

**Final Report**

**Bacterial Source Tracking Using Antibiotic Resistance  
Analysis of Fecal Coliforms**

**Stevenson Creek, Clearwater, Florida**

**June – December, 2000**

**Valerie J. Harwood and John E. Whitlock**

**University of South Florida**

**Department of Biology**

([vharwood@chuma1.cas.usf.edu](mailto:vharwood@chuma1.cas.usf.edu))

March 20, 2001

## **Introduction**

Various bacteria are found in the digestive tracts and feces of wild and domestic animals and humans. Some of these bacteria including fecal coliforms and fecal streptococci are used as indicators of fecal contamination when present in natural waters. Unfortunately, the mere presence of these indicator bacteria is not informative as to the source of fecal pollution, an important factor for risk-assessment and remediation. Consequently, several methods collectively known as bacterial source tracking (BST) methods have been developed to “fingerprint” fecal indicator bacteria in order to determine their host or source (e.g. human, dog, wild animal) and may be based on either the genetic make-up of the bacteria, or on physiological characteristics such as resistance to various antibiotics. Using antibiotic resistance analysis (ARA), it is possible to assess the source of indicator organisms based on a much larger subset of the bacterial population than is currently possible using genetic methods. ARA was used to determine the source of fecal contamination in this study essentially as described in Harwood et. al 2000.

In any BST method, a database of fingerprints, or patterns, of bacteria isolated from the feces of known source animals (e.g. humans, dogs, wild animals) must first be constructed and tested for its predictive accuracy. This database is called the calibration data set. Discriminant analysis, a multivariate statistical test, is used to analyze the data. The ability of the database to accurately predict the source of indicator organisms is assessed using isolates from known sources as “unknown” or test isolates. The database can also be self-crossed, that is, the database isolates are used as both the calibration data set and the test data set.

The database used in this study consists of 3309 fecal coliform isolates from six sources, humans (domestic wastewater), dogs, cattle, chickens, pigs and wild animals (mainly raccoons and birds). The average rate of correct classification (ARCC) is a measure of the predictive accuracy of the database, and is obtained by self-crossing the database, adding the number of correctly classified isolates in all source categories, and dividing by the total number of isolates. The ARCC of this data set is 57.2% when 6 source categories are used in the analysis. The chance of an isolate falling into one of 6 categories by chance if categorization were random is 16.7%, therefore the antibiotic resistance patterns of fecal coliforms have substantial predictive capacity with respect to bacterial sources (Harwood *et al.*, 2000).

In any database, some percent of isolates from other sources will misclassify as human isolates. The rate of misclassification of isolates into the human category can be used to develop a cut-off point for significant levels of human isolates. In this database, about 20% of wild animal isolates are misclassified as human, and wild animal isolates have the highest rate of misclassification as human of all sources. The conservative rate of 25% is used as the cut-off point for identification of a significant percentage of isolates from human sources in any sample.

## **Stevenson Creek**

This study was initiated in order to identify the dominant source(s) of fecal contamination to Stevenson Creek in Clearwater, Florida. Five sites were chosen to represent areas where routine monitoring has shown high levels of fecal coliforms. Two of the sites were located at the composite samplers previously installed for the project (Comp1 and Comp2). Comp1 is located at the golf course on the main branch of Stevenson Creek; Comp 2 is located on Spring Branch off King Highway. Sites previously designated for monitoring efforts were sampled: STC1 at Spring Branch, STC2 at Hammond’s Branch and STC5 at Evergreen.

Sampling dates were June 27, July 25, August 22, September 19, October 23, November 13, and December 27, 2000. Isolation of fecal coliforms was poor from November with samples yielding only an estimate of population density and no information as to source of contamination. Consequently, additional samples were collected in December.

## Results

The dominant sources of fecal coliform isolates obtained from the five sites over the course of this study were wild animal, dog and human. The overall trend was for wild animal isolates to comprise the majority of fecal coliforms obtained when colony forming units (CFU) counts exceeded the acceptable limit of 200CFU/100ml. STC1 and STC2 sampled on 6/27/00 were the only observations where exceedingly high CFU counts co-occurred with a majority of human isolates. On 8/22/00 at STC1, the majority of isolates were dog and the CFU count was 300/100ml. Samples from the month of September yielded a majority of human isolates from all sites while densities of coliforms were low, ranging from 7-26 CFU/100ml. The above results suggest that source most frequently contributing to excessive fecal coliform counts was wild animals followed by human and dog isolates.

Some statistically significant relationships were apparent between the CFU counts and classification of isolates. The percentages of isolates from specific sources were compared to CFU/100ml using a regression analysis. There was a significant, inverse relationship between the percentage human isolates and the CFU count for Comp2, i.e. as the CFU count went down over the study period, the percentage of human isolates went up,  $P=0.019$ ,  $r^2=0.87$  (Fig. 9). While this relationship was not observed for the other four sites examined individually, it was observed when data from all sites were pooled,  $P=0.001$ ,  $r^2=0.34$  (Fig 11). This indicates enough of an overall trend among the sites to maintain a significant inverse relationship between CFU and percent human. Likewise, when the percentage of wild isolates was compared to CFU counts in a similar test, a significant direct relationship was observed at STC 1,  $P<0.001$ ,  $r^2=0.96$ . The CFU count decreased along with the percent of wild isolates, Fig 10. Again, a significant relationship was not observed for any of the other four sites when examined individually. However, when the five sites were pooled the regression was significant,  $P<0.001$ ,  $r^2=0.60$  (Fig. 12). These results support the notion that wild animals are the predominant contributors to fecal contamination marked by elevated fecal coliform levels.

The relative importance of specific wild animals as contributors to high fecal coliform numbers is difficult to assess with confidence but is likely related to factors such as population size and density and their utilization of the territory adjacent to the sample sites. During the course of the study, birds were the most frequently observed wild vertebrates. Great white egrets, snowy egrets, little blue herons, and anhingas were frequently present in low densities during sample collection i.e one or two individuals. Waterfowl such as gallinules, coots and ducks, were observed in slightly higher densities (three or four individuals), but not as frequently. The largest aggregates of wild animals observed during the study period were flocks of migratory birds such as European starlings and boat-tailed grackles. These birds, however, were observed during the late fall months when fecal coliform densities were low. Gulls common to the coastal areas such as the black-headed gull and herring gull were not observed frequently near the sampling sites.

While human input may not be the major cause of elevated fecal coliform levels for most of the samples analyzed for this study, the domination of small populations by human isolates suggests that human sources contribute to low-level background contamination. This occurs when FC populations are low, near the transition to dry season and perhaps few isolates are washed into surface waters from draining storm water. Lowering water tables may also draw wastewater from small, otherwise innocuous leaks. Overall, there was little evidence of acute human fecal contamination on a large scale across the five site examined. However, there may be considerable human source influencing STC1 and STC2, which is detectable despite the presence of fecal coliforms from other sources. These sites were impacted by human fecal sources with the highest frequency and magnitude of the five sites. They were more frequently over 25% human and exhibited a higher mean % human than the other sites. The most pronounced human contamination of STC1 and 2 occurred in June where 56.7% and 47.7% of isolates examined were human. The density of human isolates at STC1 and STC2 may be approximated from the fecal coliform density using the percent classified as humans. This yields 11400 'human'CFU/100ml for STC1 and 2400 'human'CFU/100ml for STC 2, both of which exceed the limit of 200CFU/ml. Consequently, unlike any other sampling event in this study, the human input for these two sites in June were high enough to cause violation considering only human isolates.

## References

- V. J. Harwood**, J. Whitlock and V. H. Withington. 2000. Classification of the antibiotic resistance patterns of indicator bacteria by discriminant analysis: use in predicting the source of fecal contamination in subtropical Florida waters. *Appl. Environ Microbiol.* 66:3698-3704.

Table 1. Density of fecal coliforms from the five sites as determined by membrane filtration  
Colony Forming Units/100ml

Site	June	July	August	September	October	November	December
STC 1	20000	13000	300	26	35	1	6
STC 2	5000	2200	68	7	1	1	70
STC 5	1600	6400	115	7	1	1	24
Comp 1	12000	18000	110	9	6	30	17
Comp 2	20000	12000	0	16	960	10	120
Mean	11720	10320	118.6	13	200.6	8.6	47.4

Table 2. Percent wild isolates at Stevenson Creek sites, June – December

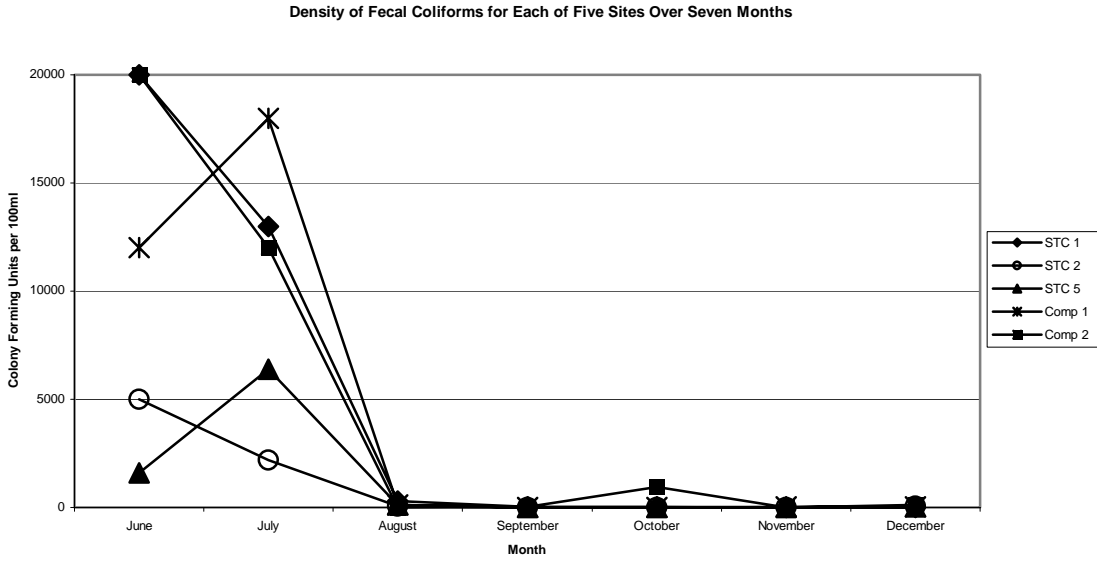
Site	June	July	August	Sept	Oct	Nov	Dec	Mean
Comp1	100.0	62.7	76.2	0	34.1	NA	0	45.5
Comp2	100.0	62.1	30.5	0	63.4	NA	0	42.7
STC1	43.3	42.1	21.7	3.1	0	NA	0	18.9
STC2	40.9	43.2	10.5	16.7	NA	NA	0	22.3
STC5	80.6	67.7	46.7	6.3	NA	NA	0	40.3

Table 3. Percent human isolates at Stevenson Creek sites, June – December

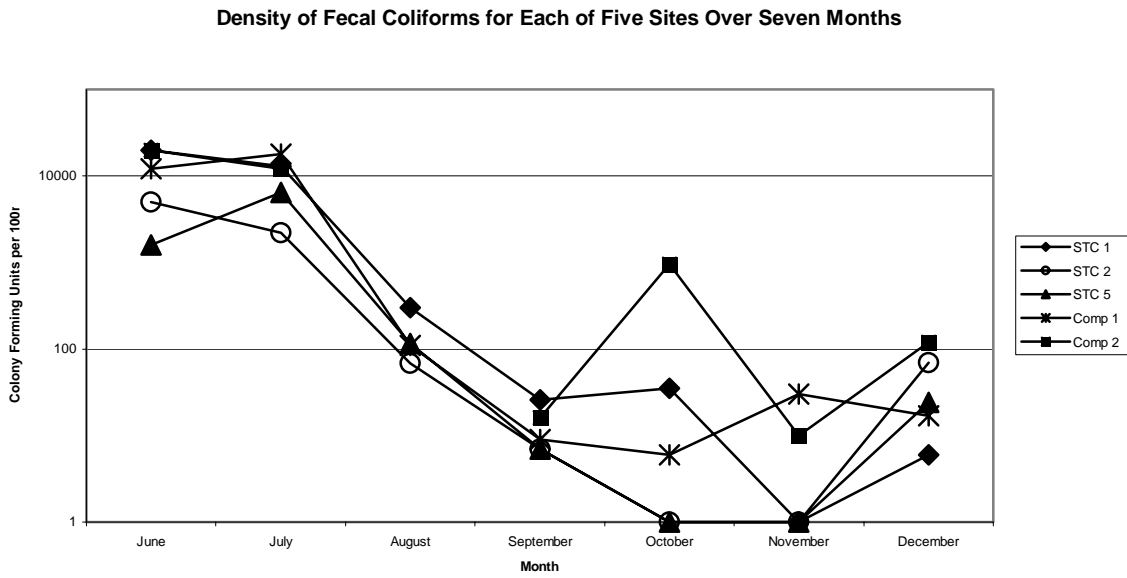
Site	June	July	August	Sept	Oct	Nov	Dec	Mean	# > 25%
Comp1	0	3.7	4.8	62.5	20.5	NA	75	27.8	2
Comp2	0	6.9	25.4	93.3	15.9	NA	90.5	38.7	3
STC1	56.7	31.6	13.3	90.6	12.5	NA	71.4	46.0	4
STC2	47.7	16.2	10.5	83.3	NA	NA	75	46.5	3
STC5	0	22.6	10	75	NA	NA	82.5	38.0	2

Table 4. Percent dog isolates at Stevenson Creek sites, June – December

Site	June	July	August	Sept	Oct	Nov	Dec	Mean
Comp1	0	0	19.1	37.5	36.4	NA	25	19.6
Comp2	0	0	42.4	6.7	2.3	NA	9.5	10.15
STC1	0	18.4	65.0	3.1	87.5	NA	28.6	33.7
STC2	0	18.9	79.0	0	NA	NA	25	24.6
STC5	0	6.5	43.3	18.8	NA	NA	17.5	17.2

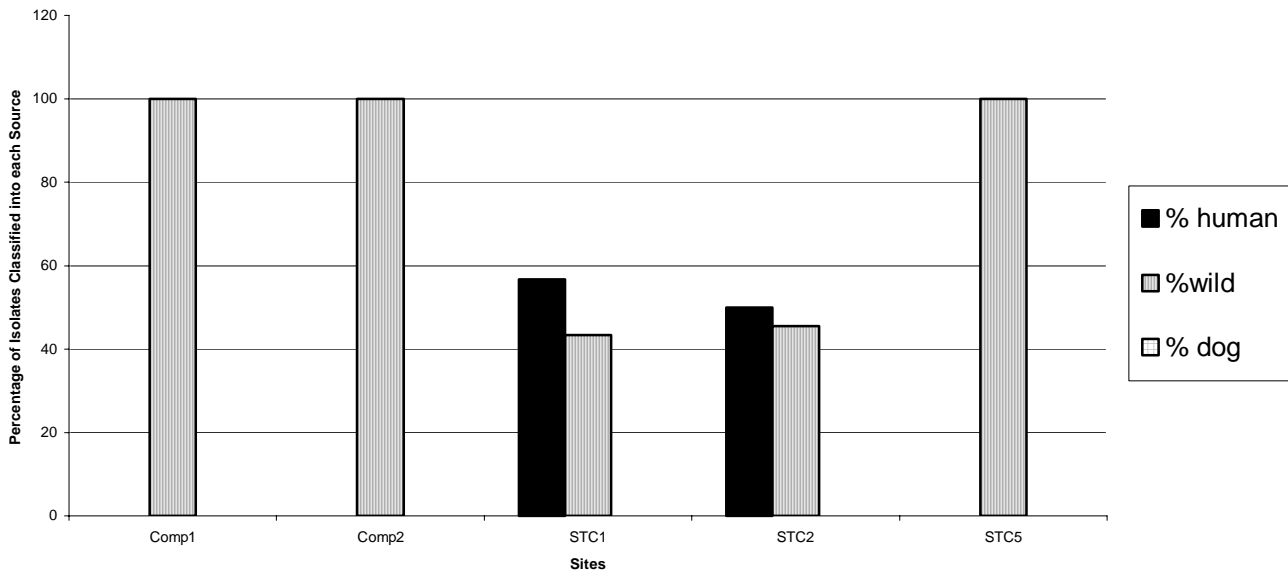


**Figure 1. CFU/100ml from the five sites over seven months.**



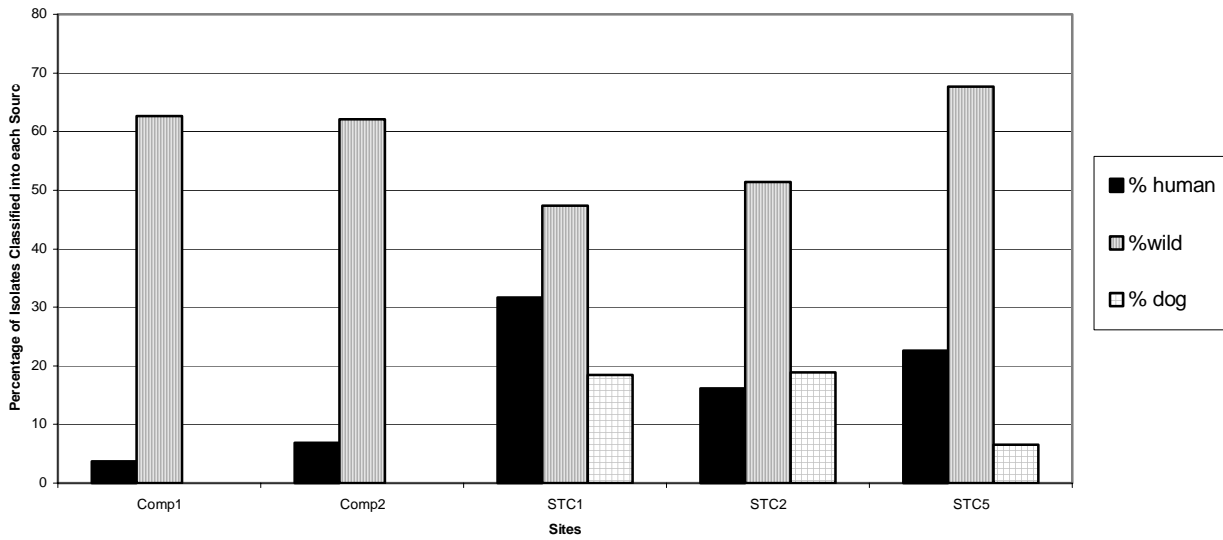
**Figure 2. CFU/100ml from the five sites over seven months, graphed on a log scale.**

**Categorization of Isolates from Each of Five Sites on 6/27/00**



**Figure 3**

**Categorization of Isolates from Each of Five Sites on 7/25/00**



**Figure 4.**

Categorization of Isolates from Each of Five Sites on 8/22/00

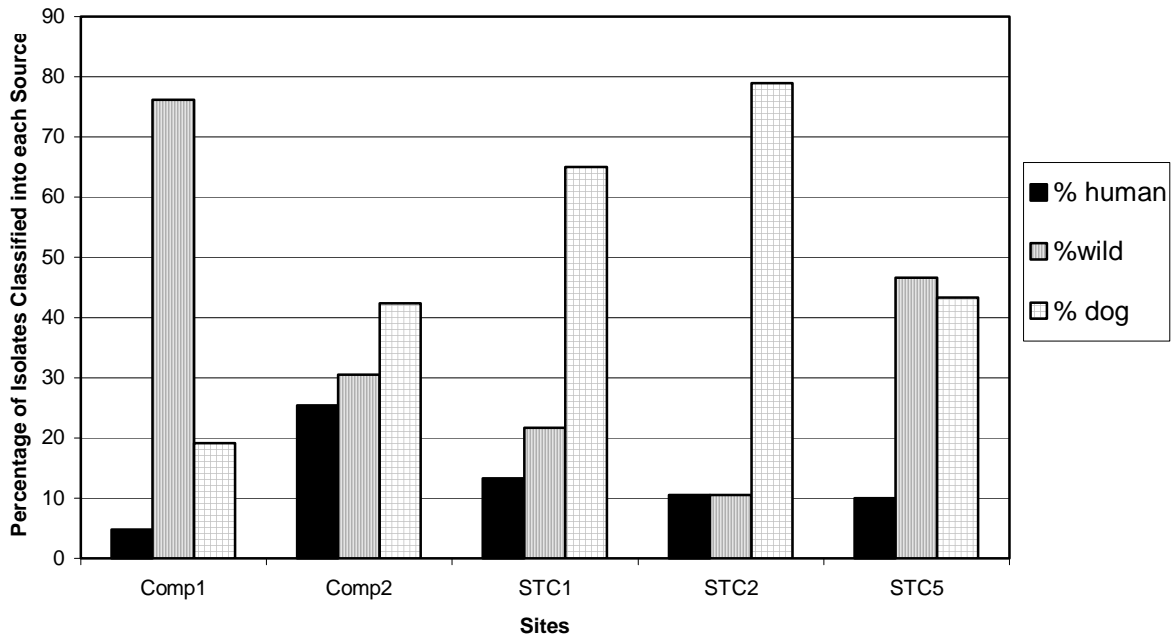


Figure 5.

Categorization of Isolates from each of Five Sites on 9/19/00

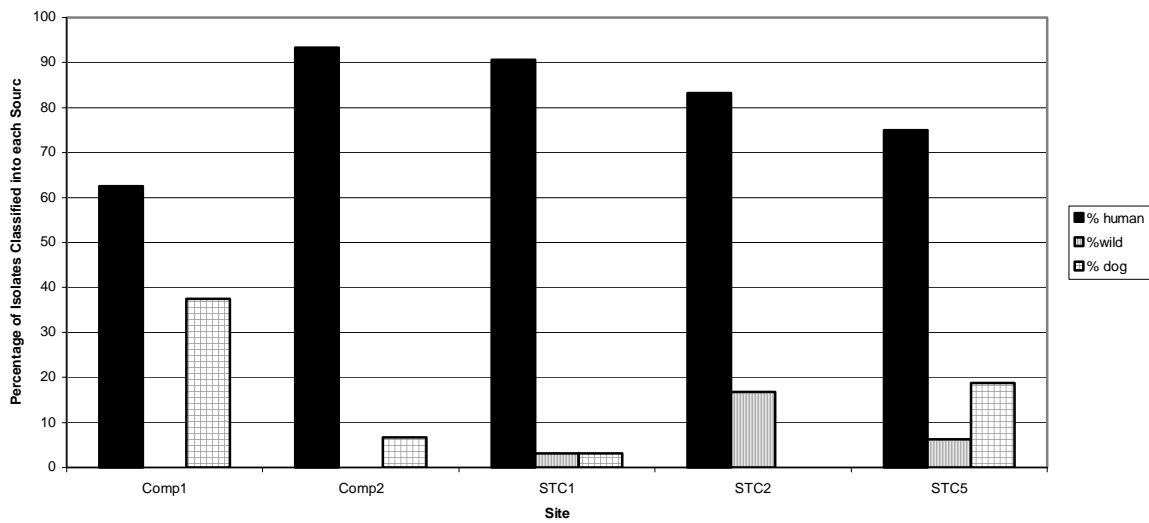
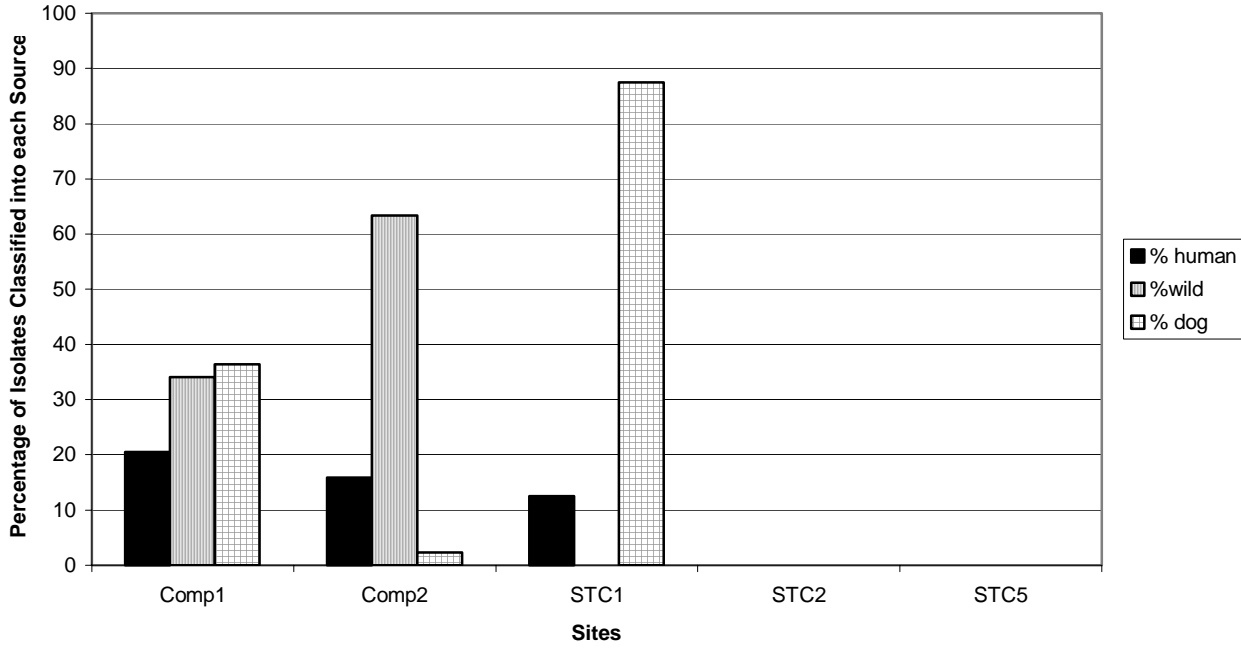


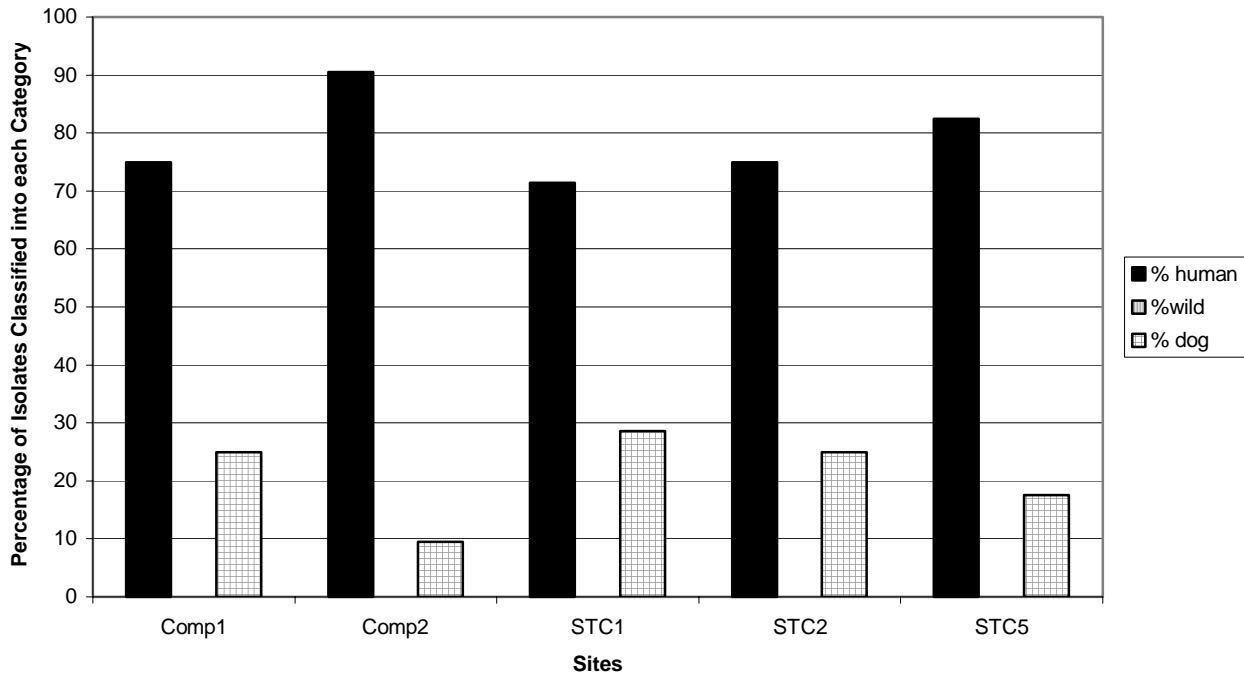
Figure 6.

**Categorization of Isolates from the Three Sites from Which Fecal Coliforms were Obtained on 10/23/00**



**Figure 7.**

**Categorization of Isolates from Each of Five Sites on 12/27/00**



**Figure 8.**

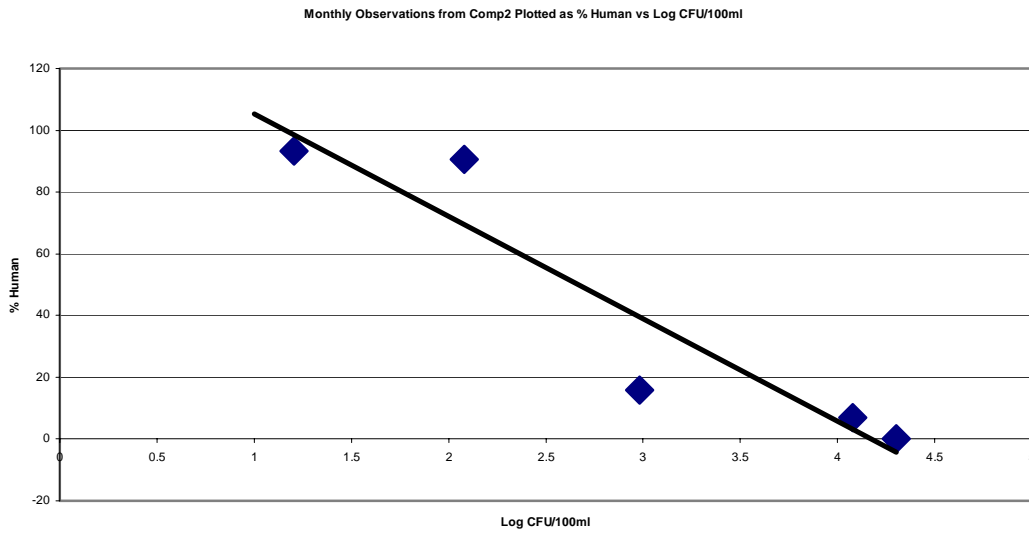


Figure 9.

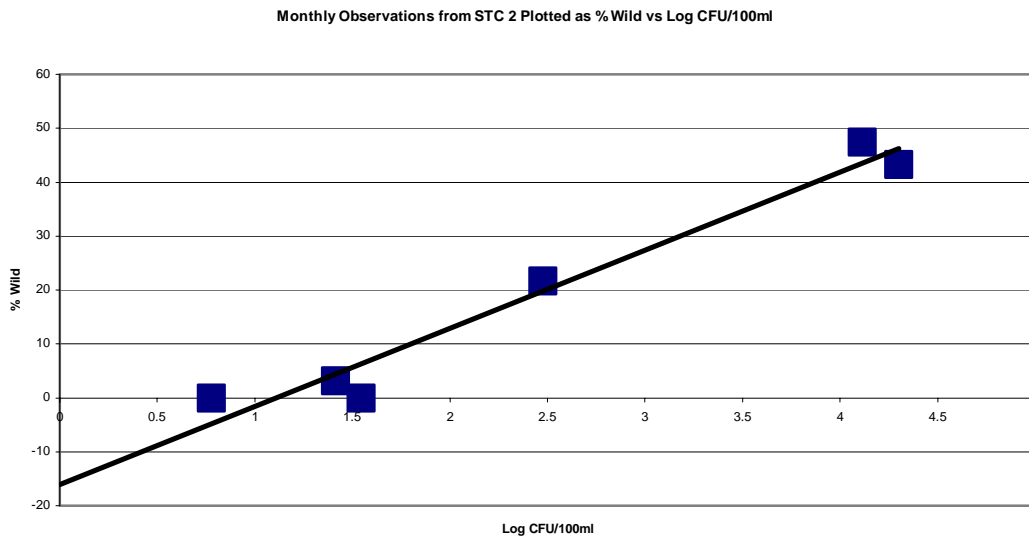


Figure 10.