FINAL REPORT

Project Title: Survey of Selected Bacteria in Irrigation Canal Water – Third Year

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Summary
Data of water quality in the irrigation canals was collected during the last three years. In this report we inform about the data obtained during the last 12 months, which were correlated with environmental factors. The objective was to determine levels of bacteria indicators, including total coliform count, fecal coliform and generic E. coli, and determine a trend throughout the year. Collection included sampling of 4 sites ("labeled" as "San Luis", "Sommerton", "Main" and "YAC" located in that order from South to North) in the Yuma Valley on a weekly basis. On the same days or sampling the populations of insects captured during the week and count of birds during the 15 minutes prior to water sampling were recorded. Temperature and relative humidity data was also computed using weather station data. The results considered for this past year showed what was observed in the two years. This meaning that bacteria indicators have higher levels before and after winter months when the winter vegetable are not harvested, which warns those companies that attempt to produce vegetable during late Spring and early Fall months in Arizona. Although in past years several spikes of E. coli population were observed during the winter this past winter we did not collect a single sample that would not pass the metrics of the recent Arizona Marketing Agreement (<126 cfu/100 ml). The reason for this is unknown however it could be associated with lack of rain during this past winter. During this period we also included the analysis of *E. coli* O157:H7 and the counts of specific insects such as various species of flies. No sample has been positive for *E. coli* O157:H7 as today (after two years). Moreover, this year we investigated the impact of different fly species that have been trapped in our collections, however, the relationship was inconsistent. In parallel study we have observed that about 25% of birds roaming fields are positive for generic E. coli, however, the impact seem low as those shed the bacteria for short time, something we observed with birds in captivity. During the last few months we started to collect information on the population of Enterococci in different sites and the survival rate of actual pathogens. This is information that we believe will be useful in combination with the data we generated during the last three years.

Introduction

The reported illnesses cases associated with lettuce are now more publicized, and despite the fact that most cases have been due to cross commodity contamination (eg.

meat to lettuce in kitchens), the cases of contaminated lettuce in the field are increasing. Eighteen outbreaks associated with consumption of lettuce were reported during the 1995-2005, due to contamination with pathogenic *E. coli.* Moreover, lettuce and leafy salad accounts for 25% of all the outbreaks traced to produce during the 1990-2003 period. After seafood, produce is the single food associated with the highest incidence of outbreaks. Consumers are becoming more aware of this information while retailers as well as Federal authorities are enforcing/stressing the importance of developing efficient food safety programs during the production and handling of lettuce. There is no doubt, that currently food safety is one of the most important concerns of the iceberg lettuce industry.

Despite the fact that no Arizona lettuce grower has been involved in any contaminated-lettuce outbreak, it is of paramount importance to determine the reasons why Arizona lettuce is regarded as safe. This can help lower possibilities of any emerging problem and prevent a catastrophic damage to the industry, as it has occurred in other regions when no control was taken to reduce risks of contaminated product.

This study was justified because more stringent food safety programs are currently requested by buyers, and the quality of surface water in Arizona is not well defined. Despite the fact that no Arizona grower has been implicated in any contaminated-lettuce outbreak, it is of paramount importance to determine the reasons why Arizona lettuce is regarded as safe, as to continue ensuring safety of the Arizona head lettuce. The objectives of this study (for all three years) are:

a) To develop a survey of bacteria indicator in irrigation canal water of the Yuma Valley;

b) To examine possible relationship between bacteria indicator population and environmental factors and/or biological agents;

c) To determine any implications of the survey on current and future food safety regulations.

Methodology

Samples of water were taken in four different sites across the Yuma Valley. Two were near the city of Yuma (labeled "YAC" and "Main), one in the Sommerton area and one in the San Luis area. The samples were taken every week, and this has been the norm during the third year as well. We submitted the samples to AgriTrend Lab were analysis of bacteria was conducted following specifications of the Membrane Filtration Method m-Coliblue 24™ (USEPA Method No. 10029). Preparation of samples, inoculation, incubation and count of microbes were performed following manufacturer's recommendation. We analyzed water for total coliforms, and fecal coliforms. We also evaluated for generic *Escherichia coli*, as this a very important human fecal indicator used for reference of pathogenic growth in the environment. Since the second year we analyzed for *E. coli* O157:H7 during 6 months of the year (October-March) to determine any correlation with any of the bacteria indicator counts with PCR techniques.

Data of waterborne bacteria were matched with data of wind speed, air temperature, solar radiation, relative humidity, sourced from the weather station. Insect traps were placed nearby the water sampling sites and the number of caught insects were counted weeks. Birds roaming in a 200 feet diameter around the water sampling site were

counted for 15 minutes before taking the water samples. We identify flies in traps and conducted correlation analysis with the bacteria counts.

This year different insect traps were used, which were stickier than the old ones, however the glue does not cover all of the squares. Therefore only counts of the center 6 squares were considered. For this reason the graphs reflect lower amounts. When samples were too dirty to count on both sides no value was added, if only one side was too dirty the value for the opposite side is used for both sides.

For the weather data, the values were taken from AZMET at the Yuma Valley station. The values were taken from the standard reports in the weekly section. The values were average of daily values for the values given 6 days prior to the date of collection and the date of collection (ie 7 days average).

Results

AILRC awarded us funds to develop a water survey study for three years. In all three years the pattern was very consistent: with coliforms and *E. coli* declining significantly during the winter showing a similar trend to that of environmental and soil temperature. This can be seen in the data collected during this past year (Figures 1-4). It is relevant to mention that the low temperature during the winter accompanied with <60% relative humidity prevalent in Yuma, may substantially decline *E. coli* as suggested by the literature.

The new metrics used for the California Marketing Agreement and adopted for the Arizona Marketing Agreement, states that acceptable levels should include <126 cfu E. Coli/100 ml). However, it is important to mention that we found that in certain weeks and locations "spikes" of the bacteria population are possible. This past year however we did not find this occurring in any of the sites. No clear relationship has been observed to this point between bacteria indicator counts and bird or insects/flies. Starting during the second year we initiated with the analysis of *E. coli* O157, and up to date no sample has been positive for this pathogen.

Final Remarks

Our results have shown a clear trend indicating decline of bacteria indicators during the season when lettuce is grown in Arizona, which correlates with a decline in average temperature. The "spikes" in population observed during the first two years were not observed during the third year. The benefit of these results is two fold. First, growers, national regulatory entities and buyers are starting to have information about the quality of the water in the Yuma valley, and how this fluctuates during the year. More importantly, the data is used to determine whether there is any connection with environmental or biological factors. In parallel study we have observed that about 25% of birds roaming fields are positive for generic E. coli, however, the impact seem low as those shed the bacteria for short time, something we observed with birds in captivity. Despite the fact that Arizona leafy greens has never been associated with foodborne outbreaks, this study is contributing with relevant information that can be used for future regulatory guidelines.

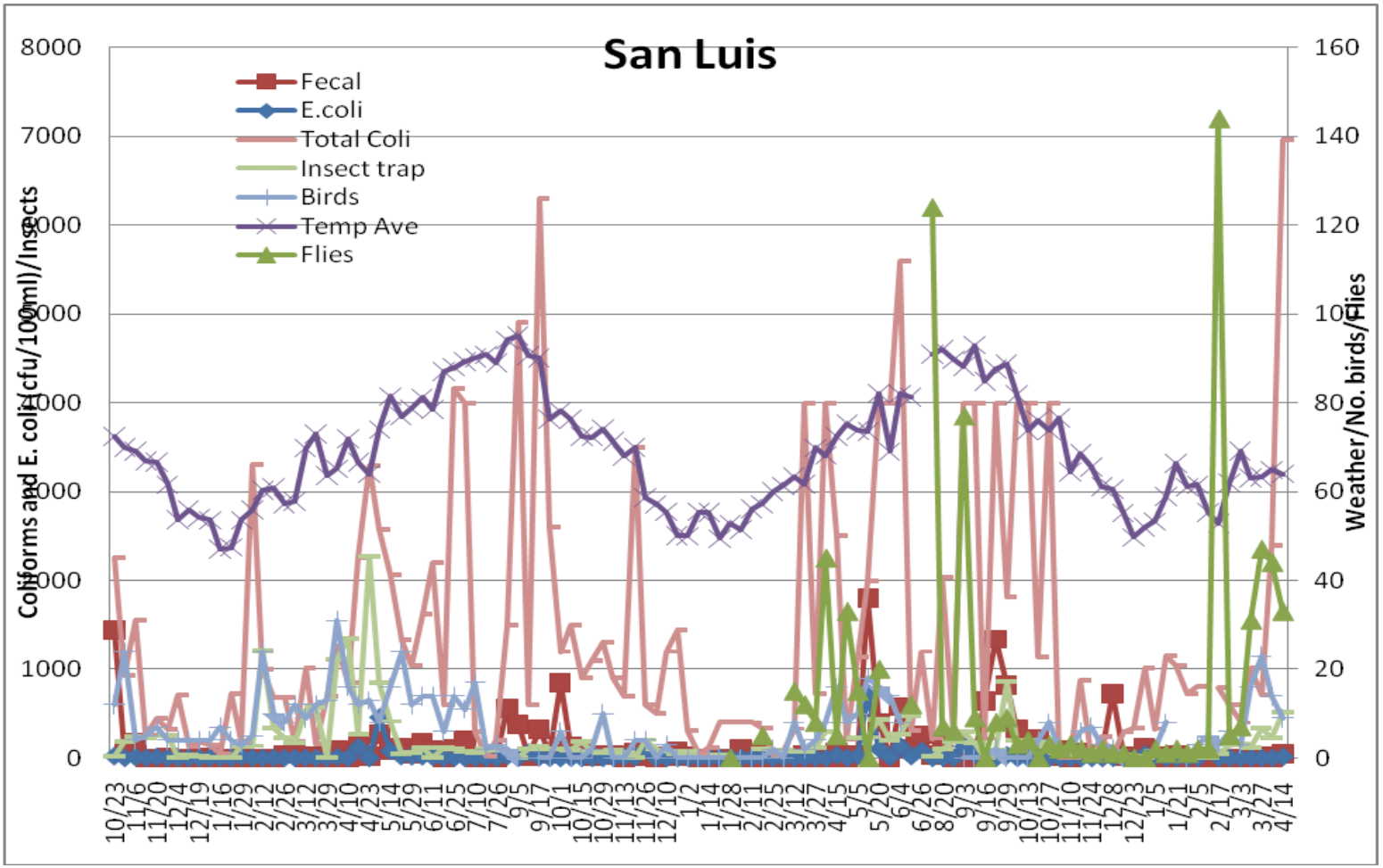

Figure 1. Bacteria, birds and insect counts in a secondary irrigation canal in the vicinity of the town of San Luis during the period October 2007-April, 2009.

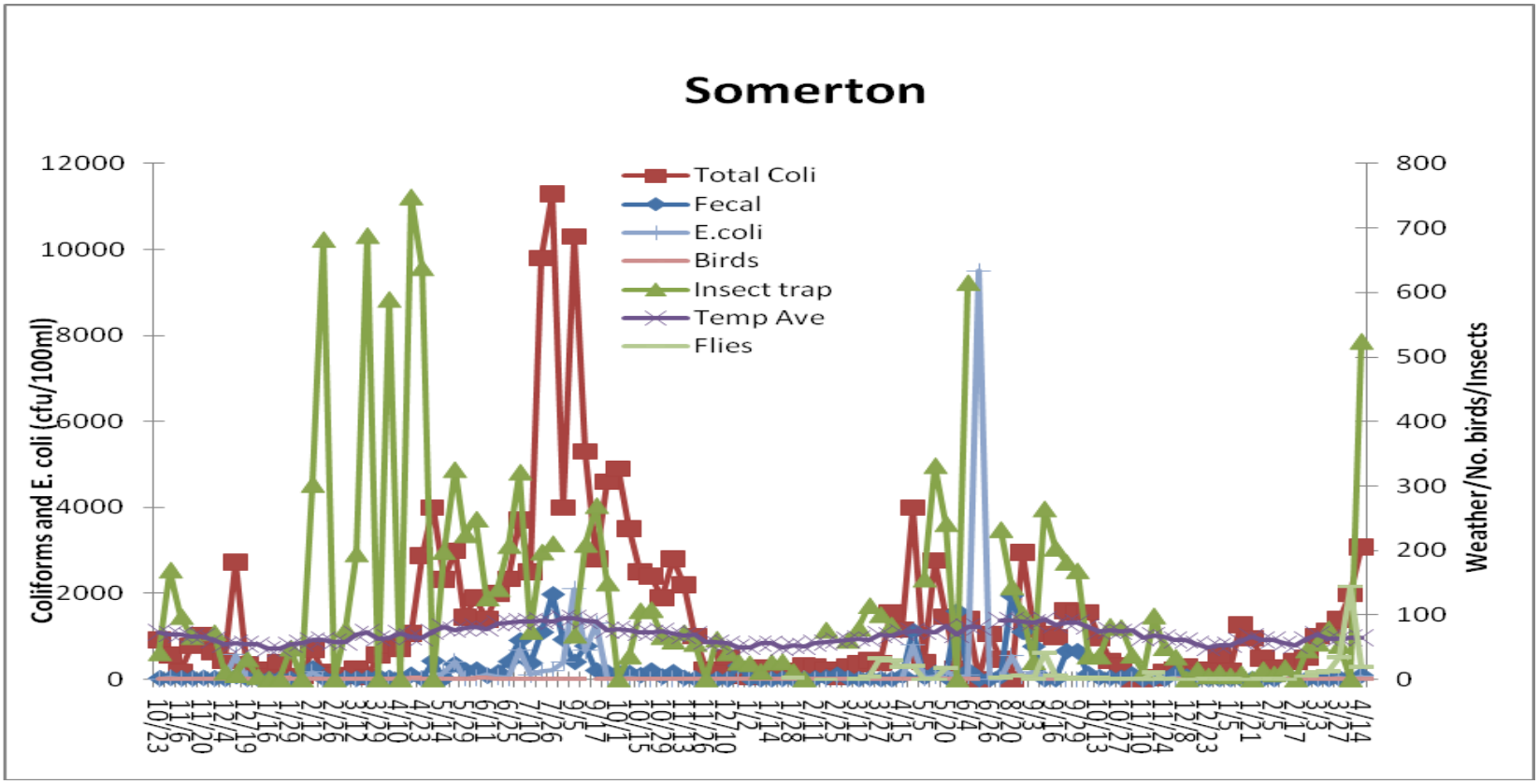

Figure 2. Bacteria, birds and insect counts in a secondary irrigation canal in the vicinity of the city of Sommerton during the period October 2007-May 2009.

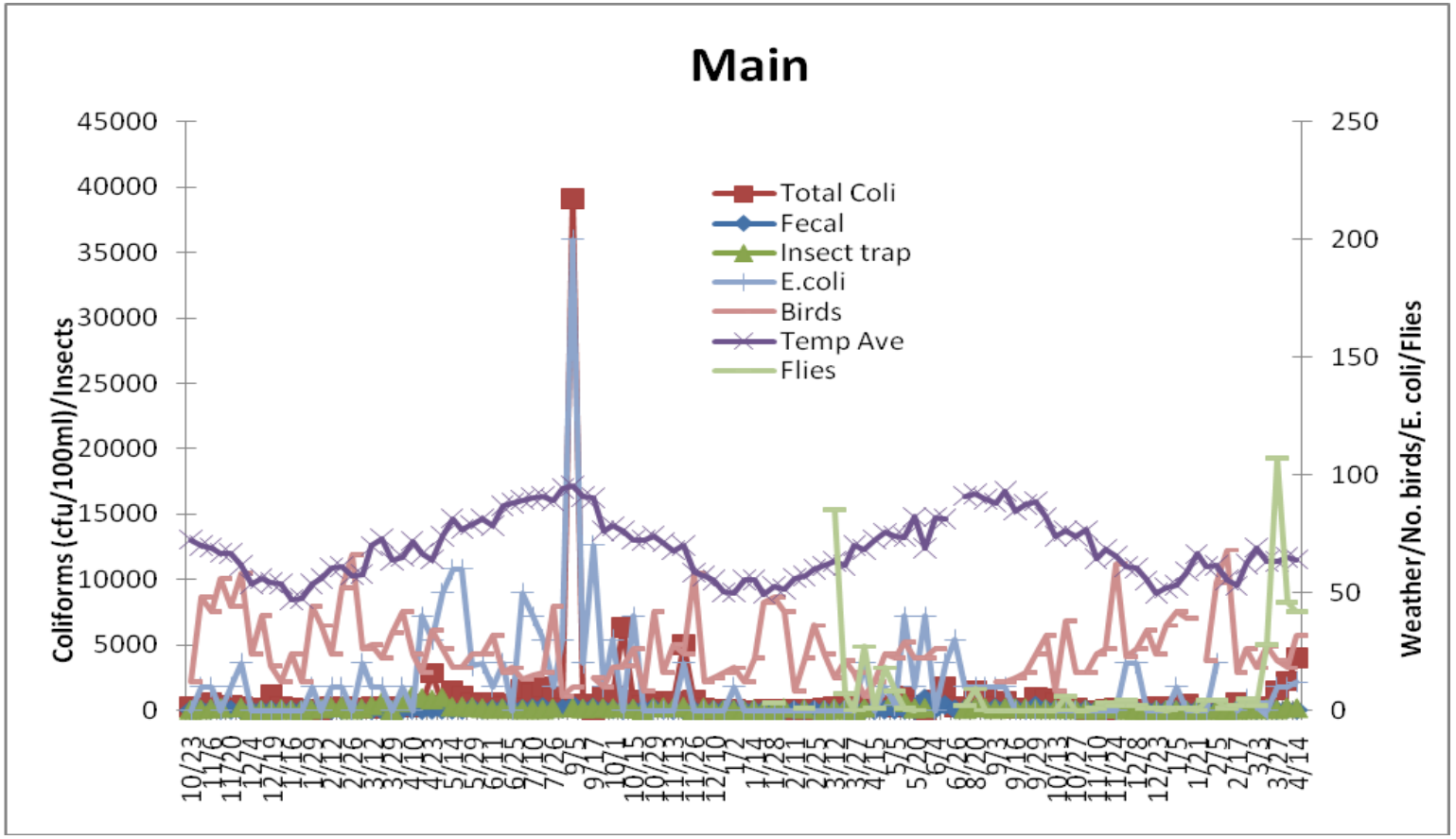

Figure 3. Bacteria, birds and insect counts in a main irrigation canal near the city of Yuma, AZ during the period October 2007-May 2009.

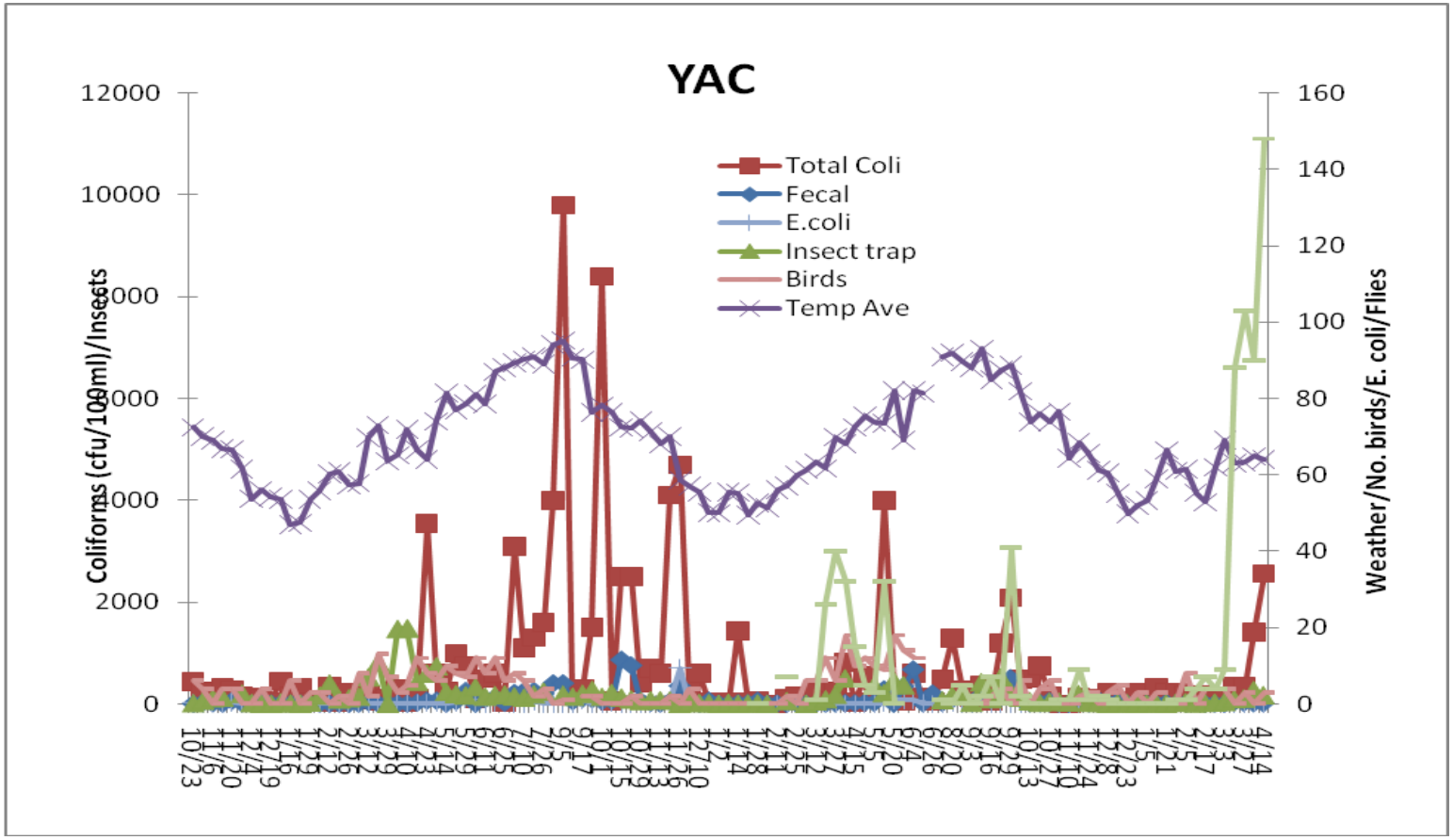

Figure 4. Bacteria, birds and insect counts in a secondary irrigation canal near the city of Yuma during the period October 2007-April 2009.