FOOD SAFETY STUDY OF LEAFY GREENS IRRIGATED WITH TILAPIA FARM EFFLUENTS

Human Health Impacts of Aquaculture/ Experiment/ 07HH02UA

Pablo Gonzalez Alanis
[1]Universidad Autónoma de Tamaulipas
Carretera Villahermosa-Cárdenas Km. 0.5
Ciudad Victoria, Tamaulipas, México. CP 86000

Kevin Fitzsimmons
Department of Soil, Water and Environmental Science
Environmental Research Laboratory
University of Arizona
2601 E. Airport Dr.
Tucson, Arizona 85706

ABSTRACT

Aquaponics has drawn interest as a sustainable farming method merging aquaculture with hydroponics. Using the effluent from fish farming to irrigate and fertilize vegetables which filter the water so that it can be reused for fish production has many benefits but unknown risks. One potential concern would be the potential for pathogens from fish effluent splashing onto the edible portions of plants and then being passed to the human consumers of the plants.

To explore this concern, we developed an aquaponics system in a newly dedicated greenhouse and planted a lettuce crop in perforated styrofoam boards floating in a raceway filled with effluent from a tilapia production unit constituting a recirculating aquaculture system. The effluent and plant leaves were tested for bacterial counts. In an additional trial we developed a simple ultraviolet treatment system in an effort to reduce the level of bacteria in the water and on the plants.

We did detect low levels of several bacteria in the water and on the plants. The UV system did reduce these bacteria to non-detectable levels in most cases. In conclusion, we did not find any health hazard in the aquaponic system tested and we developed a simple UV treatment system that lowered the levels of the bacteria that were observed.

As an additional effort we developed an associated system utilizing tilapia and vegetable culture at the Conrado Castillo, a farming cooperative near Ciudad Victoria. The farm has been technically and economically profitable according to the farmers.

INTRODUCTION

In 2006, several nation-wide epidemics of *Escherichia coli* or related gastro-intestinal pathogens were traced to consumption of fresh vegetables (spinach, lettuce, green onions). In several of these cases the vector was thought to be contamination from human or animal wastes applied through irrigation water. As we encourage more organic farming methods and re-use of composted wastes and effluent waters from animal operations to irrigate field crops, we increase the opportunities for these types of contamination. One alternative is to increase the multiple-use of water for production of fish and use of these effluents for irrigation of vegetables that are bound for human consumption.

Across much of Asia, fish is the primary protein component of the diet and integrated fish and vegetable farming has been practiced for centuries. However, very few studies have been conducted to determine any health hazards that may result from this practice. The assumption is that fish being heterotherms (cold-blooded) and obviously not mammals, they are unlikely to be vectors for intestinal pathogens. However, fish farms do have human workers and ponds can be visited by farm animals and pets. Examination of typical aquaculture farm effluents and any residual potential pathogens that might be left on leafy vegetables irrigated with fish farm effluents, bound for direct human consumption would be an advisable precaution.

Bacteria have been identified in aquaculture systems that are considered human pathogens, such as fecal coliforms including *Escherichia coli* (Ogbondeminu, 1993; Flick, 1996; Del Rio-Rodriguez et al., 1997; Pullela et al., 1998), *Clostridium botulinim* (Pullela et al., 1998), *Pseudomonas* species (Nedoluha and Westhoff, 1995), Aeromonas hydrophila (Nedoluha and Westhoff, 1997) and *Salmonella* species (Ogbondeminu, 1993) to name a few. Some typical fish pathogens have also been known to cause illness in humans. The microflora of the fish gills, skin, and digestive tract have been shown to reflect the microflora of the water they inhabit and may also pose a threat to humans (Reilly and Käferstein, 1997; Nedoluha and Westhoff, 1997; Ogbondeminu, 1993).

Preliminary studies at the University of Arizona demonstrated that total coliforms levels of 10^4 CFU's/100ml and fecal coliform levels of 10^3 CFU's/100ml can be found in two separate recirculating systems rearing grass carp and tilapia (McKeon et al., 2000; McKeon, 1998). Samples from this system were found to contain total coliform levels as high as 10^4 CFU/100 ml. Fecal coliform levels were found to be variable over a test period of two months. Counts were as low as 1 CFU/100 ml to as high as 10^3 CFU/100 ml. These levels indicate the possible presence of human enteric pathogens that could cause illness in fish handlers and consumers if proper precautions are not observed. However, using the Colilert rapid detection test we had a negative result for E. coli presence. The source of the coliforms is unknown.

MATERIALS AND METHODS

We proposed that complementary lab and field studies be performed to examine the bacterial flora typically encountered in in-door and out-door aquaculture operations. Along with this we would grow lettuce and spinach and in greenhouses and in the field which would be irrigated with effluents from attendant fish (tilapia) production facilities. An indoor – outdoor system was evaluated in a newly dedicated research building constructed to support the AquaFish Research program. Six round fiberglass tanks containing 5,000 liters of water were stocked with 200 tilapia (*Oreochromis niloticus*), with an average weight of 140 grams each and a total length of 20 cm. The fish were fed a 30% protein diet at a rate of 5% of the biomass of fish per tank, split between three feedings by hand per day. Water temperature was maintained at 22 degrees C. during the trials. The six tanks were contained inside a building.

The effluent water from each tank was fed to a biofilter containing plastic media to capture solids and increase dissolved oxygen before the water entered the hydroponics beds. Lettuce (*Lactuca sativa*) and spinach (*Spinacia oleracea*) were stocked onto floating sheets of styrofoam (floating bed technique) placed on top of the water in the hydroponics bed. 287 plants were transplanted from a seed starting table to the growing boards in each

recirculation raceway for each trial (lettuce in summer and spinach in winter). The hydroponic raceways were 7,500 liters each and were constructed adjacent to the fish production building containing the tanks.

Water and plants samples were collected from the University of Tamaulipas UV system, Veterinary College, fish and plants were grown during summer, fall and winter during 2008 and 2009 at different intervals. Leaf samples are removed randomly from the growing lettuce and spinach plants. Samples were collected and stored in sterile plastic bottles and bags. They were immediately placed in an ice chest and transported to the Veterinary college water quality analysis laboratory for analysis.

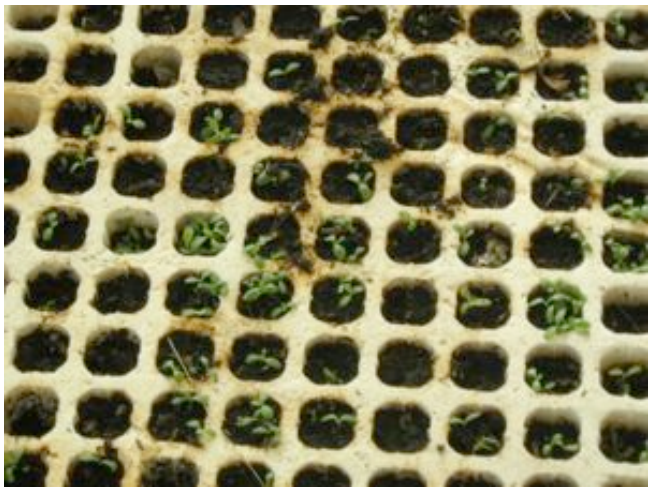


Fig. 1 Lettuce plants germinating

1. Greenhouse studies

Tilapia production facilities at the Universidad Autonoma de Tamaulipas utilize recirculation technology to rear fish. These systems incorporate biological filtration, which includes nitrifying and heterotrophic bacteria to treat water so that it can be recycled to the fish tanks for continuous rearing of fish in the same water.

Fig. 2. Student from the UAT planting lettuce in styrofoam floating on the trough of effluents.

The effluent water and a control water supply, tap water from the city water source, used for irrigation would be sampled during each irrigation event (every 3rd day for the first three weeks and 5th day for the final nine weeks). Crops cycle times for the lettuce and spinach were 7 weeks and 10 weeks respectively.

At the end of the growing cycle, leaves from the portions of the plant typically harvested for commercial sale will be collected and sampled for total fecal coliforms, E. coli, enterococci and Salmonella. Total colony forming units (CFU's) will be determined and reported on a per 100 ml basis.

2. Field Studies

Tilapia production facilities at the Ejido Conrado Castillo utilize a pond system with source water from wells passing through

two ponds stocked with fish before use as irrigation water for spinach and lettuce. These farmer cooperators agreed to provide matching support to the project and provided ponds, liners, plumbing and field preparation in addition to the care and feeding of the fish and tending the vegetables.

The field plots required more frequent irrigation in the dry field environment, with the exact irrigation schedule dependant on weather conditions. Sample schedule was not as frequent as the university lab testing but did encompass five sample dates during the irrigation schedule. At the end of the growing cycle, leaves from the portions of the plant typically harvested for commercial sale were collected and sampled for total fecal coliforms, E. coli, enterococci and Salmonella. Total colony forming units (CFU's) was be determined and reported on a per 100 ml basis.

LAB ANALYSES.

Fecal coliforms will be isolated by membrane filtration of the sample and direct plating of the filter on mFC agar according to Standard Methods (APHA, 1998). For E. coli isolation, a presumptive test involving lauryl sulfate tryptose (LST) broth will be employed. Positive presumptive tests (gas production) will be followed by the tests with E. coli (EC) broth (Hitchins et al., 1992).

Enterococci will be isolated according to Standard Methods (APHA, 1998) using membrane filtration and m Enterococcus agar for determining the presence of fecal streptococci. Confirmation will be performed after transferring typical colonies from a membrane to the surface of a brain-heart infusion (BHI) agar and incubation. A catalase test and gram stain of the BHI isolate will be done as well as observations of growth on bile esculin agar and growth in BHI broth containing 6.5% NaCl to confirm the presence of enterococci (APHA, 1998; Hartman et al., 1992).

Salmonella pre-enrichment will be accomplished using lactose broth (Flowers et al., 1992) and selective enrichment will subsequently be performed using a ducitol selenite broth. After enrichment subcultures will be performed on Salmonella Shigella (SS) agar (Difco Laboratories, 1984) and Xylose lysine desoxycholate (XLD) agar (APHA, 1998). Following incubation, an oxidase test and indole test will be performed. A short set of biochemical tests will be followed by inoculation of an API 20E strip as per directions by API (Flowers et al., 1992; APHA, 1998). Testing with Salmonella antiserum will conclude the confirmation tests (APHA, 1998).

STATISTICAL ANALYSES

We compared total fecal coliform, enterococci and Salmonella densities (Colony Forming Units per 100 ml) among treatments with a two-way ANOVA where season and irrigation treatment were the two independent factors. There were three levels for the Season factor (winter, spring, and summer) and two levels of the Irrigation-treatment factor (tap water/well water, and effluent water). Analysis for statistically different levels of potential pathogenic bacteria in the fish effluent and control water used to irrigate vegetables will be determined. Statistically different levels of potential pathogenic bacteria actually collected off the edible portions of the plants were examined. SysStat software will be utilized to facilitate analysis.

POTENTIAL IMPACTS

If levels of pathogenic bacteria in fish effluent are determined to be low enough for safe use as irrigation water, these results would be useful for developing farm management

strategies that will insure production of high-quality crops in integrated production systems based on aquaculture effluents.

The aquaculture program of the UAT (Universidad Autónoma de Tamaulipas, Facultad de Medicina Veterinaria y Zootecnia "Dr. Norberto Treviño Zapata") has developed aquaculture systems which are being adapted from the US (University of Arizona models) and also incorporating ideas from Indonesia, Italy, Korea, Egypt and other Central American countries. As implementation of these systems proceeds in these locations, information about health and environmental issues have to be monitored and recorded in order to report the presence of human bacterial pathogens in the aquaculture system water and the potential for contamination and consequently, negative impact to consumers and environment.

The aquaculture systems studied in this research were conducted in both indoor closed and outdoor environments and accessible by birds and small mammals. Feces from these animals can contaminate the water with coliforms and other pathogenic bacteria. This research evaluated water and plants samples from both systems over a 2 year period to determine:

a. The presence of total and fecal coliforms, salmonella and enterococci
b. And if the UV treatment provided any significant difference.
c. And reported the number of organisms.

METHODOLOGY

The project first needed to develop and operate a new aquaponics system at UAT. The building and greenhouse addition with tanks and growing beds were built with state funds in specific support of the AquaFish CRSP grant. The system was operated for several months before the trial started in order to develop the biofilter and other biotic community in the aquaponics unit.

Standard Methods were utilized to identify and enumerate the bacteria in the system water and on the roots and leaves (APHA 1998). The specific tests were:

NOM 112-SSA1-1994 for coliforms
NOM 092-SSA1-1994 for aerobics bacteria
NOM 114-SSA1-1994 for Salmonella

In all standard techniques (SSA), the laboratory performed the presumptive portion of the multiple-tube test. This test uses 9 tubes of lauryl tryptose broth, each of which was inoculated with a different dilution of UV aquaponics system water.

The estimation of bacterial density, was made positive results from the fermentation

Table 1:

INDOORS WATER SAMPLES					
TOTAL COLIFORMS			FECAL COLIFORMS		
Tank	Mean	SD	Tank	Mean	SD
1	0.207	0.265	1	0.112	0.066
2	0.609	0.348	2	0.599	0.105
3	0.157	0.092	3	0.118	0.076
4	0.748	0.328	4	0.452	0.060
5	0.192	0.165	5	0.137	0.097
6	0.854	0.372	6	0.618	0.206

Fecal coliforms procedure was used to determine the presence and number of fecal coliforms. This test is conducted after Total coliform technique has confirmed the presence of coliforms. The presence of bacterial density is determined by the most probable numbers (MPN) reported organisms in a 100 mL, sample. The results of these methods are included in Table 2.

INDOORS Lettuce			Mean	SD	Spinach			Mean	SD
UV	Roots	TOTAL COL	0.145	0.098	UV	Roots	TOTAL COL	0.100	0.011
		FECAL COL	0.095	0.085			FECAL COL	0.090	0.016
	Leaf	TOTAL COL	0.14	0.087		Leaf	TOTAL COL	0.025	0.057
		FECAL COL	0.075	0.034			FECAL COL	0.115	0.064
NT	Roots	TOTAL COL	0.09	0.040	NT	Roots	TOTAL COL	0.115	0.077
		FECAL COL	0.105	0.075			FECAL COL	0.010	0.011
	Leaf	TOTAL COL	0.06	0.034		Leaf	TOTAL COL	0.105	0.010
		FECAL COL	0.06	0.034			FECAL COL	0.155	0.086

Fig. 4. Newly transplanted lettuce plant in aquaponics trough.

Fig. 5. Fully grown lettuce on the floating bed, ready to be harvested.

RESULTS

Figure 6. TOTAL COLIFORMS IN SYSTEM WATER

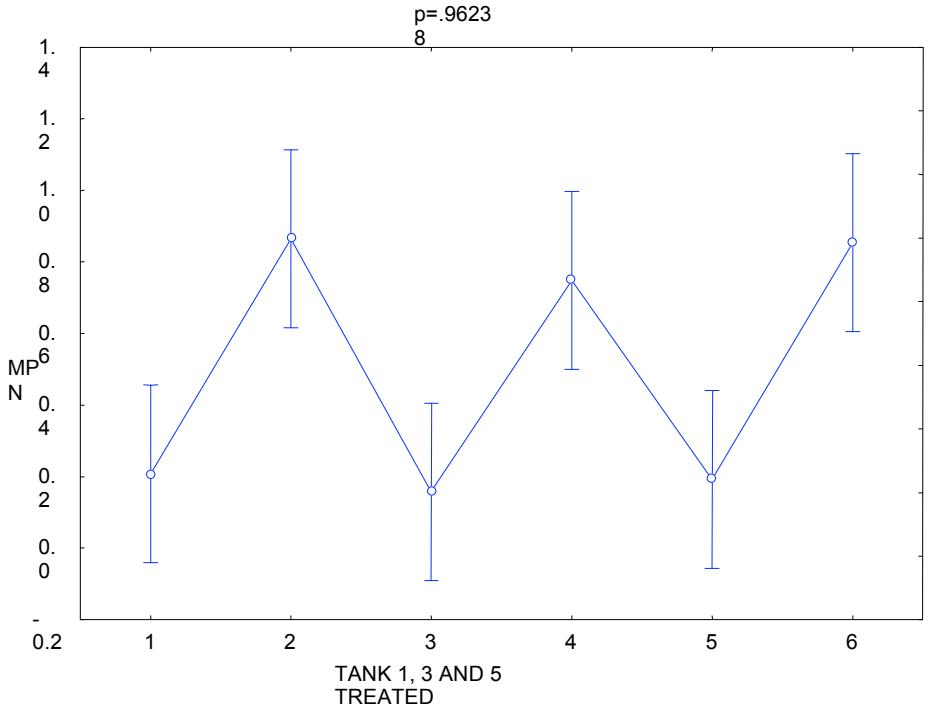


Figure 7. FECAL COLIFORMS

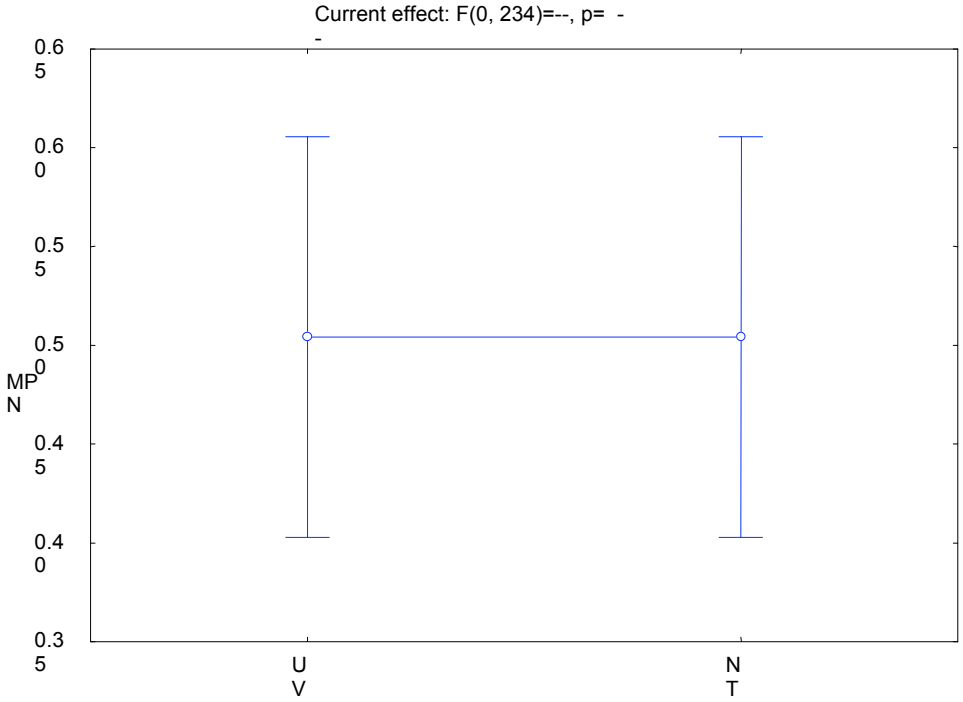


Figure 8.

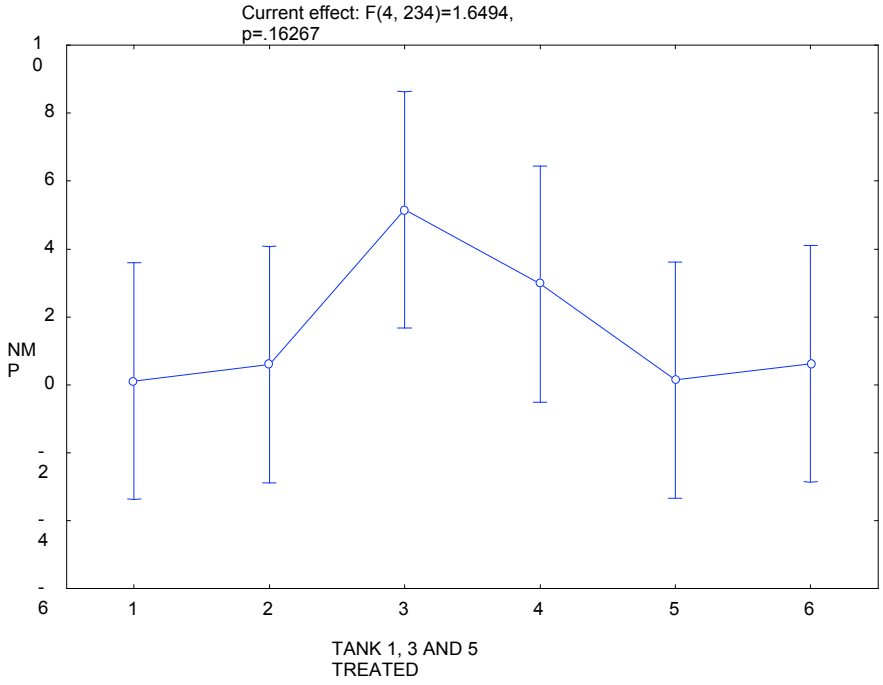
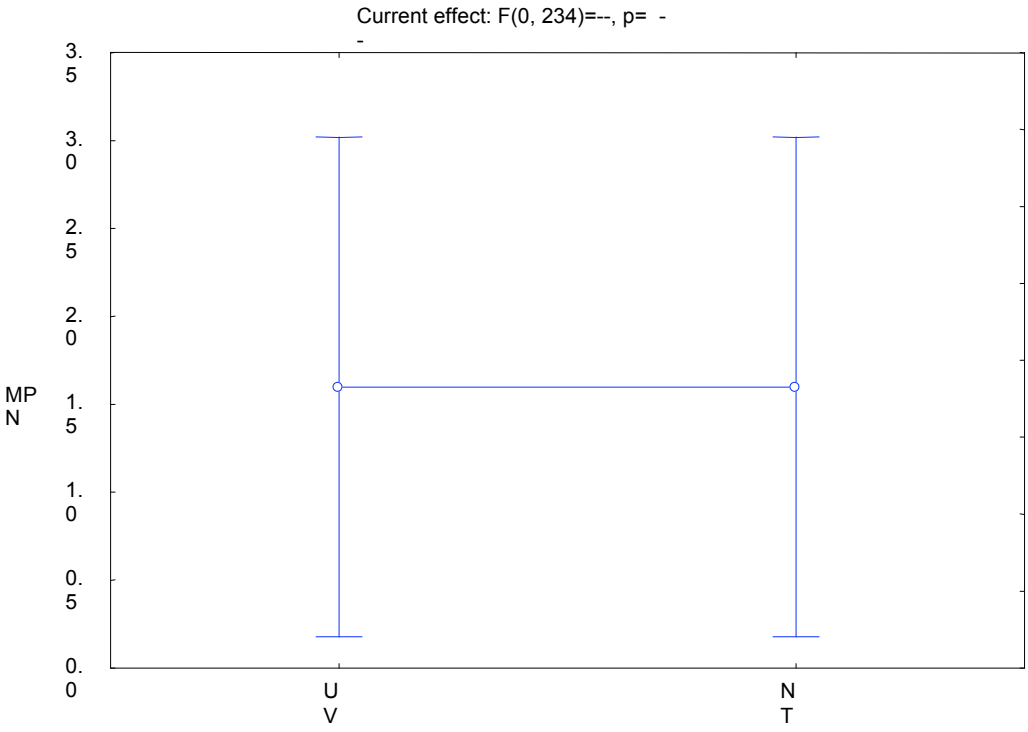
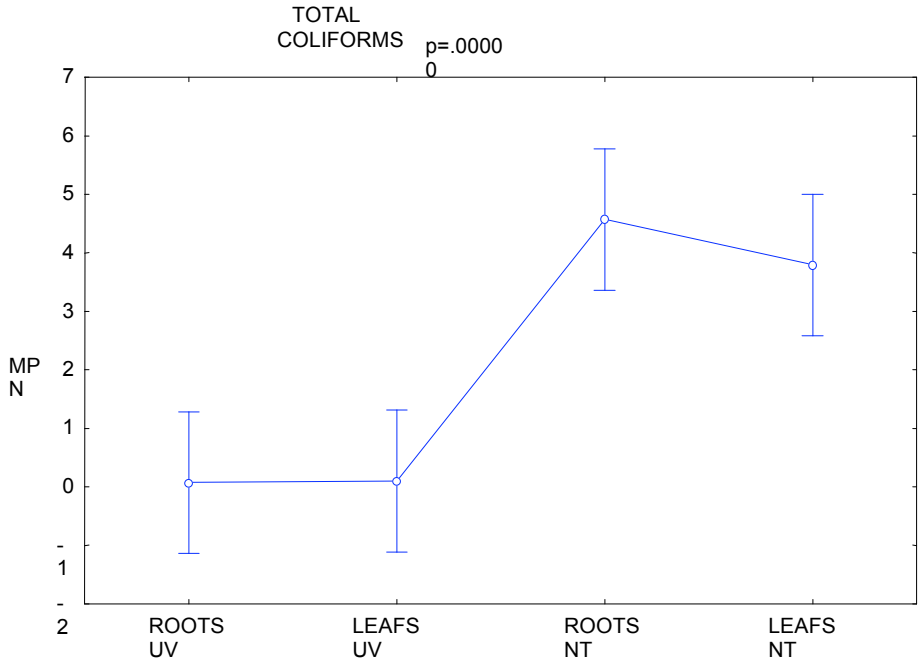


Figure 9. Coliform counts on spinach leaves.



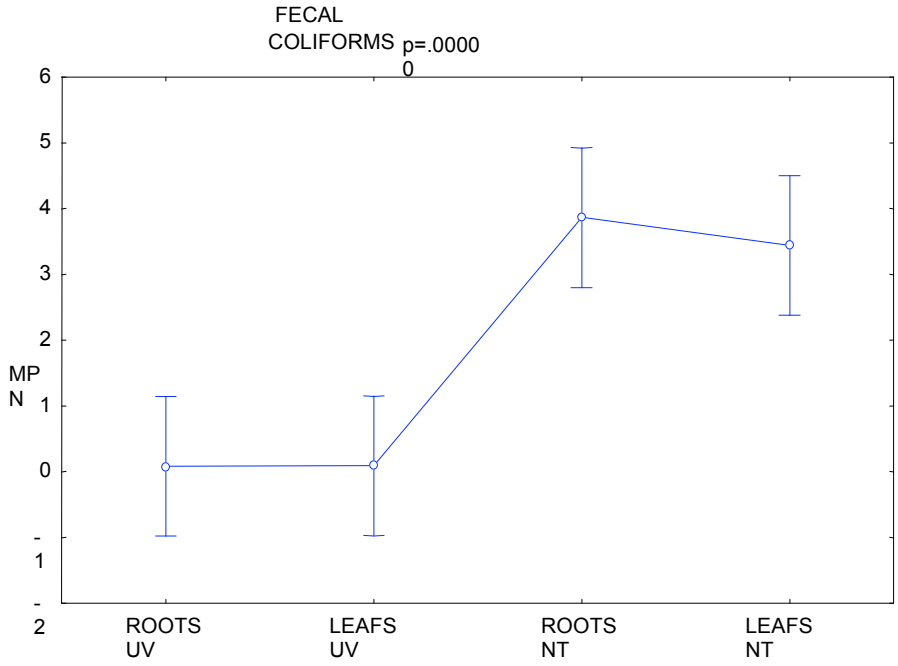
SPINACH - TOTAL COLIFORMS

Figure 10. Total COLIFORMS



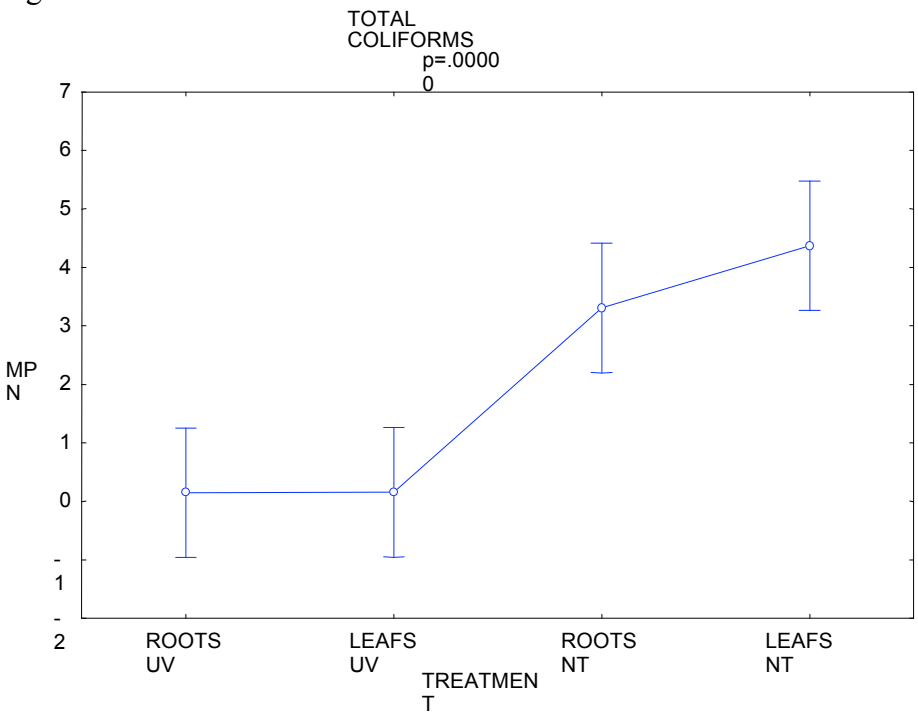
Effect	SS	Degr. of Freedom	MS	F	p
Intercept	364.2311	1	364.2311	49.21482	0.000000
TRATAMIENTO	341.8653	3	113.9551	15.39759	0.000000
Error	562.4640	76	7.4008		

Figure 11. FECAL COLIFORMS



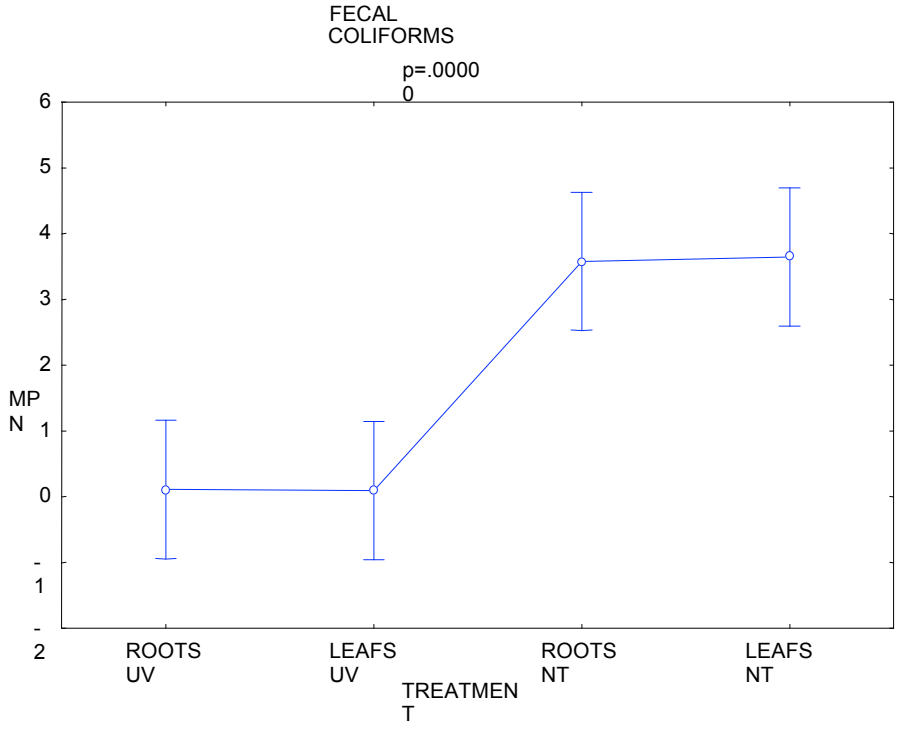
Effect	SS	Degr. of Freedom	MS	F	p
Intercept	279.4903	1	279.4903	49.18566	0.000000
TRATAMIENTO	256.0980	3	85.3660	15.02300	0.000000
Error	431.8588	76	5.6824		

Figure 12. LETTUCE - TOTAL COLIFORMS



Effect	SS	Degr. of Freedom	MS	F	p
Intercept	318.5217	1	318.5217	51.60458	0.000000
TRATAMIENTO	283.4483	3	94.4828	15.30742	0.000000
Error	469.0988	76	6.1724		

Figure 13. FECAL COLIFORMS



Effect	SS	Degr. of Freedom	MS	F	p
Intercept	276.4333	1	276.4333	49.70261	0.000000
TRATAMIENTO	246.6937	3	82.2312	14.78514	0.000000
Error	422.6927	76	5.5617		

Figure 13. TOTAL COLIFORMS

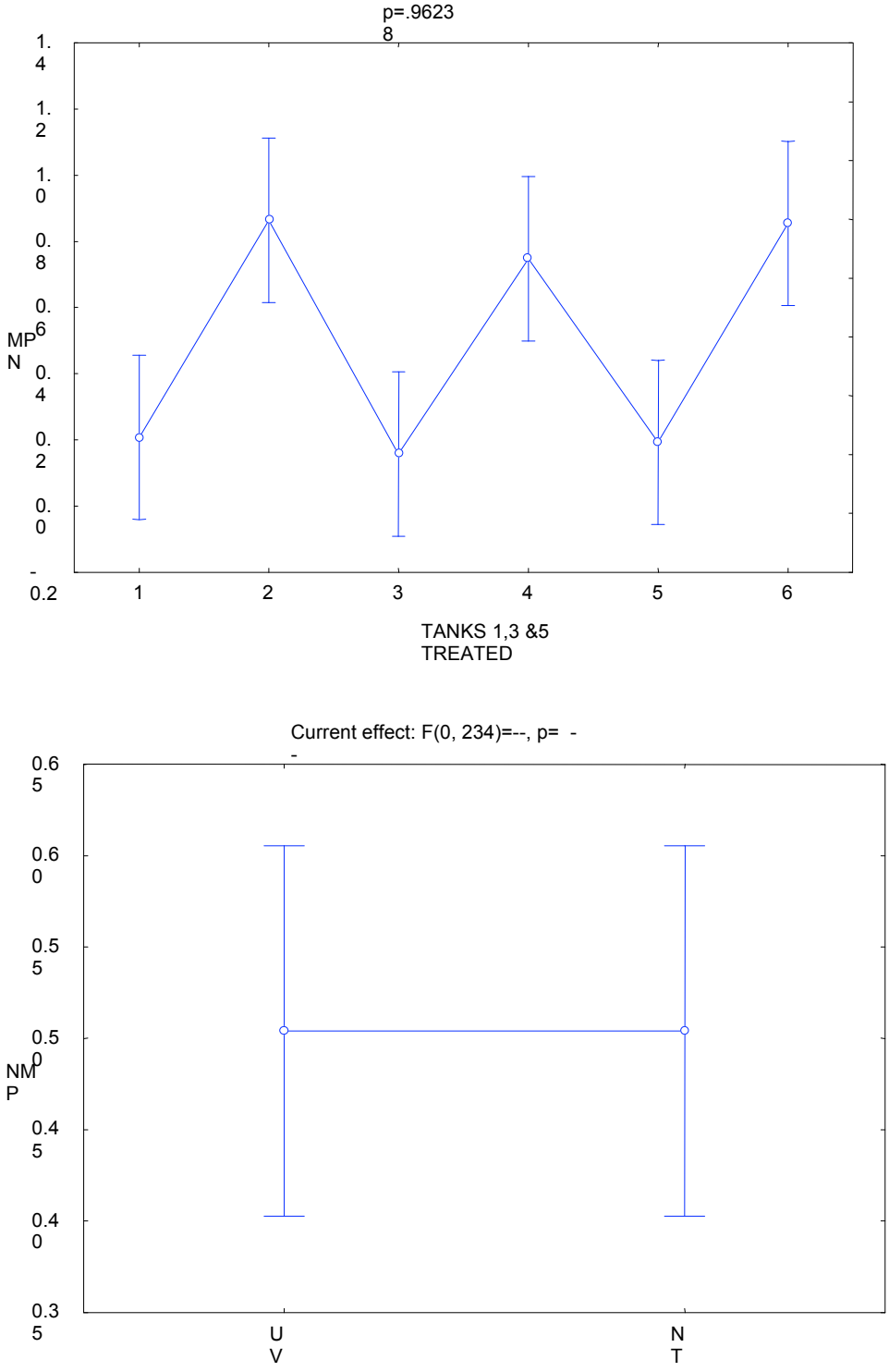
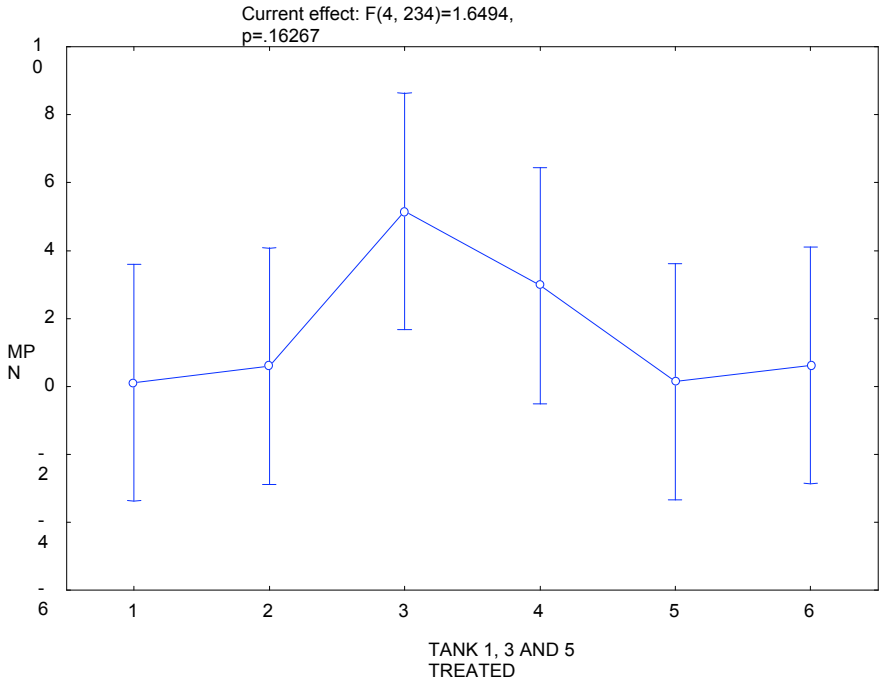


FIGURE 14. FECAL COLIFORMS



PRINCIPAL FINDINGS AND CONCLUSIONS

In the aquaponics system constructed at the UAT, the system was found to be technically feasible with good growth of fish, lettuce and spinach. Water samples from each sampling date and each system found total, and fecal coliforms in measurable numbers. However, Salmonella, E. coli, and Enterococci were negative.

The levels of the coliforms were all in the range of typical background numbers found in nature and not indicative of contamination. However, as any level could be seen to be undesirable, a system to lower the level was devised. This UV Treatment provided a significant reduction of one order of magnitude in total coliform counts in the recirculating aquaponics system. The second system constructed at the Ejido Conrado Castillo was also technically successful. The farmers reported producing commercial quantities of tilapia and vegetables in addition to their household consumption.

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