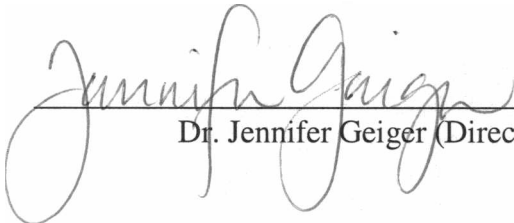


*Water Quality Survey of Nitrate Levels and Coliform/Coliphage Presence
in the Helena Valley Compared with Helena City Water*


Submitted in partial fulfillment of the requirements for graduation with honors from the
Department of Natural Sciences at Carroll College, Helena, MT

Emma Layton Swingle
April 4, 2005

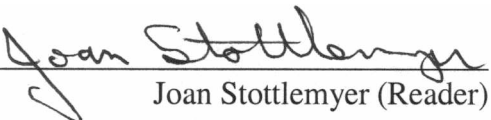
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TABLE OF CONTENTS

Acknowledgements	iii
Abstract.....	v
List of Tables.....	vi
List of Figures.....	vii
Introduction.....	1
Materials and Methods.....	5
Results.....	10
Discussion.....	14
Literature Cited.....	19

Abstract

The well water of the Helena Valley has shown several significant problems in recent years. High nitrate levels and/or the presence of coliform and coliphage have been identified in numerous wells. These problems are likely the result of a combination of faulty sewage systems and regional agriculture. To effectively monitor and evaluate problem wells, data were collected and then statistically compared from thirteen Helena Valley wells with nine city water samples. The highly monitored city water contained minimal nitrate concentrations and no microbial contamination while the Helena Valley samples showed great diversity in contamination levels. Additionally, no correlations were evident between high nitrate levels and bacterial or viral presence in Helena Valley wells. Larger, more detailed studies of the region are needed to continue monitoring problem wells and to search for links between coliform and coliphage presence and nitrate concentration.

List of Tables

Table 1. Abbreviated sampling results.11

List of Figures

Figure 1. Map of well collection sites in the Helena Valley.....	8
Figure 2. Map of city well sites, Helena, MT.....	9
Figure 3. Comparison of nitrate levels v. well depth.....	12
Figure 4. Comparison of the average nitrate levels in the Helena Valley and Helena city water.....	12
Figure 5. Comparison of nitrate levels and coliform positive samples.....	13

Introduction

Diseases potentially transmitted through ground water include Shigellosis, Giardiasis, Cryptosporidiosis, Gastroenteritis, and other gastro-intestinal illnesses as well as a variety of viral pathogens (Madigan et al., 2002). In fact, between 1991 and 1996 “126 outbreaks, 429,021 cases of illness, 653 hospitalizations, and 58 deaths were reported in public and individual water systems in 41 states and three U.S. territories” (Craun, 2002). The transmission of water-borne organisms occurs when the waste of an infected human or animal is able to enter a water supply and infect other people through a fecal/oral route (Madigan et al., 2002). Most water-borne diseases are preventable as long as sewage and waste are separated from sources of drinking water and proper treatment of water systems occurs (The Center for Disease Control, 2003). On a global scale, outbreaks often occur in underserved and developing regions (The Center for Disease Control, 2003). However, these problems persist even within the borders of the United States and pose a serious problem to a nation in which nearly half the citizens depend on ground water as their source for drinking water (Sun, 1986).

High levels of nitrates may also compromise ground water quality. Although most healthy adults tolerate elevated nitrate levels, such levels may interfere with oxygen binding in infants in a condition known as “blue baby syndrome,” or methemoglobinemia (Mitchell and Harding, 1996). Additionally, studies have indicated that nitrates may be carcinogenic (Tsezou et al., 1996) and related to several high-risk conditions during pregnancy (Tabacova et al., 1997). Other possible health risks associated with high nitrate levels have been suggested, but most of these have not been substantiated

(Swistock et al., 1993). Nitrates may enter groundwater through the improper disposal of animal waste, incorrectly installed or maintained septic systems, or through the overuse of nitrogen-based fertilizers (Swistock et al., 1993). This agricultural contamination may take years to appear due to the time necessary for the nitrates to leach through the soil to the groundwater table (Burkart, 2004).

To combat the inherent danger created by enteric pathogens and high nitrate levels in ground waters the EPA adopted a maximum contaminant level of zero for coliform and coliphage and less than 10 mg/L for nitrates (USEPA, 2004). Coliform and the viruses that infect them are not pathogenic to humans, but they serve as indicators of fecal contamination due to agricultural or septic pollution (USEPA, 2004). It is assumed that if pathogens of similar structure were introduced into a water supply, they could be transmitted in the same manner as coliform and coliphage (USEPA, 2004).

According to Kathy Moore (pers. comm.) of the Water Quality Protection District of the Lewis and Clark County Health Department, there are about 8,000 wells within the 252 square miles that make up the Helena Valley. Each year nearly 350 wells are added to the area due to continued regional expansion and development (Montana Bureau of Mines and Geology, 2005). As part of the normal monitoring of ground water systems in the Helena Valley, the Lewis and Clark County Health Department has noted several widespread water quality problems in recent years (K. Moore, pers. comm.). High levels of nitrate have been observed at several Helena Valley locations without readily identifiable causes, and previous monitoring has revealed numerous wells in the Helena Valley that have tested positive for coliform and coliphage (K. Moore, pers comm.).

Current laws require public notification if wells exceed the minimum coliform allowance for the number of people served by the system or if wells test positive for coliform more than one time in a month. Over a longer time frame, sanitary surveys must be collected every five or ten years depending on the size and use of the system (USEPA, 2000). Monitoring of existing wells allows state and county health officials to evaluate the practicality and cost-effectiveness of new groundwater rulings before they are implemented. Additionally, studies that examine the levels of nitrates, coliform, and coliphage in the Helena Valley assist in the management of current problem wells and aid in future development and planning.

The monitoring of wells in the Helena Valley is especially important at this time because the U.S. Environmental Protection Agency may institute stringent new requirements regarding the quality of private wells (Kilbreath et al., 2004). If the Ground Water Rule (GWR) is implemented, these regulations will require strict monthly monitoring of wells for eight different groundwater components including the following: “hydrologic assessments” of wells that could be contaminated by fecal matter, “source water monitoring” of potentially problematic wells, detailed well treatment programs for those facilities that have demonstrated previous fecal contamination, and supervision to ensure that the necessary treatment methods are conducted properly (USEPA, 2000).

For those wells that have already demonstrated water quality problems, the United States Environmental Protection Agency (2004) recommends several treatment methods. Initially, it is essential to determine and control the source of the contamination. Filtration and treatment systems are often effective options. Chlorination is able to kill

most bacteria, but it cannot eliminate chemical contaminants (Sun, 1986). If a well is seriously compromised, often it is more cost-effective for an owner to simply construct a new well or find an “alternative water source” (USEPA, 2000). Fortunately, when ground water has been contaminated by heavy fertilizer use, nitrate readings will usually return to previous levels due to natural reclamation processes and are not hindered by the presence or absence of domestic animals on the land (Comis, 2004).

To effectively examine the water quality in the wells of the Helena Valley, a secondary water source was used for comparison. Strict monitoring and treatment of public water systems should ensure that city residences have low nitrate levels and no coliform or coliphage present. Water provided by the city of Helena undergoes a stringent process that includes a polymerization procedure to remove sediment, filtration using coal and silicate, and chlorination (Hahn et al., 2003). Alternatively, ground water in the Helena Valley does not endure the same processing and water from wells in this area has previously demonstrated numerous water quality problems (see above; K. Moore, pers. comm.). Therefore, the present study was designed to investigate the differences in levels of nitrates as well as differences in the occurrence of coliform and coliphage between city water sources and well water sources from the Helena Valley. Because of the differences in water treatment between the two groups, I hypothesized that samples taken from city water sources would show significantly lower levels of nitrates and less frequent presence of coliform or coliphage than those taken from the Helena Valley.

Materials and Methods

Locations Sampled:

To accurately sample the water quality of the Helena Valley, it was necessary to include sampling sites that spanned the entire region (Figure 1). Eight private wells and five public wells were selected and sampled three to four times in a three-month period (see Table 1 for sampling amounts). Before the initial sampling, each well was evaluated based on the land usage and geological setting. The land usage at most sites was characterized by rural subdivisions surrounded by small agricultural plots of land primarily used for grazing cattle and raising crops such as hay and alfalfa. All selected private and public wells were geologically classified as alluvial aquifers with the exception of one well located in bedrock (Figure 1). Additionally, samples were collected from nine city water sites using similar methods to those used to sample private wells. All city water collections were made at private residences dispersed widely through the area served by the public system (Figure 2).

Collection Procedures:

All collection procedures corresponded with standard Montana Department of Environmental Quality and Lewis and Clark County Health Department protocol. Before samples were collected at private wells, the well depth was measured and recorded. A depth was not recorded for city water or public wells. In both public and private wells, the system was flushed for at least five minutes to ensure a standard pH and electrolyte reading. When the system stabilized, the faucet was sterilized using a 95% ethanol

solution. An additional minute of flushing ensured that all traces of ethanol were removed. Next, sterile containers were used to collect the samples from the faucet. After the collection was complete, all samples were kept at about 1 degree Celsius, and the necessary microbial tests were performed immediately upon return to the laboratory to ensure specimen integrity.

Sample Processing:

Coliform samples were tested using the techniques described in the EPA method 1604 (USEPA, 2002). Procedural changes included the following: quality and control procedures were not followed in the interest of conserving time and materials. Instead of being cultured in MI broth, samples were grown in both M-FC and M-endo broths. Colonies on these media were counted in natural light without the aid of a florescent lamp as specified for the MI media. Although section 11.5 describes the use of sterile dilution water, this procedure was omitted. Additionally, between samples filters were autoclaved or treated with 95% ethanol because a germicidal UV light box was not available.

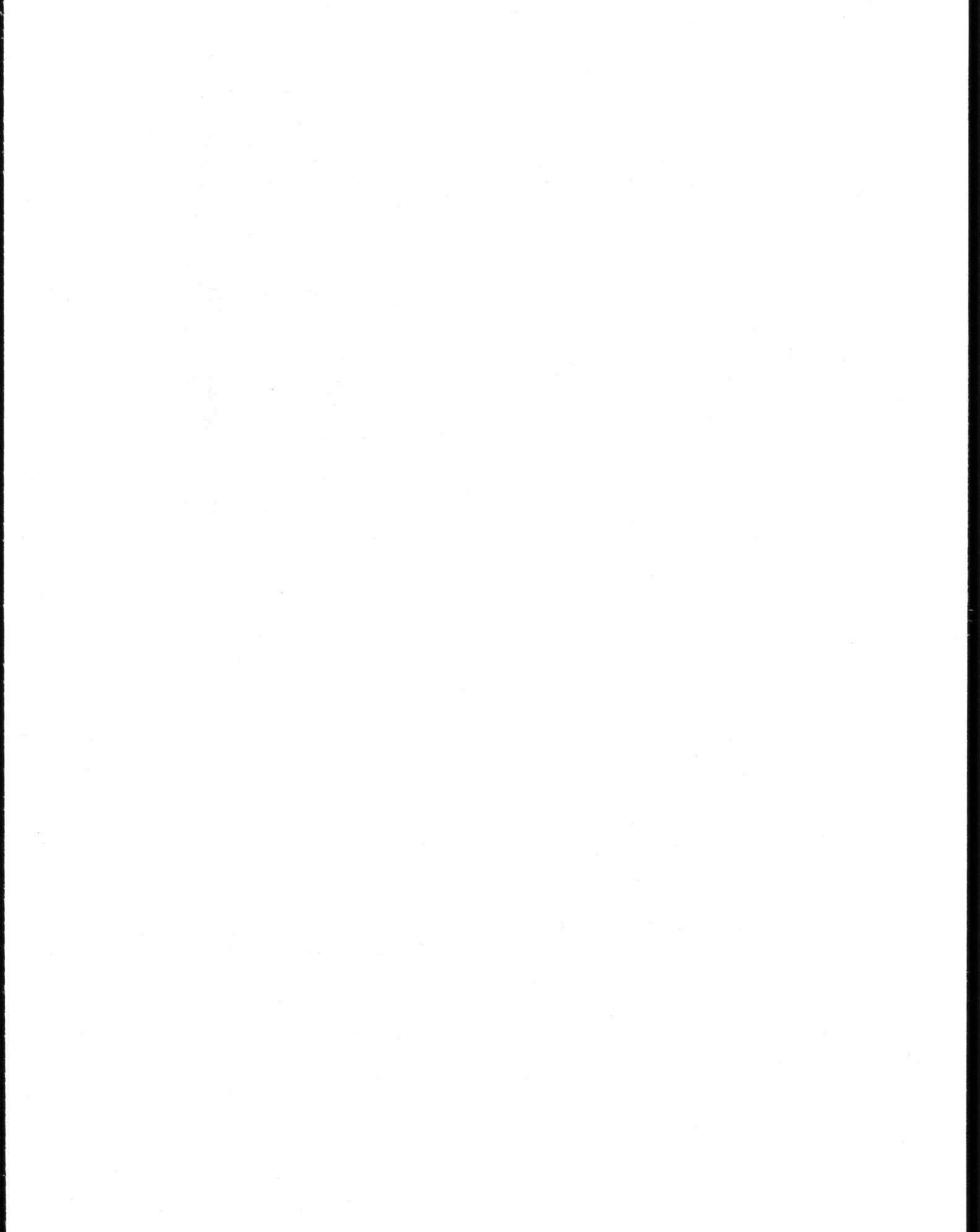
Coliphage samples were processed in accordance with the EPA laboratory method 1601 with the following changes (USEPA, 2001): quality control procedures were omitted again. Additionally, 500 mL samples were collected instead of 100 mL or 1000 mL as suggested. All added substrates were adjusted accordingly. Sections 12.1.2.8 and 7.3.1.2 suggest using stock nalidixic acid in plates and media for somatic cultures, but stock streptomycin and ampicillin were used instead. Finally, spread plating of the host

organism was used instead of the top-agar procedure described in the EPA document.

All nitrate samples were processed at Energy Labs, a local commercial laboratory, using EPA method 353.2.

Data analysis:

Each well was classified by its average nitrate levels as a high, medium, or low nitrate containing well. Wells with nitrate levels below three mg/L were classified as low nitrate wells; wells with levels between three and eight mg/L were classified as medium nitrate wells; and wells showing greater than eight mg/L were considered high nitrate wells. Each classification was compared to the number of coliform positives for the samples included in that category. Additionally, well depth was correlated with the average nitrate levels of each well. Finally, a T-test was used to determine whether the nitrate levels in the city and valley were statistically different. All data analysis was completed using *Statistica* and *Excel* software programs.



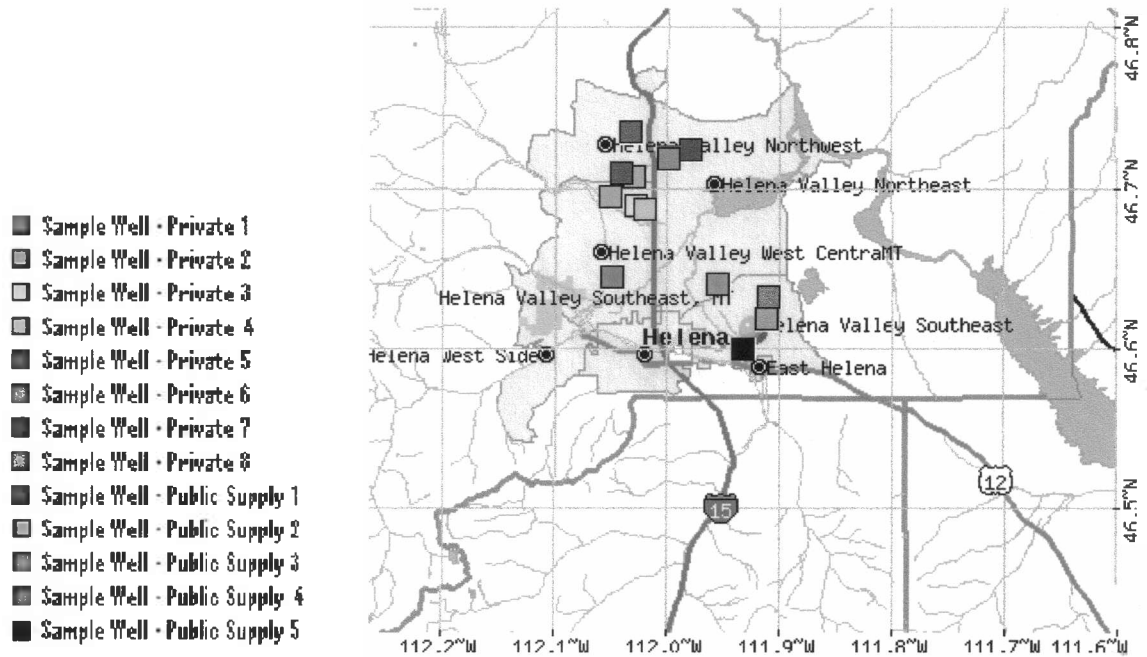
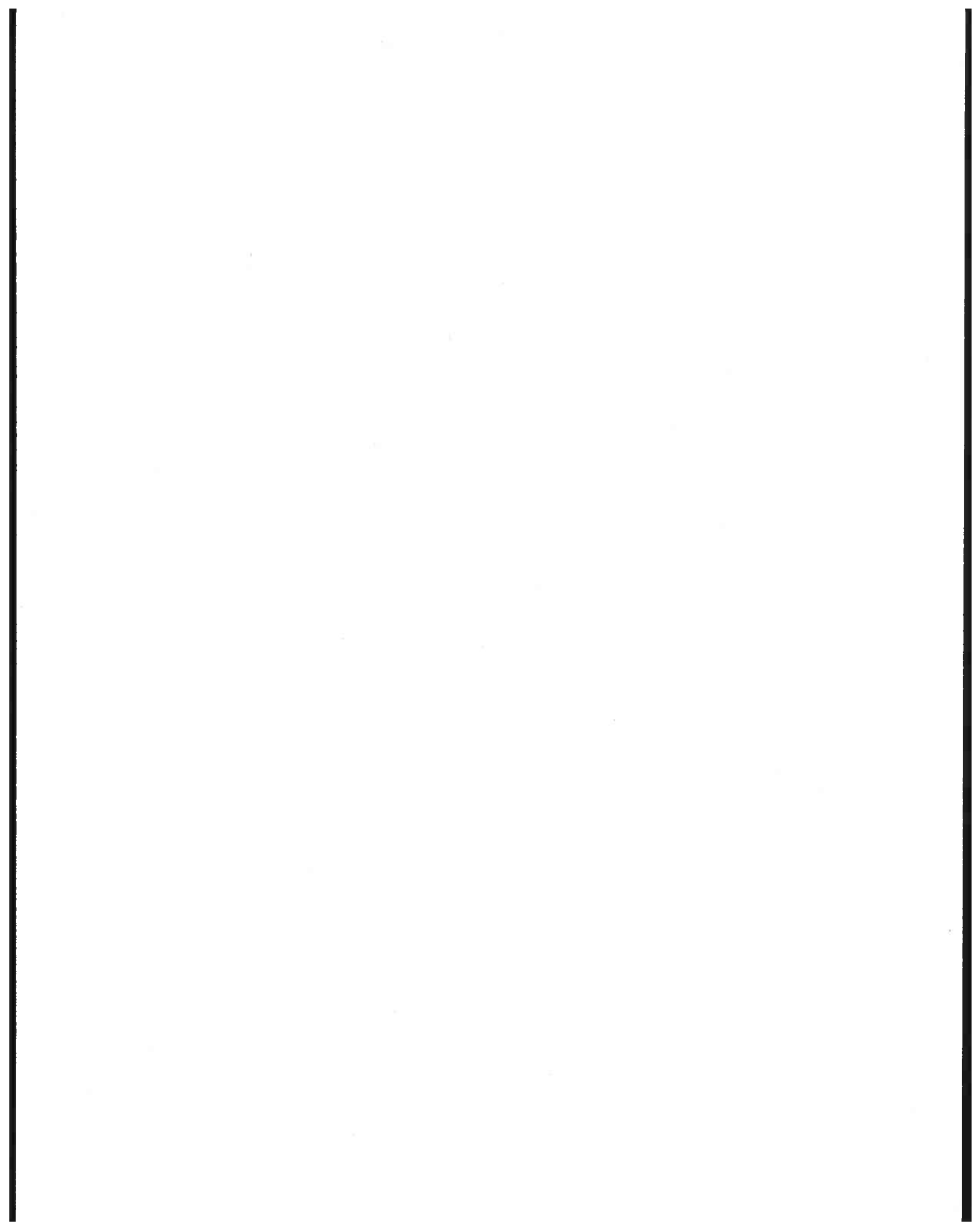


Figure 1. Map of well collection sites in the Helena Valley (adapted from <http://maps.yahoo.com>)



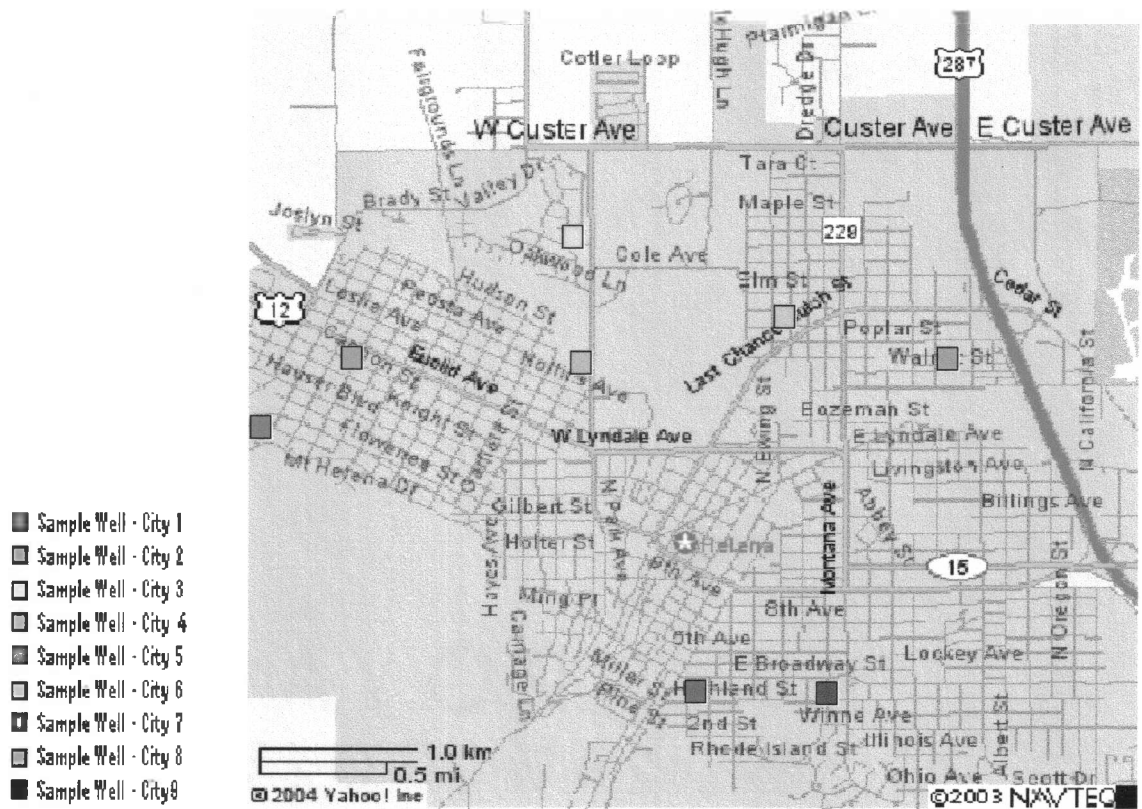
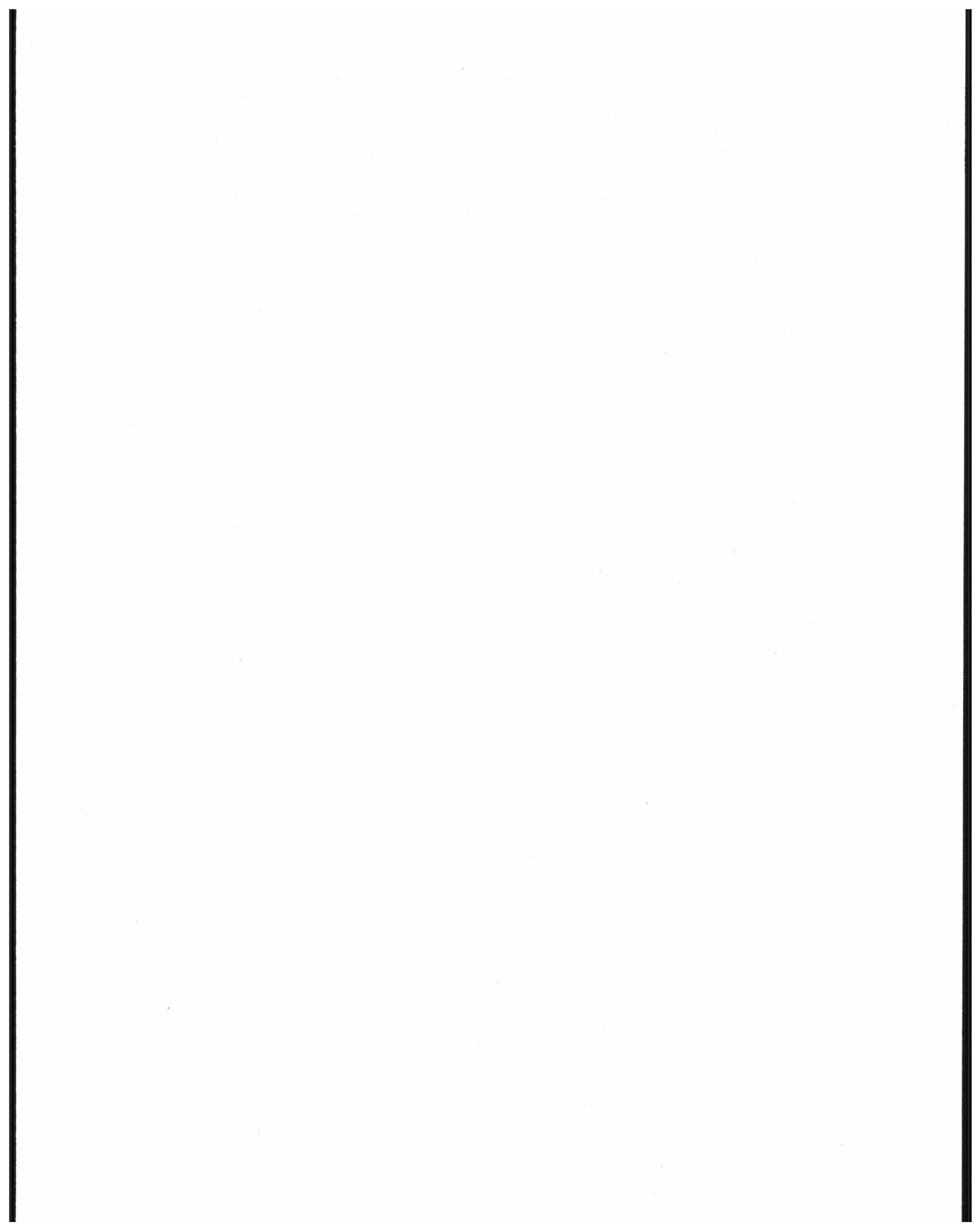


Figure 2: Map of city water system sampling sites, Helena, MT (adapted from <http://maps.yahoo.com>)



Results

Each water source was evaluated for the presence or absence of coliform, the number of times the site tested positive for coliform, the average nitrate levels, and the presence or absence of coliphage. The nitrate and microbial results of each water source sampled are listed in Table 1. The Helena city water system showed no microbial growth and minimal nitrate concentrations while many Helena Valley wells were laden with microbial contaminants and/or high nitrate levels. All of the coliform positive wells were located in a high-density population area in a central region of the Helena Valley.

Correlation analysis suggested that well depth was not a conclusive indicator of the nitrate levels in each well (Figure 3). Additionally, a T-test revealed that the nitrates in the Helena Valley were statistically significantly higher than the Helena city samples with p-value of 0.017 and a t-value of 2.60 assuming 20 degrees of freedom (Figure 4).

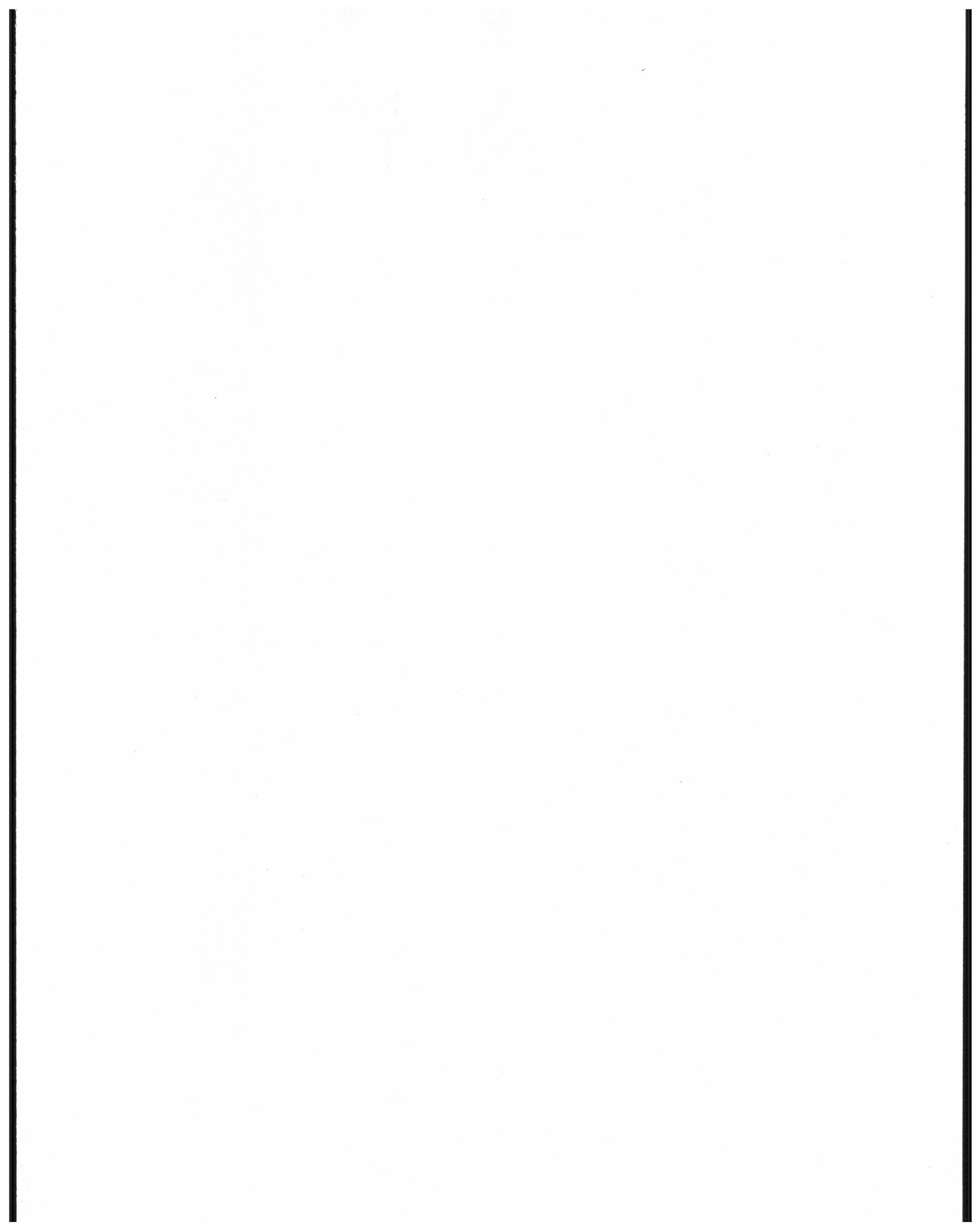
To simplify microbial and nitrate comparisons, wells were categorized as having high, medium, or low nitrate levels. The number of coliform positive wells was then determined for each category of nitrate level (Figure 5). High nitrate levels were not necessarily indicative of the presence of coliform or coliphage nor was the reverse true. This result may have been due to the small number of wells in the study. The nitrate levels remained fairly consistent in each individual well across samplings, but neighboring wells (often less than 300 meters) frequently demonstrated vastly different concentrations.

Table 1. Abbreviated sampling results. Private and public wells are numbered according to their geographic location spanning the valley from west to east. This arrangement shows a coliform positive trend in the central region of the sampled area (private well 5 through public system 2).

	Presence or Absence of Coliform (+/-)	Number of Positive Coliform Samples/Number of Times Sampled	Average Nitrate Levels (mg/L)	Presence or Absence of Somatic Coliphage (+/-)	Geological Classification
Private Well 1 ^a	-	0/3	3.23	+	Alluvial Aquifer
Private Well 2 ^a	-	0/3	0.74	-	Alluvial Aquifer
Private Well 3 ^a	-	0/3	1.38	-	Alluvial Aquifer
Private Well 4 ^a	-	0/3	1.20	-	Alluvial Aquifer
Private Well 5 ^a	+	1/3	4.28	-	Bedrock
Private Well 6 ^a	+	1/3	2.86	-	Alluvial Aquifer
Private Well 7 ^a	+	2/3	10.14	+	Alluvial Aquifer
Private Well 8 ^a	+	3/3	0.55	-	Alluvial Aquifer
Public System 1 ^a	+	2/3	0.23	-	Alluvial Aquifer
Public System 2 ^a	+	2/3	9.24	-	Alluvial Aquifer
Public System 3 ^a	-	0/3	0.50	-	Alluvial Aquifer
Public System 4 ^a	-	0/3	1.57	-	Alluvial Aquifer
Public System 5 ^a	-	0/3	2.49	-	Alluvial Aquifer
City Water 1 ^b	-	0/1	0	-	N/A
City Water 2 ^b	-	0/1	0	-	N/A
City Water 3 ^b	-	0/1	0	-	N/A
City Water 4 ^b	-	0/1	1.39	-	N/A
City Water 5 ^b	-	0/1	0.07	-	N/A
City Water 6 ^b	-	0/1	0.09	-	N/A
City Water 7 ^b	-	0/1	0	-	N/A
City Water 8 ^b	-	0/1	0.06	-	N/A
City Water 9 ^b	-	0/1	0	-	N/A

^a = see Figure 1 for geographic location

^b = see Figure 2 for geographic location



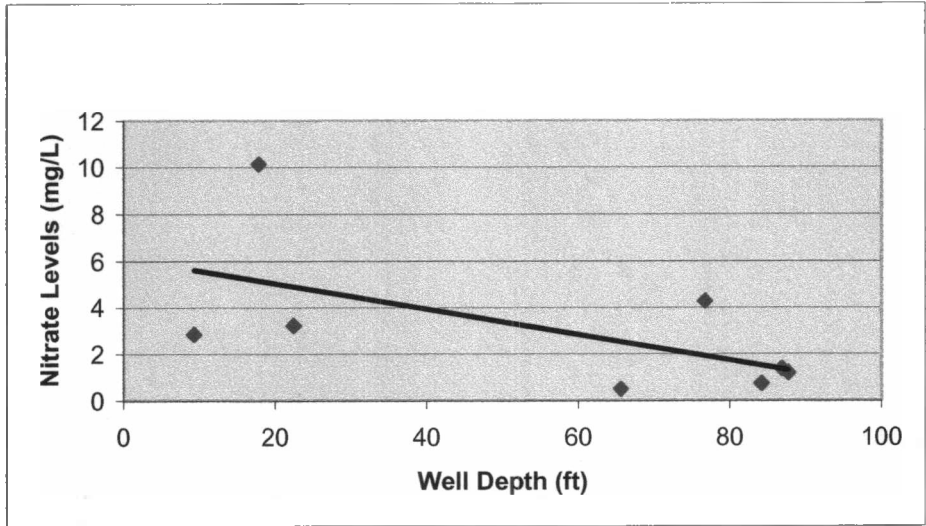


Figure 3. Comparison of nitrate levels v. well depth. R^2 value of 0.3484 ($y = -0.055x + 6.1395$).

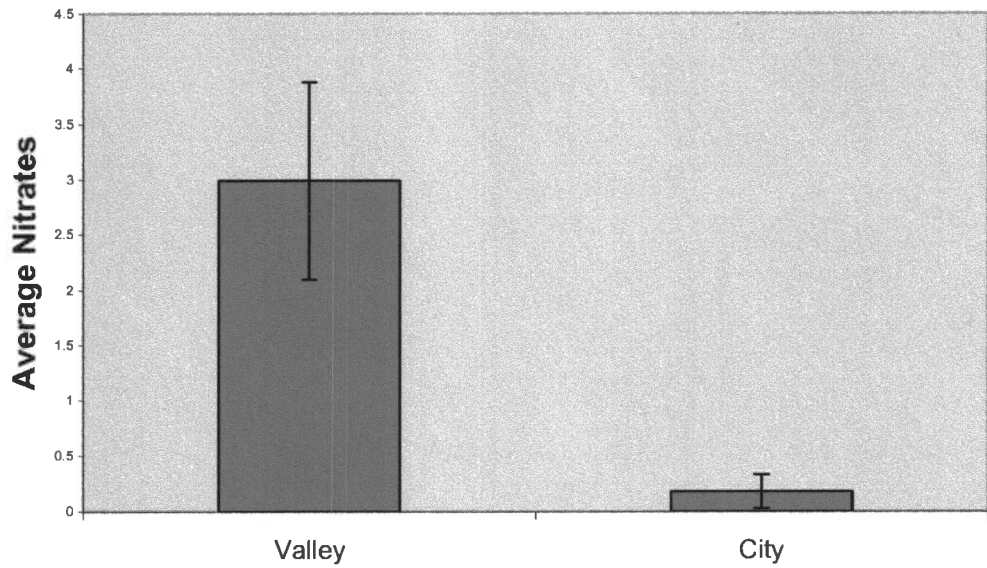
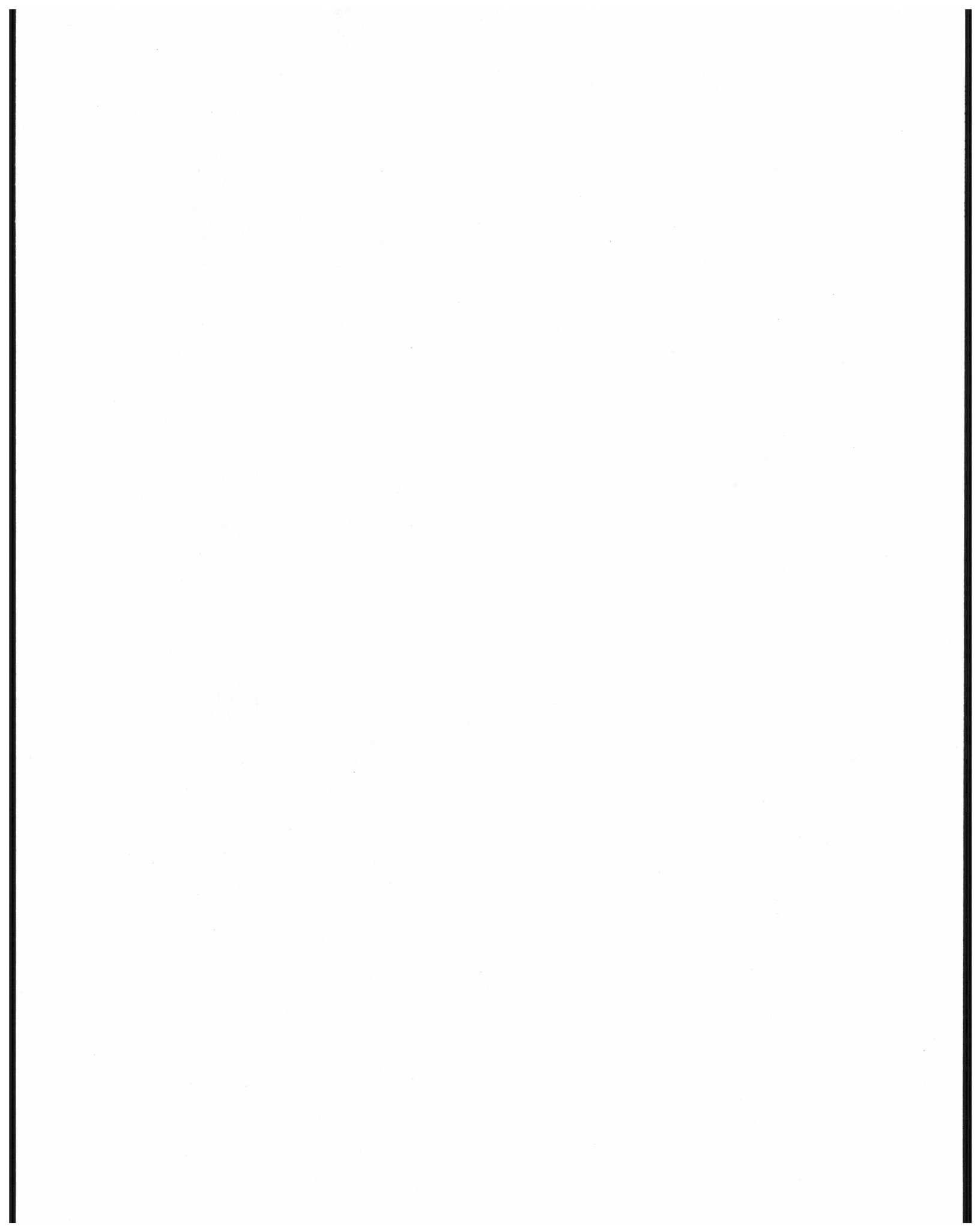


Figure 4. Comparison of the average nitrate levels in the Helena Valley and Helena city water samples. Error bars are indicative of two standard errors of the mean.



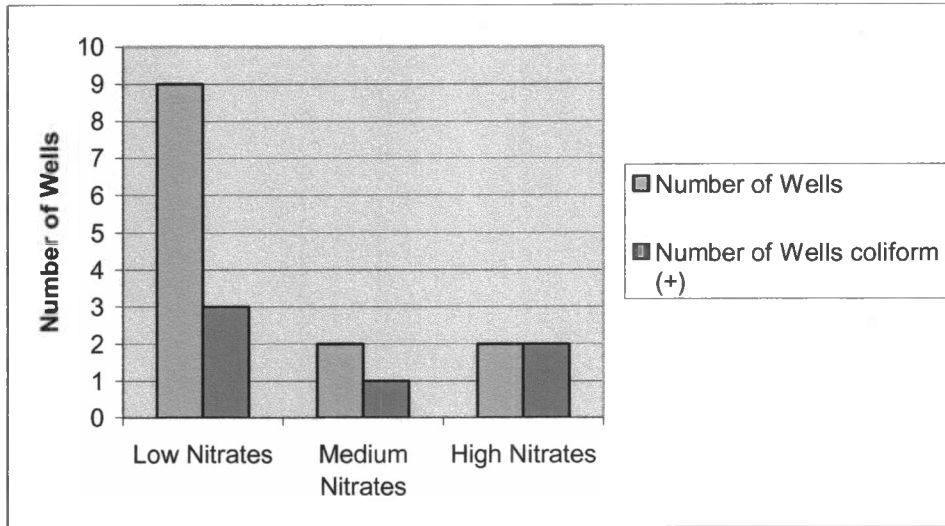
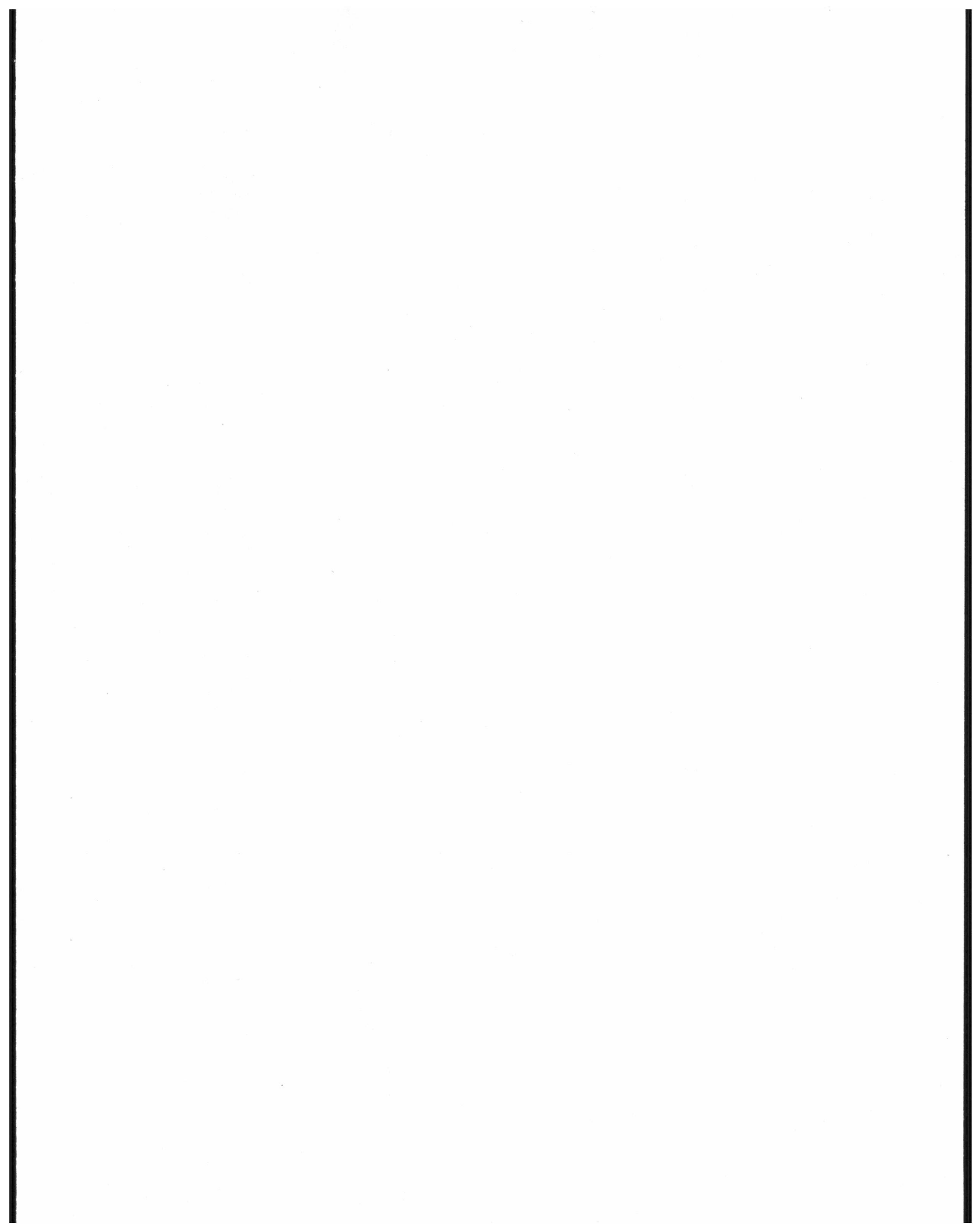


Figure 5. Comparison of nitrate levels and coliform positive samples.



Discussion

By comparing Helena city water with water obtained from the Helena Valley, this study essentially compared a highly monitored and non-treated source of water with a minimally monitored and treated region. In accordance with the stated hypothesis, the results show that the Helena city water provides a higher level of public safety than the Helena Valley well water. Not only did the results show an overall increase in the presence of coliform, coliphage, and nitrates in valley wells, but also the individual wells of the Helena Valley varied widely in the amount of each contaminant. For example, average nitrate levels varied from 0.23 to 10.14 mg/L. This variation is reflected in the standard error of the Helena Valley mean as shown in Figure 4.

No correlations among the tested variables were found because the localized microbial contamination and nitrate concentration were specific to each individual well and the surrounding conditions. When data were examined as a whole, there was no reportable relationship between well depth and nitrate levels or between nitrates and microbial presence or absence. Despite these larger inconsistencies, on an individual level, sometimes the causes of the poor water quality were evident. For example, private well 7 was positive for coliform multiple times, positive for coliphage once, and consistently showed nitrate levels above 10 mg/L (Table 1). Examinations of the site showed that the household septic system could feasibly be contaminating this well. Specifically, the well is very shallow in nature, may be located “down stream” from the septic waste flow, and is located close to the septic system itself.

Although septic contamination of wells is likely a contributing factor at some of

the private residences, the problem is complicated when one compares public well (PW) 1 and public well 2. These two wells are within sight of each other and are separated only by a field, yet the nitrate levels at PW1 averaged 0.23 mg/L while PW2 averaged an astounding 9.24 mg/L. Steve Kilbreath (pers. comm.) of the Montana Department of Environmental Quality suggests that this discrepancy may be the result of drainage from an alfalfa field in which cattle are periodically grazed near the high nitrate well and is probably not influenced by the facility's distant septic system. These two examples illustrate how the individual influences on each well make standardized correlations very difficult to discern. Because nitrate contamination can come from leaky septic systems or agricultural runoff and our methods did not allow differentiation between human and animal microbial or nitrate contamination, the search for relationships is very complex.

The quality of ground water is not an issue unique to the Helena Valley. Many other regions of the United States have also begun to study and address water quality issues specifically associated with private wells. In Oregon's Lower Umatilla Basin, Mitchell and Harding (1996) found that almost a quarter of the local population was consuming water with nitrate levels above the recommended 10 mg/L. This particular study is relevant because the project included an examination of the demographics of the local population. By determining the age distribution of the residents, the researchers could assess the number of children in the region who could potentially suffer negative consequences due to poor water quality.

In 1998, Tuthill et al. found more definitive correlations between septic systems and water quality in a study conducted in Maryland. Researchers found a negative

correlation between the length of the septic casing (a measurement indicative of the distance between the residence and the septic system) and the presence of coliform. The study also noted that the smaller the property lot is, the more likely the drinking water would be contaminated with coliform and show high levels of nitrates. The relative success of this research further suggests that a diversified study in the Helena Valley could also identify correlations.

A study conducted by Swistock et al. (1993) in Pennsylvania tested almost 1,600 homes for the presence of nitrates. Of these wells, just over nine percent of the wells showed levels above the 10 mg/L allowed by the EPA. One of the most significant findings of the study was that these high nitrate levels appeared to be clustered in specific areas and ninety-six percent of those exceeding EPA standards were located in agricultural areas. The researchers also found a correlation in which the distance of a residence to a cornfield predicted the likelihood of recording high nitrate levels in the well of that home. Other research suggests that Iowa, one of the nation's chief corn producers and a state in which three-quarters of the population depends on consumable ground water, is also struggling with widespread nitrate contamination (Sun, 1986).

As seen in the present study, multiple factors may contribute to high nitrate levels in different wells and identifying the role of each factor can be difficult. Nolan (2001) used statistical analysis to determine six primary predictors of high nitrate concentrations in well water. As defined by Nolan (2001), the six predictors are as follows: (1) the amount of "nitrogen loading" in the area due to the use of fertilizer; (2) the proportion of cropland in the region; (3) the "natural log of population density"; (4) the proportion of

“well-drained soils”; (5) the “depth to the seasonally high water table”; and (6) the “presence or absence of a fracture zone within an aquifer.” The larger implication of this study is that nitrate-contaminated groundwater is usually the result of a combination of circumstances, not a single cause (Nolan, 2001).

While in the Helena Valley study no single factor seemed to predict the presence or absence of another variable, correlations among the different contaminants cannot be ruled out. The quality of the well water in the Helena Valley is probably influenced by multiple factors that were not tested in this preliminary examination. To further explore this issue, it is imperative that future studies include a larger sampling size and examine site-specific factors such as well distance from local septic systems, age of septic systems, local geological variations, ground water flow, and regional population density. It is also possible that correlations between nitrate levels and microbial presence are influenced more strongly by the individual sources of contamination (either septic or by agriculture) than by each other.

Demographic studies of the Helena Valley may provide impetus for further research on groundwater quality because nearly 31% of the population is under the age of 18 (U.S. Census Bureau, 2000). Two of the public well sites regularly sampled in this study were elementary schools, one of which showed levels of nitrates just below the maximum EPA recommendations. These findings could generate increased public interest in more research, but the impact of nitrates on children remains unclear. New research conducted in association with the World Health Organization suggests that nitrates may not be directly responsible for methemoglobinemia but instead may be

unrelated or only a contributing factor to the malady (Fewtrell, 2004). Even if the connection between “blue baby syndrome” and nitrates proves to be erroneous, nitrate monitoring will probably continue to serve as an indicator of septic contamination.

On a broader spectrum, this study suggests that without intervention, the water quality in the Helena Valley may deteriorate further as the regional population increases and more septic and well systems are installed. According to the 2000 U.S. Census (U.S. Census Bureau, 2000), the regions of the Helena Valley described as Helena Valley North East, North West, South East, and West Central house over 18,328 people. This population has shown enormous growth in recent years with an increase of 17.3% from 1990 to 2000 for Lewis and Clark County as a whole (United States Census Bureau, 2005). Because this growth trend is likely to continue, at some point it may even be necessary to consider expanding the current city water system to serve at least a portion of the Helena Valley. It is imperative that research continues in the region to monitor problem areas and to contribute to the body of knowledge specific to the region.

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