

## REPORT

**Project Title:** Preliminary Survey of Coliform Bacteria in Irrigation Canal Waters: A first attempt to answer why Arizona head lettuce is safe

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### Summary

Data of water quality in the irrigation canals was collected during 10 months (this an on-going study in its second-year stage ) and 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 through the year. Collection included sampling of 4 sites in the Yuma Valley on a weekly basis. On the same days 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 recorded. The results considered for this first-year report have shown that bacteria indicators have higher levels before and after winter months. For example total coliforms in one site dropped from levels of over 2000 cfu/100 ml to levels below 500 cfu/100 ml, then increasing again to higher than 2000 cfu/100 ml during early April. However, several spikes were observed during the winter which is a concern because some of those levels would not be acceptable using the metrics of the new Arizona Marketing Agreement. In this study we aim to collect results during a three-year period, which will allow enough data to draw conclusions that will be helpful for establishing future food safety regulations.

### 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, 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 second year as well. We submitted the samples to Silliker-AgriTrend Lab where analysis of bacteria was conducted following specifications of the Membrane Filtration Method m-Coliblu<sup>TM</sup> 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. For the second year (currently) we also 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.

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 weekly, regardless of the species. Birds roaming in a 200 feet diameter around the water sampling site were counted for 15 minutes before taking the water samples.

## **Results**

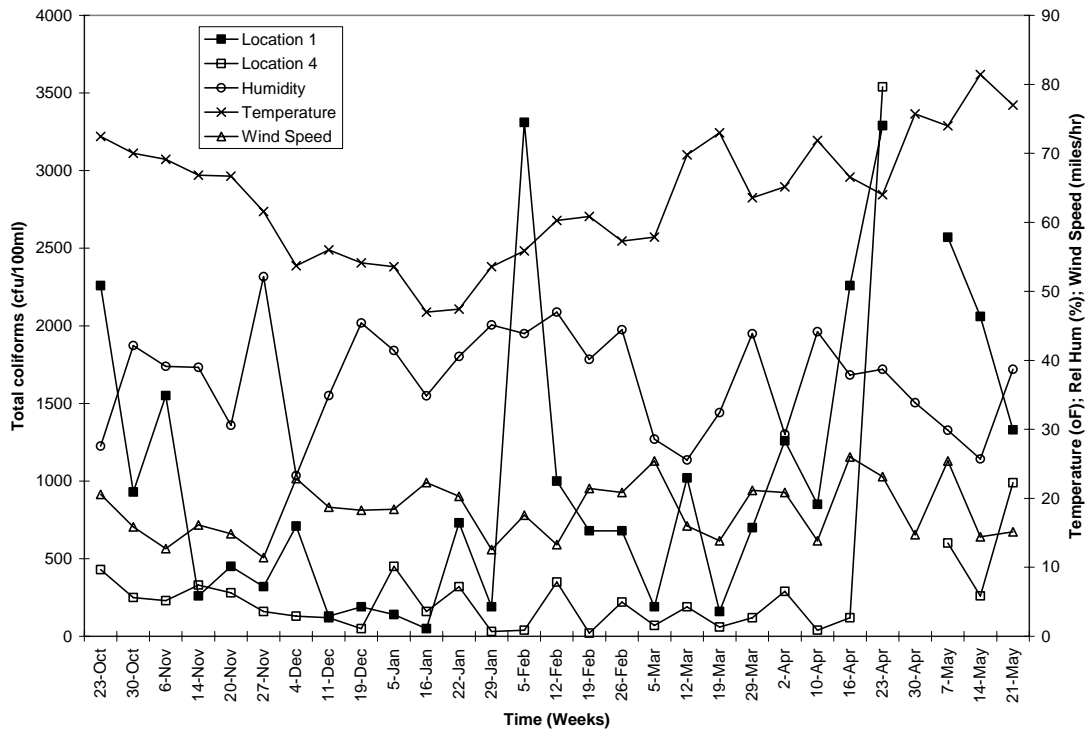
AILRC awarded us funds to make a first step of this water survey study. During the first stage of this project we have observed that coliforms and *E. coli* decline significantly during the winter showing a similar trend with that of temperature (Figure 1). It is relevant to mention that the low temperature during the winter accompanied with <60% relative humidity prevalent in Yuma, may substantially decline *E. coli*.

The new metrics used for the California Marketing Agreement and adopted for the Arizona Marketing Agreement, states that acceptable levels should include <126 cfu

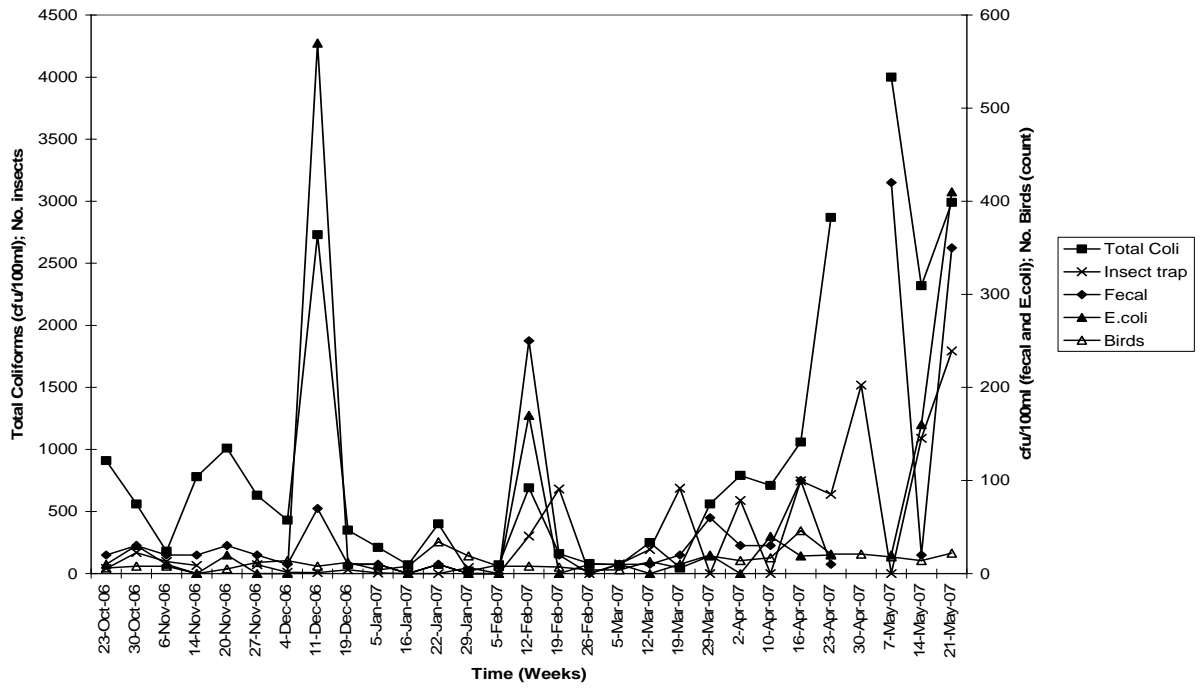
E. Coli/100 ml). However, we found that in certain weeks and locations “spikes” of the bacteria population are possible (Figure 2). This is a concern because it would imply exhaustive subsequent sampling under the new Arizona Marketing Agreement. No clear relationship has been observed to this point between bacteria indicator counts and bird/insect counts (Figure 3).

The results from the first year showed a trend that indicates that all indicator levels decline during the winter time. However, it is yet too early to make conclusive statements, since climatic conditions can vary from one year to another (e.g. no rainfall event was recorded last year in the Yuma Valley during the harvest season).

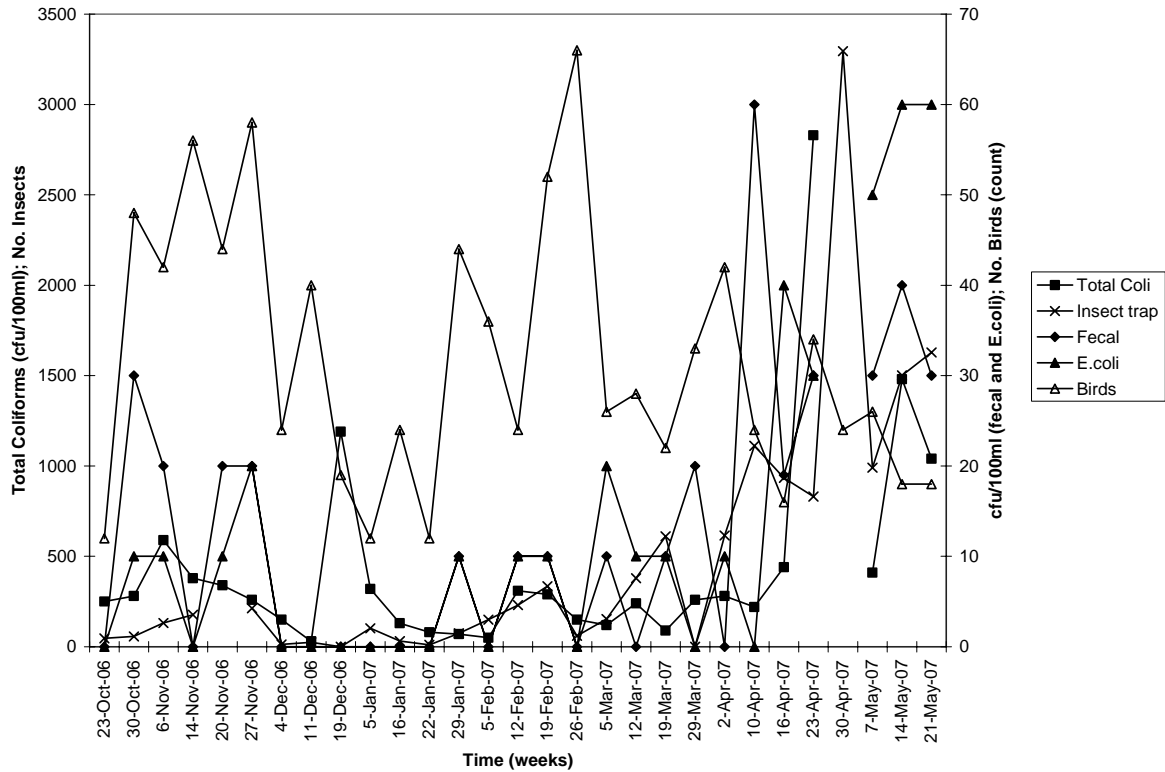
Lately, we have continued the study with funds from a second year AILRC award. The intention is to complete a 3-year study including sampling. Starting during the second year we have initiated with the analysis of E. coli O157. The goal is to compile the data of all three years and make it available to the AILRC, buyers and regulatory entities.



**Figure 1. Total Coliform counts in two sites of the Yuma Valley and environmental conditions prevalent during the period of sampling**



**Figure 2. Generic *E. coli*, fecal and total coliform in water, bird and insect in surrounding areas data obtained in a selected site.**



**Figure 3. Generic *E. coli*, fecal and total coliform in water, bird and insect in surrounding areas in a selected site.**

### 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. However, we have also observed “spikes” in the data that alert growers as this would involve more sampling (following the spike) according to the new Arizona Marketing Agreement. The benefits of the results from this project 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. Despite the fact that Arizona head lettuce has traditionally not been associated with foodborne outbreaks, this study is contributing with knowledge that can be a foundation for future prevention programs, and keep the industry growing.