check all that apply

☐ Winter
☐ Spring
☐ Summer
☐ Autumn

28. Do you use a cover crop?

☐ Yes
☐ Yes, but no manure was applied
☐ No

29. Is manure treated or composted before being applied to the field?

☐ Yes
☐ No

30. What is the form of manure spread on the field?

☐ Liquid manure
☐ Manure with bed pack
☐ Piled manure without bed pack
☐ Other, please specify

31. How do you apply manure to land?

☐ Applied to land and left alone
☐ Applied and immediately tilled into the soil
☐ Knifed in
☐ Other, please specify