

Study Title: Addressing Current and Proposed Requirements for Good Agricultural Practices for Adoption by Established and New Growers in Massachusetts and Educating Growers about Food Safety Production and Handling Practices.

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Introduction: Between June 2014 and October 31, 2014, microbial samples were taken from the University of Massachusetts Research Farm in South Deerfield, MA to identify potential food safety risks which may be present on the facility. Water samples were also taken from the nearby Connecticut River and compared to new agricultural water regulations outlined in the Food Safety Modernization Act (FSMA). This report serves as a summary of the data collected as well as to identify potential risk sites.

Water Testing

Beginning on June 13, 2014 water samples were collected from two spots on the Connecticut River each week and tested with the Quanti-Tray 2000 MPN system. The first site, designated “R1”, is directly over the Connecticut River on the bridge leading into South Deerfield, MA. The second site, “R2” is a small area 2.5 miles north of R1 and seems to serve as a popular fishing and canoeing site and is up river from the “R1” site.

Methods

Five samples were collected at each site using autoclaveable 125 mL samples jars. Each sample jar was fitted with two 2 ounce fishing weights and tied to the end of a fishing pole. The bottles were cast into the water, allowed to sink to the bottom, and reeled in to be sealed and place in a cooler until testing.

The new FSMA water regulations require that agricultural water have no more than 250 CFUs (or MPN) of generic E. coli in any 100 mL water sample or no more than 126 CFU/MPN for a rolling geometric mean of five samples. These FSMA rules would require water found with any more E. coli than mentioned above to be discontinued from agricultural use.

<u>Date</u>	<u>Average MPN</u>	<u>Average E. coli MPN</u>
6/13/2014	1028.92	35.19
6/20/2014	1625.58	38.07
6/27/2014	2500.00	1950.14
7/3/2014	2500.00	1160.59
7/10/2014	1265.81	32.86
7/17/2014	824.18	305.16
7/24/2014	1559.13	93.73
7/31/2014	2500	109.73
8/8/2014	819.59	38.01
8/15/2014	2500.00	1090.69
8/22/2014	559.41	15.52
8/29/2014	653.88	115.74
9/19/2014	501.9125	22.50
10/3/2014	1756.457143	173.89
10/31/2014	586.17	35.79

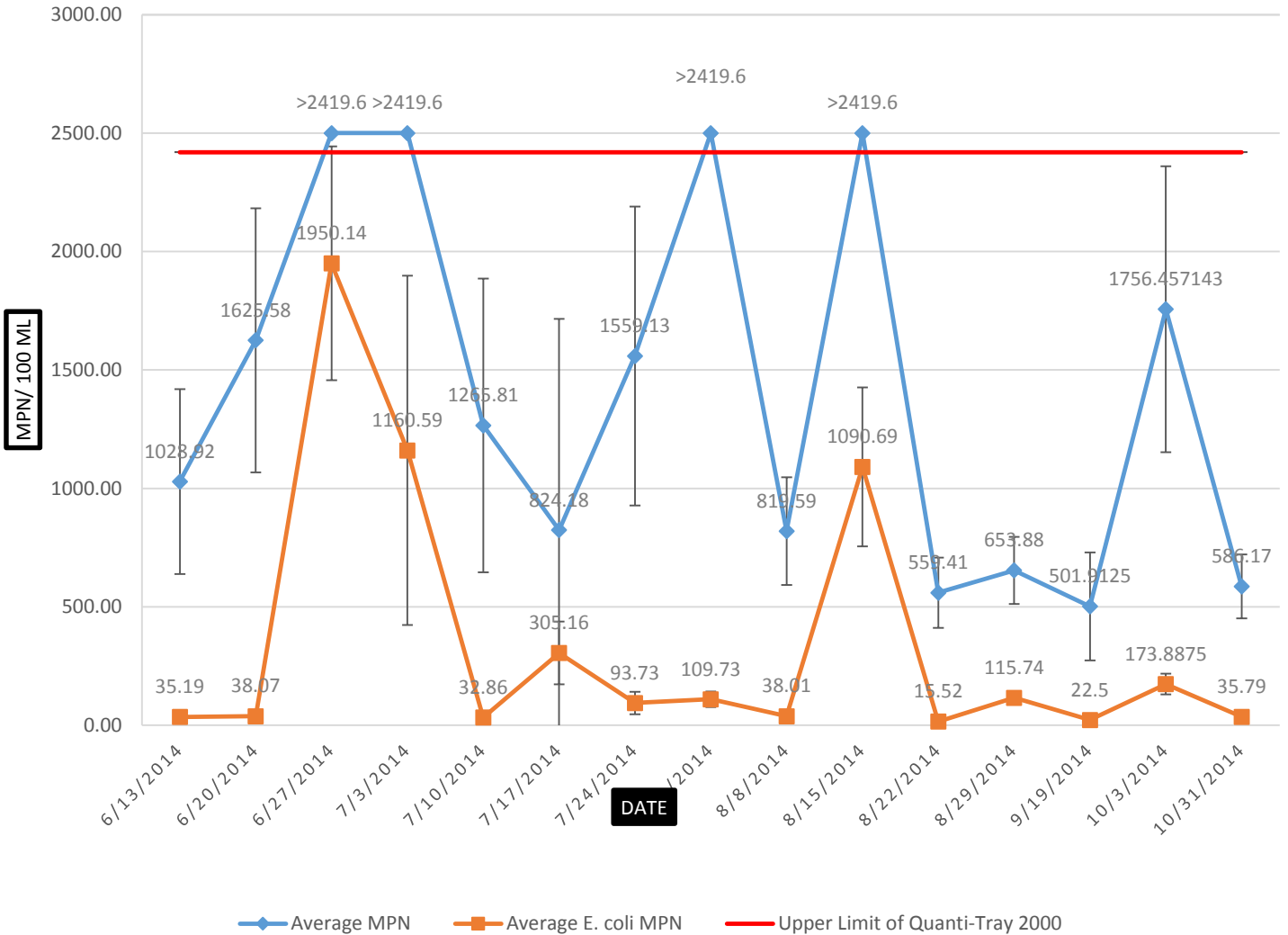
Date	R1 Average MPN	R1 Average E. coli MPN
6/13/2014	1137.26	47.16
6/20/2014	1373.16	33.08
6/27/2014	2500.00	2105.16
7/3/2014	2500.00	1486.10
7/10/2014	1320.88	31.04
7/17/2014	2137.92	394.64
7/24/2014	1907.76	124.74
7/31/2014	1849.02	85.96
8/8/2014	945.5	50.00
8/15/2014	2500	1228.62
8/22/2014	531.16	16.58
8/29/2014	653.88	115.74
9/19/2014	398	21.55
10/3/2014	2094.65	200.88
10/31/2014	524.22	31.72

Date	R2 Average MPN	R2 Average E. coli MPN
6/13/2014	920.58	23.22
6/20/2014	2195.60	43.06
6/27/2014	2500	2108.96
7/3/2014	2500.00	1383.20
7/10/2014	1210.74	34.68
7/17/2014	1424.94	215.68
7/24/2014	1382.6	62.72
7/31/2014	2500	133.50
8/8/2014	693.68	26.02
8/15/2014	2500	952.76
8/22/2014	587.66	14.46
8/29/2014	0	0.00
9/19/2014	605.825	23.45
10/3/2014	1604.15	146.90
10/31/2014	648.12	39.86

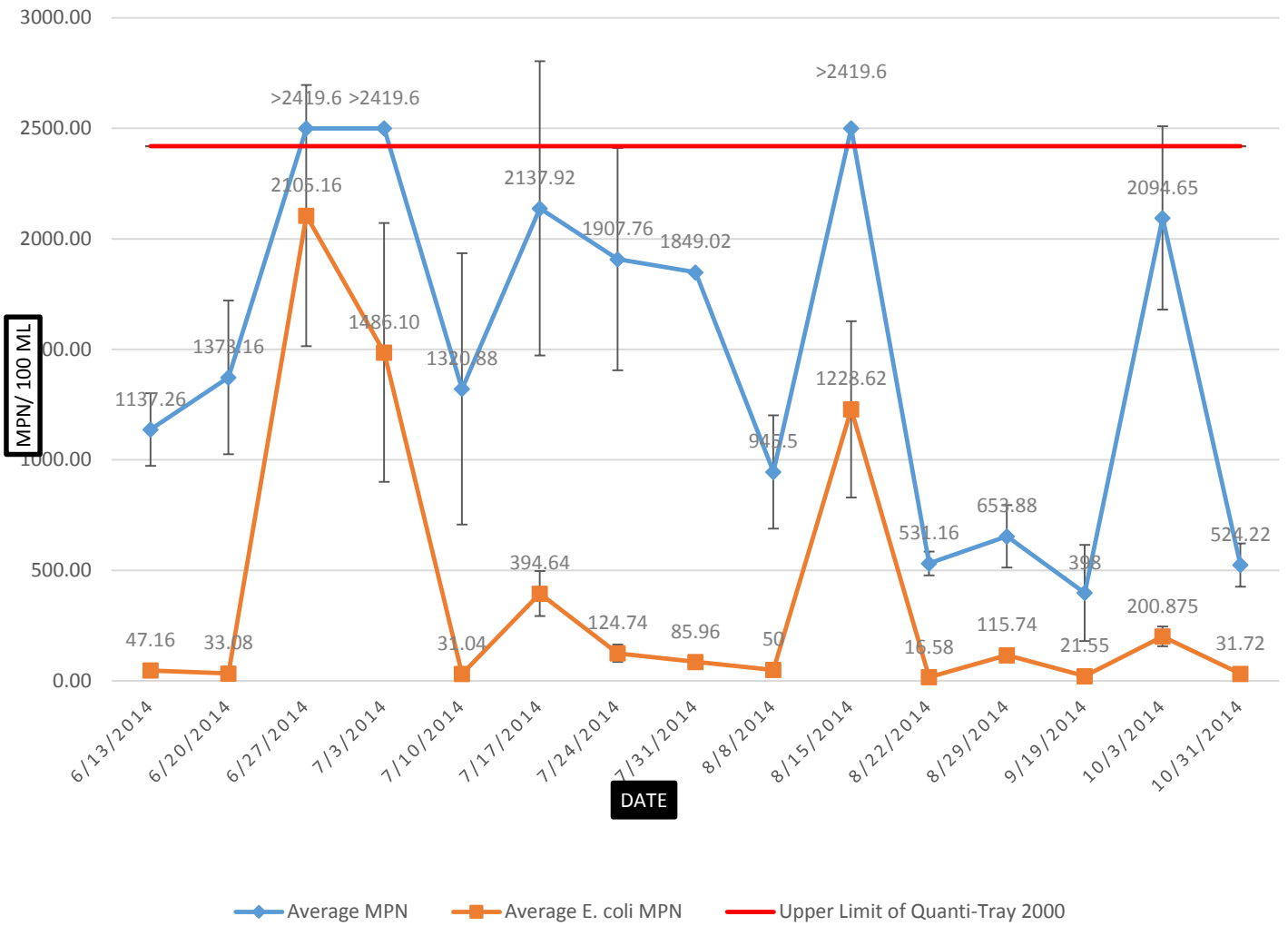
Summary of MPN from samples taken from the Connecticut River. E.coli MPN was determined with fluorescent wells in the Quanti-Tray 2000 system.

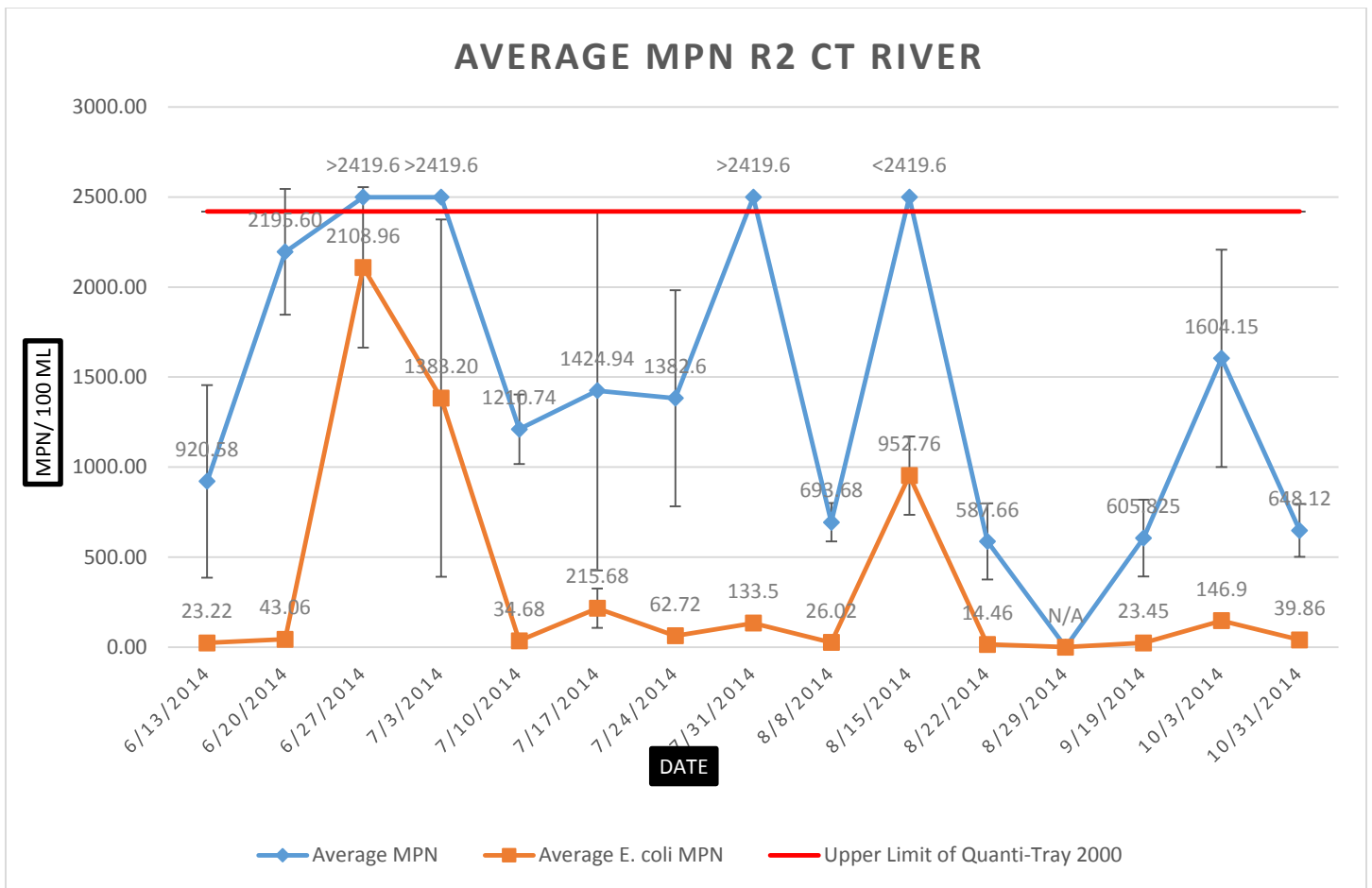
Values in yellow would violate the new FSMA rules as proposed January 2013 in Subpart E regarding agricultural water and would have to be discontinued for agricultural and corrective action be required.

AVERAGE MPN FROM CT RIVER



AVERAGE MPN R1 CT RIVER





Discussion

Environmental conditions on sampling days have also been monitored and recorded over the course of sampling. The days where the *E. coli* concentration of the water exceeds FSMA requirements saw high rainfall in short periods of time prior to samples being taken. *E. coli* concentration and river height did not show a definite correlation other than through observational information on the height of the river. It appears more likely that *E. coli* concentration is dependent on several factors including the rate of rain fall during a given period of time. It is possible that combined sewage overflow may contribute significantly to the *E. coli* levels of the Connecticut River following heavy rainfall due to sewage runoff. Agricultural runoff from farm manure may also be a contributing factor to the increase in *E. coli* concentrations after high rainfalls. Corrective action for farms using this water source could include a chemical treatment with a sanitizer such as chlorine, a heat treatment, or any other scientifically verifiable method. Discontinuation of the water source for a determine amount of time until the *E. coli* levels go below maximum levels is another potential corrective action.

Farm Samples

Beginning June 6, 2014 microbial samples were collected from the University of Massachusetts Research Farm in South Deerfield, MA. Three main types of samples, Swab, sponge and soil samples were taken weekly from a variety of sites on the facility. Areas tested include the packing area, soil and gravel from the large tobacco barn, the repurposed milking area connected to the packing area, any doors and floors used to navigate between areas, compost as well as the bathroom facilities provided for individuals in the packing area. Hand samples were also taken on three occasions from consenting individuals in both the packing house as well as out in the field.

Methods

Swabs were used to take samples from difficult to reach and irregularly shaped areas such as small crevices, door knobs, and inside processing machinery. Swabs were taken and placed in a cooler for transport, placed in a refrigerator until use and tested within 24 hours of collection. Swabs were added to a whirl-pack bag and 10 mL of peptone water was added on top of the swab. The swab was massaged inside the liquid for one minute, the liquid was expelled and the swab was discarded. 1 mL samples of the resulting liquid were diluted appropriately and plated on 3M APC and *E. coli*/ Coliform petrifilm.

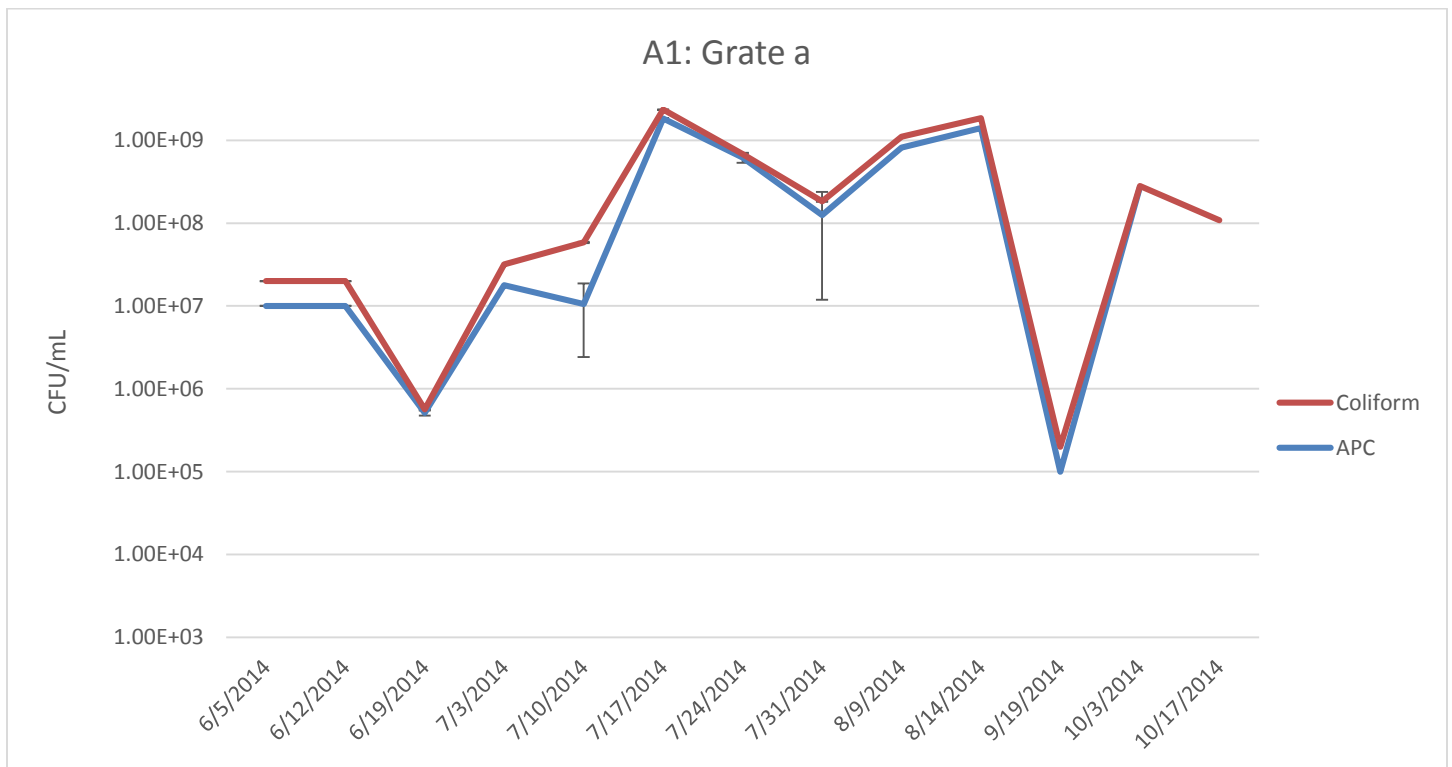
Sponges were taken, transported in a cooler, and refrigerated until use for no more than 24 hours. Sponges were massaged in sample bag for 60 seconds, liquid (10 mL) was expelled and tested the same as sponges. Soil and compost samples were collected using autoclaved aluminum soil corers, placed in a cooler for transport, and refrigerated no more than 24 hours before use. Samples of soil weighing 25 grams were measured and added to 250 mL of peptone water in a filter bag. Samples were then tested the same as previous samples.

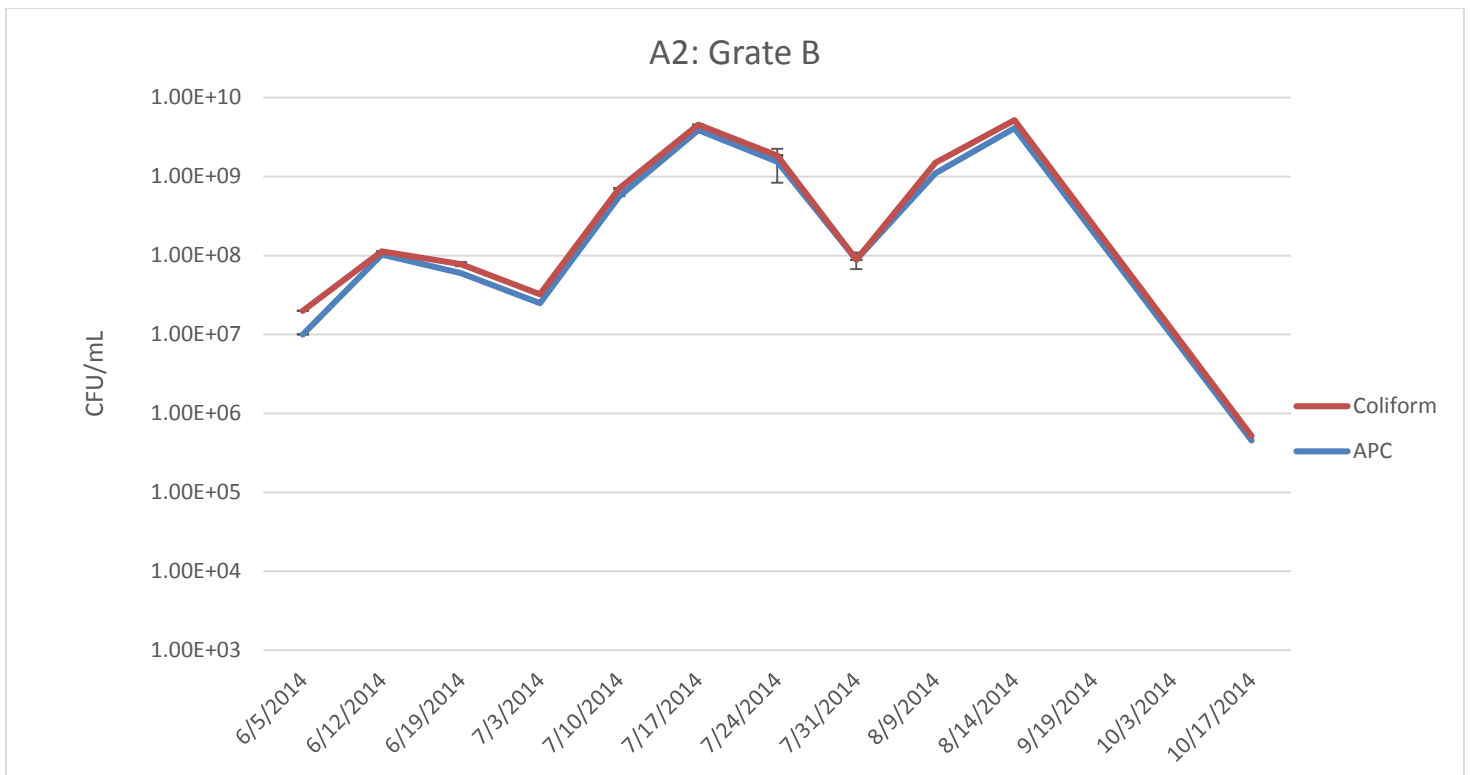
Potential Risk Areas

Throughout monitoring, several sites were sample frequently as they showed the potential to be contaminated with *E. coli* at very high or very variable amounts. These sites include the two drains in the packing area, sink drains and handles, various parts of the produce washer machine, worker hands, and compost samples. Some other areas showed little risk or relatively stable levels of organisms.

Packing Area Floor Grates

Two floor grates in the packing area were sampled very frequently using the sponge method described above. One grate (A1) was located on the ground closest to the front door while the other (A2) was located at the back of processing area closer to the door to the old milking area. Both grates were covered by removable bars which were sampled once and shown to have lower levels of organisms than the grate directly above the drain. These two drains in the grates seemed to collect large amount of debris from processing and packing which lead to a variable and often high level of organisms including the occasional E. coli as noted by blue colonies on the E. coli/coliform petrifilm. E. coli was found on 7/3/2014 on both A1 and A2 and 7/10/2014 on A2. Results from the sampling of these grates are expressed graphically below.





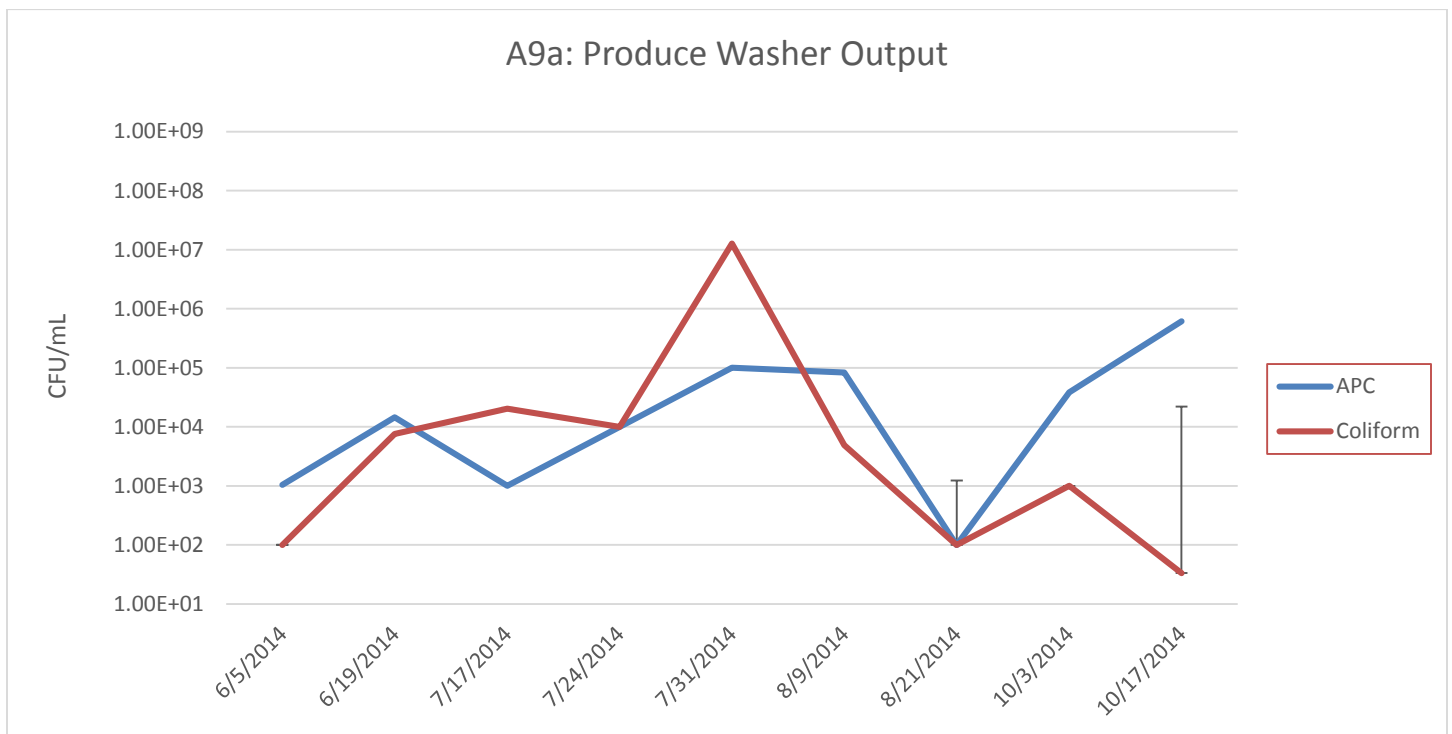
Note: Values from 9/19/2014 are from the grate covering the drain and not the drain itself as other times. Numbers are lower but potentially show how organisms from the drain can be spread to the top of the grate and potentially to produce being processed

Evaluation

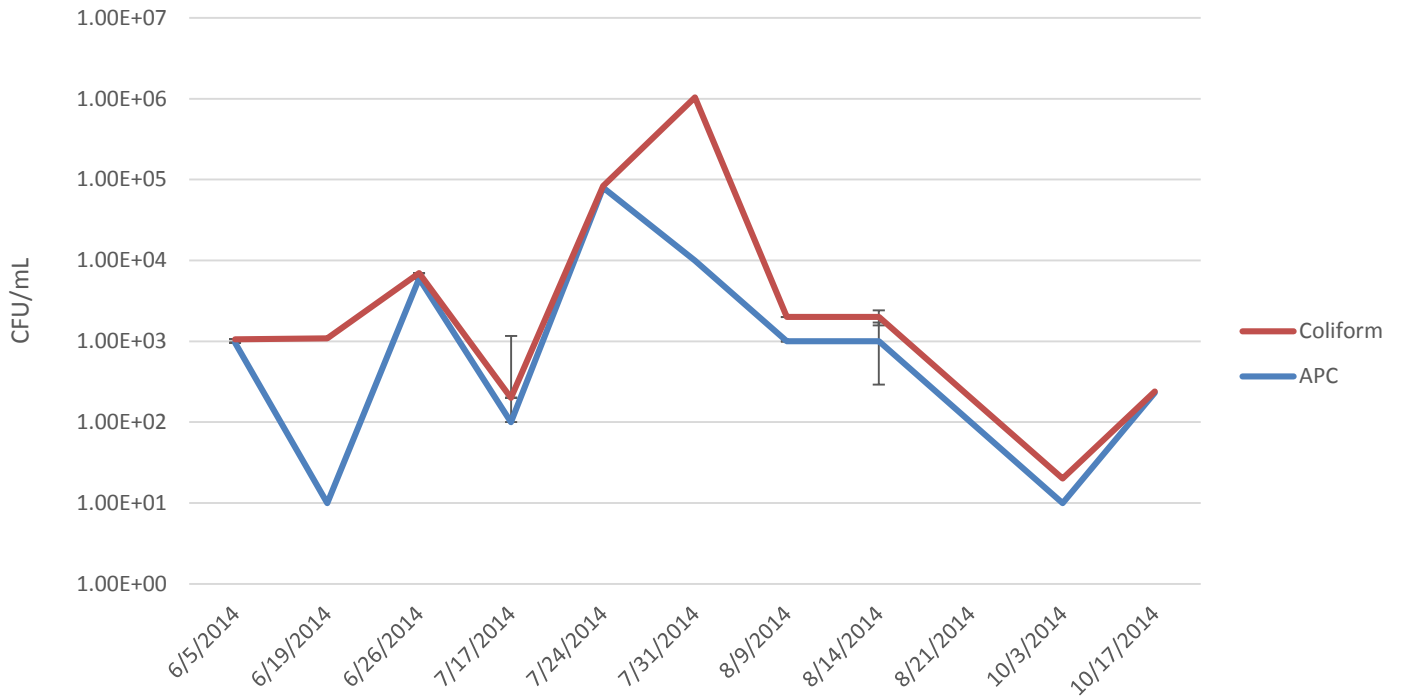
The two grates sampled in the packing area were the most variable in their CFU counts as well as having the highest coliform counts of any other sample, sometimes exceeding 10^9 CFU just on the area of the small drain grates. Note that counts from 9/19/2014 were taken from the larger removable grate from above. While the grate covering prevents produce from coming in direct contact with the drain, there is potential for organisms to be transferred via splashing of water going into the drain during processing. A standard operating procedure for cleaning these grates should be established to limit the risk that these two drain grates pose to contamination of produce during processing. Further improvement could include replacing the drain as they are rusted and filled with small crevices which could potentially harbor *E. coli* that could be moved to fresh produce during processing.

Produce Washer

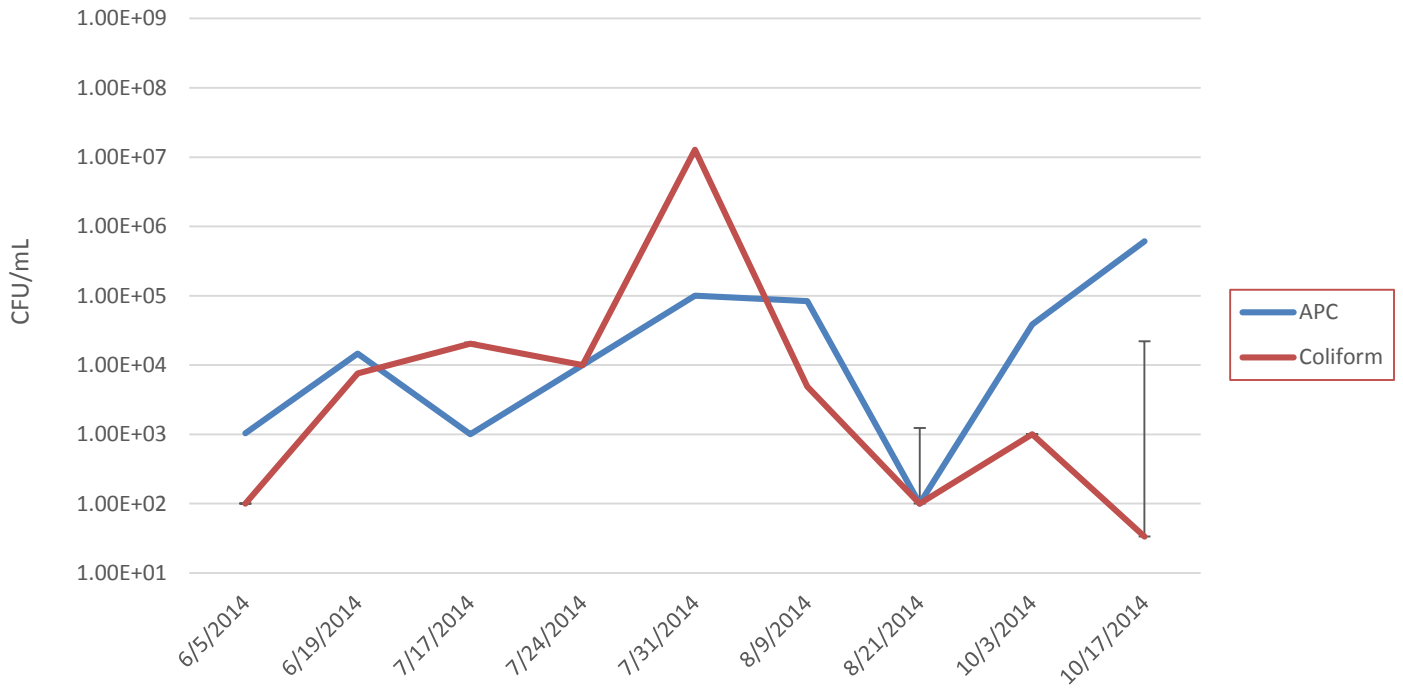
In the processing area, various parts of a produce washer made by Oesco, Inc. was sampled with both the sponge and swab methods described above. The produce washer was used primarily to wash tomatoes and peppers and consists of an input area, several rolling brushes on the inside, and output area and a drain for any water runoff. Other vegetables such as zucchini, summer squash and eggplant were also run through the washer. The brushes were sampled using the swab method while the other sites were sampled with the sponge method. Microbial counts, especially coliform were very variable and at times were very high and at other times relatively low. A standard operation procedure for cleaning the washer should be established to ensure that contamination does not occur during processing. Another potential standard operation procedure could include a user and sanitation log for the produce washer to allow the washer to be maintained and use tracked to help limit potential contamination. Counts from the samples are expressed graphically below.

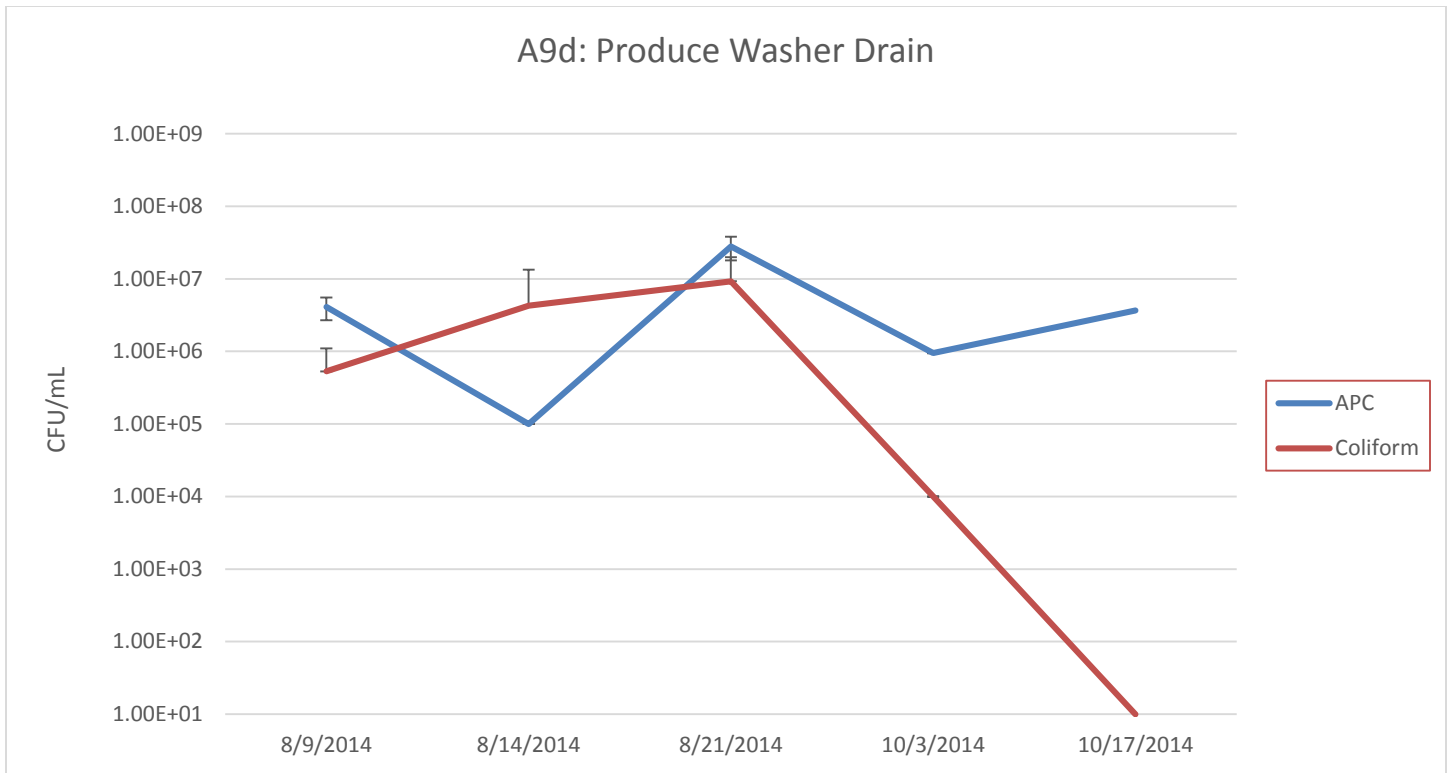


A9b: Produce Washer Brushes



A9a: Produce Washer Output





Evaluation

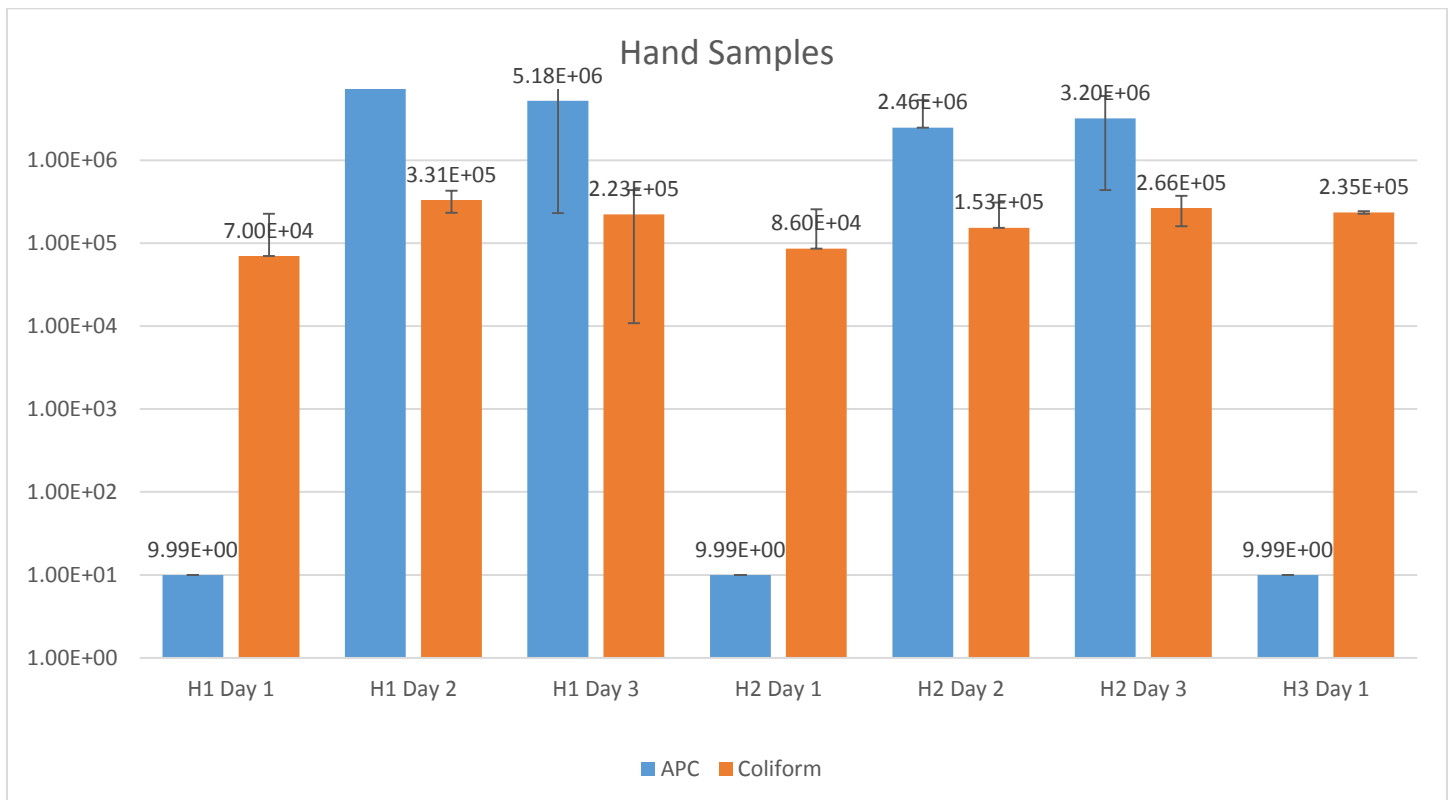
Counts from the produce washer seem to be very variable and reached their peak around the end of July. It is possible this is a result of higher frequency of tomato and pepper processing which gave more opportunities for potential contamination to occur. From that point, counts seem to drop off gradually until becoming very low by October, likely due to inactivity.

These sites, particularly the brushes, pose a potential risk of contaminating large amounts of produce. If such high numbers of coliforms can be found on the brushes after use, there is a high possibility that produce run through following this could become contaminated. Further tests must be done on the produce washer to determine an adequate standard operating procedure to clean the machine, as well as inoculation studies to quantify transfer of microorganisms from the brushes and intake areas of the machine. Potential corrective actions could include the use of a chemical treatment for the water such as chlorine, a standard operating procedure for allowing the washer to run a determined amount of time to shed any potential contamination or another scientifically verifiable method to minimize contamination.

Hand Samples

Consenting individuals both working in the field and in the packing area had their hands sampled. Participants placed their hand in a sterile filtered blender bag filled with 200 mL of sterile peptone water. The hand was massaged through the bag for thirty seconds and then removed. The second hand was then placed in the bag and massaged as above. Bags were stored in a cooler for transported and were testing within 24 hours using both APC and *E. coli*/ coliform petrifilm.

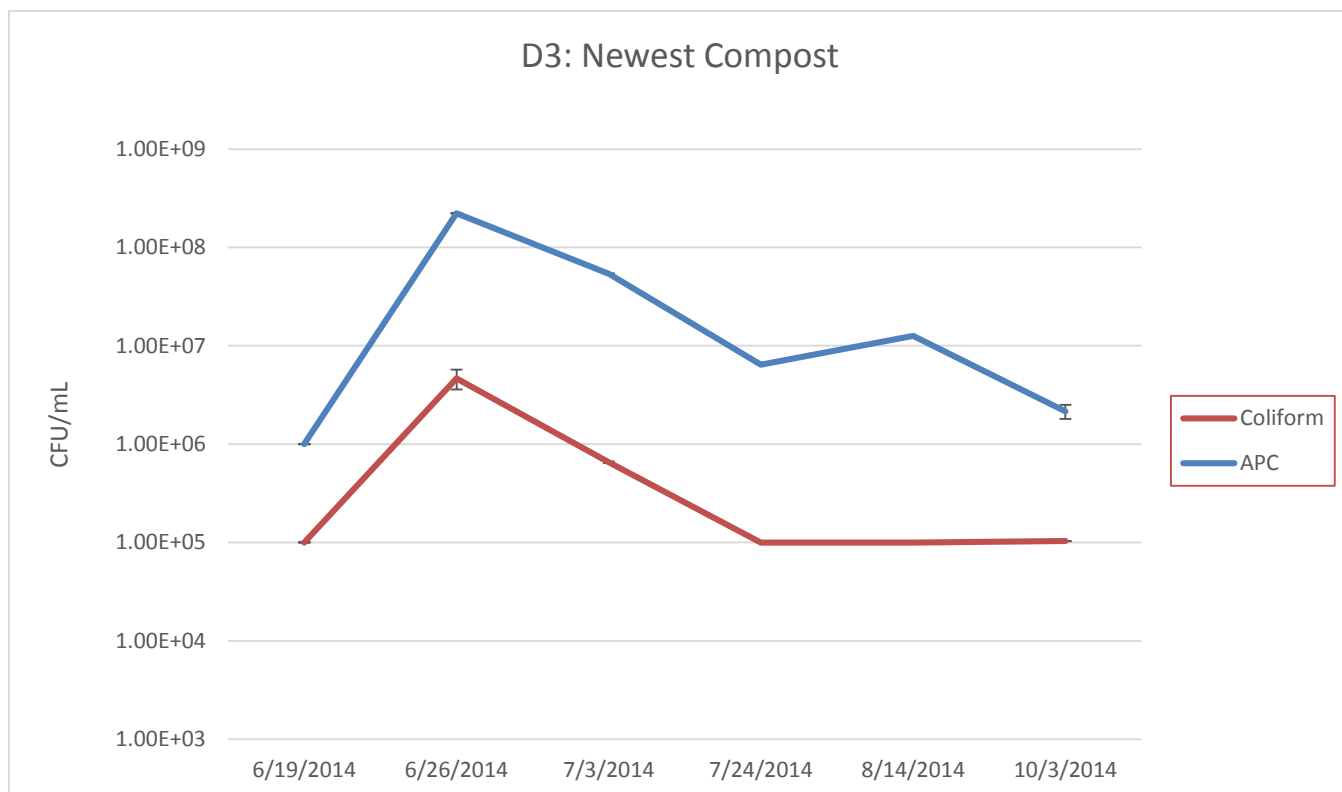
While only a small amount of hand sample were taken, it is obvious that worker contamination could be a potential risk factor for contamination during processing or harvesting. Coliform counts were relatively high on most samples compared to a typical baseline for food handlers of 10^2 CFU as found by one study (Sheth et al). and *E. coli* was found on one sample on 10/3/2014 (Day three). The results from hand sampling are represented graphically below.

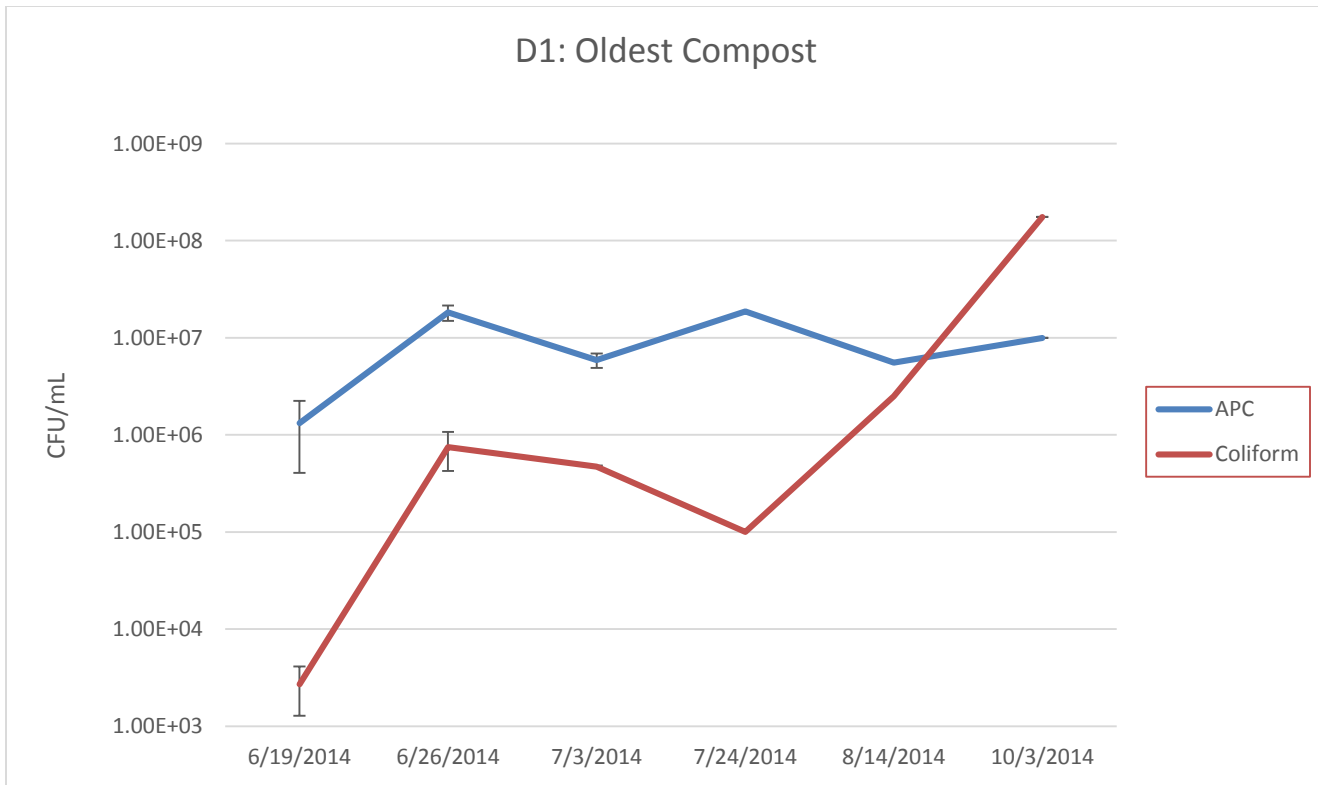
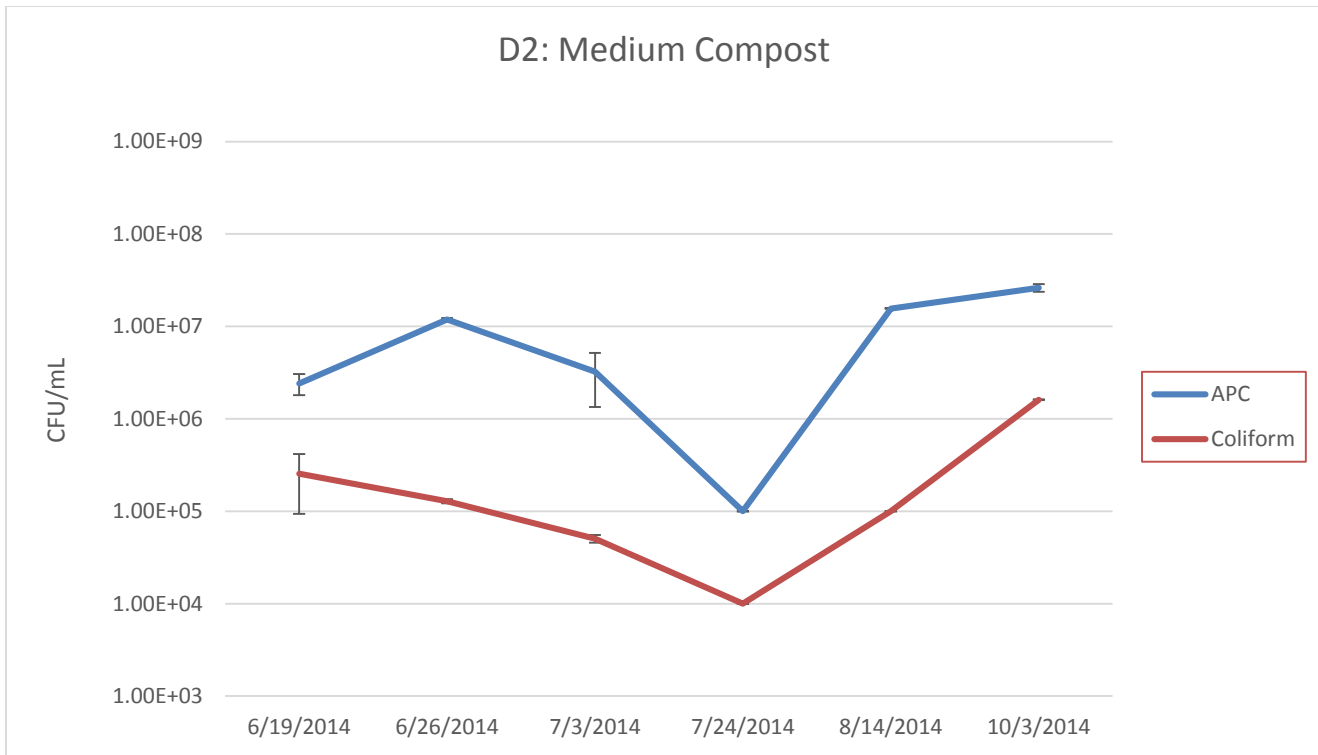


Worker contamination is a risk factor in any facility which handles food. Contamination of hands can occur due to inadequate hand washing when using the bathroom, from already contaminated produce during harvesting or processing or from soil and compost. Proper hand washing is an important step in minimizing this risk. Hands should be washed after harvest and during processing and packing, especially when moving to a different crop to prevent the spread of contamination. A potential corrective action could include a field staff hygiene training to ensure that all workers follow a standard sanitary program when harvesting and processing produce.

Compost

Compost at the facility was divide into three piles; newest material, medium and oldest composted material. Samples were taken from each compost pile with a sterile aluminum soil corer and placed into a sterile sample bag. Samples were placed in a cooler for transport and tested within 24 hours of collection. A 25 gram of the compost sample was weighed and added to 250 mL of sterile peptone water in a filtered blender bag. Sample was diluted and plated on 3M APC and E. coli/coliform petrifilm.



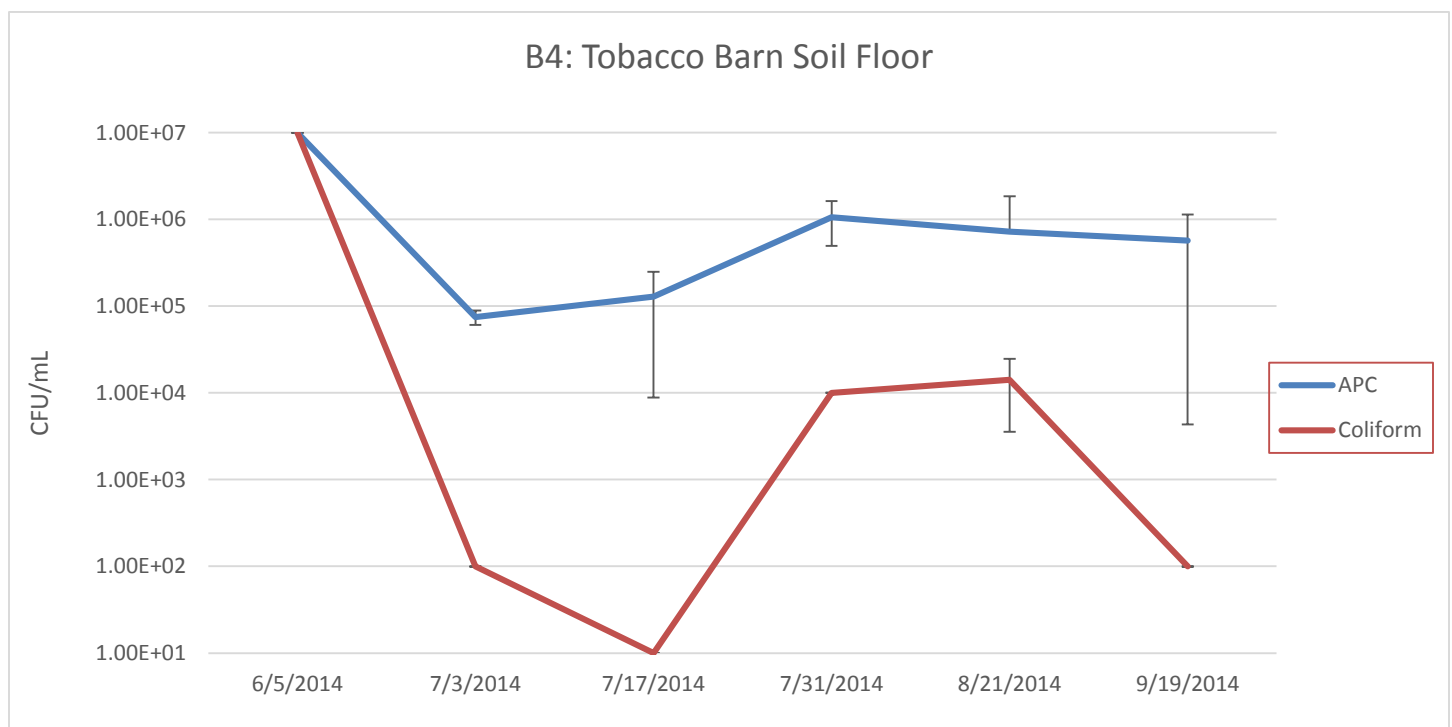


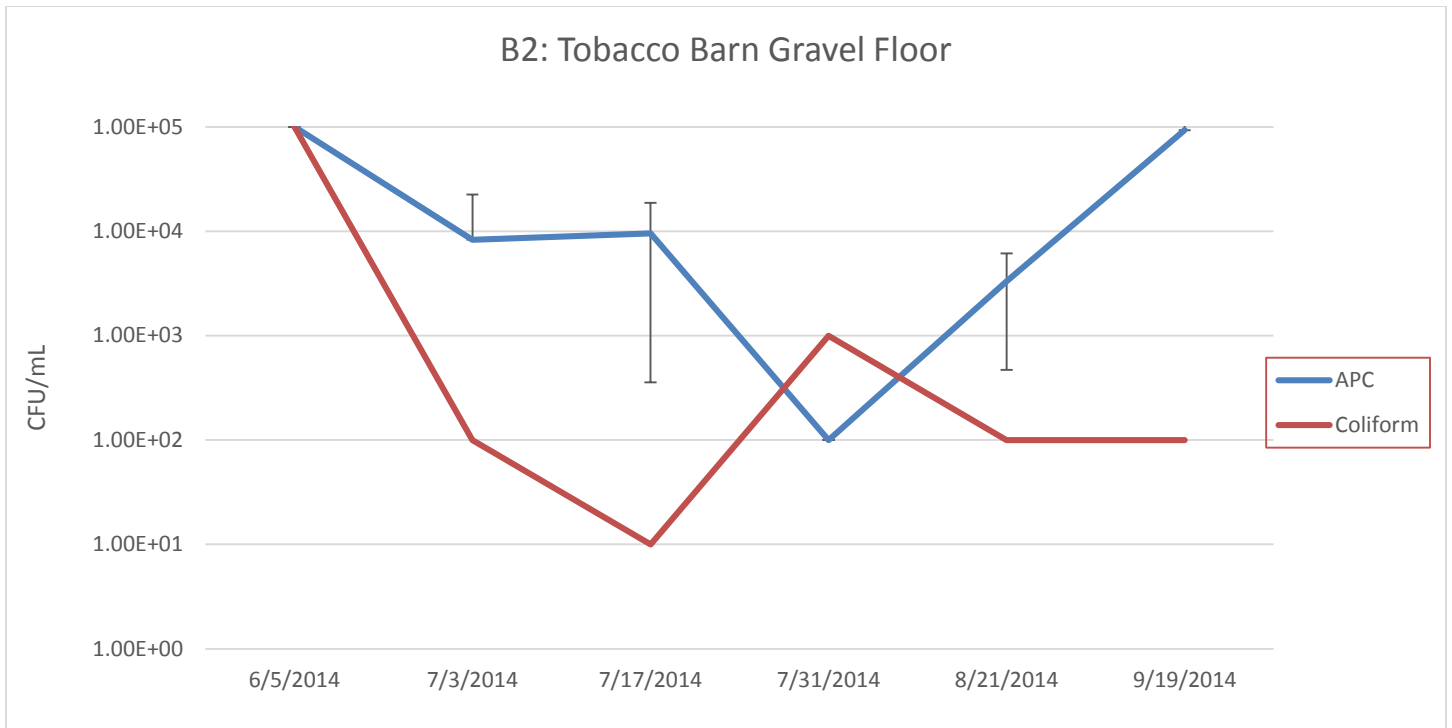
While for the majority of the duration of the sampling only minimal changes were observed in the microbial counts from the compost samples, the oldest compost, which should have lower coliform counts began a steady

increase starting at 7/24/2014. E.coli was found in the oldest compost on 10/3/2014 and on 7/3/2014 for the newest compost. Compost temperature logs should be taken with a large thermometer to ensure adequate temperatures are achieved to kill off potentially harmful microorganisms. Cycle of material records should also be kept to track when material at different stages of composting should be moved to another pile. Applying compost which has not reached adequate temperatures to kill off organisms could potentially contaminate produce on the field as well as worker hands which could contaminate produce in the packing area.

Tobacco Barn

Soil and gravel samples were taken from the tobacco barn and tested as described above with the compost samples. This site was sampled to check potential risk if this area were to be used as a packing or processing area. Soil samples were taken from the front of the barn where equipment and other materials were stored while gravel samples were taken from the back of the barn which had much less equipment as well as several dunk washers which could be used should this site become used for packing and processing.

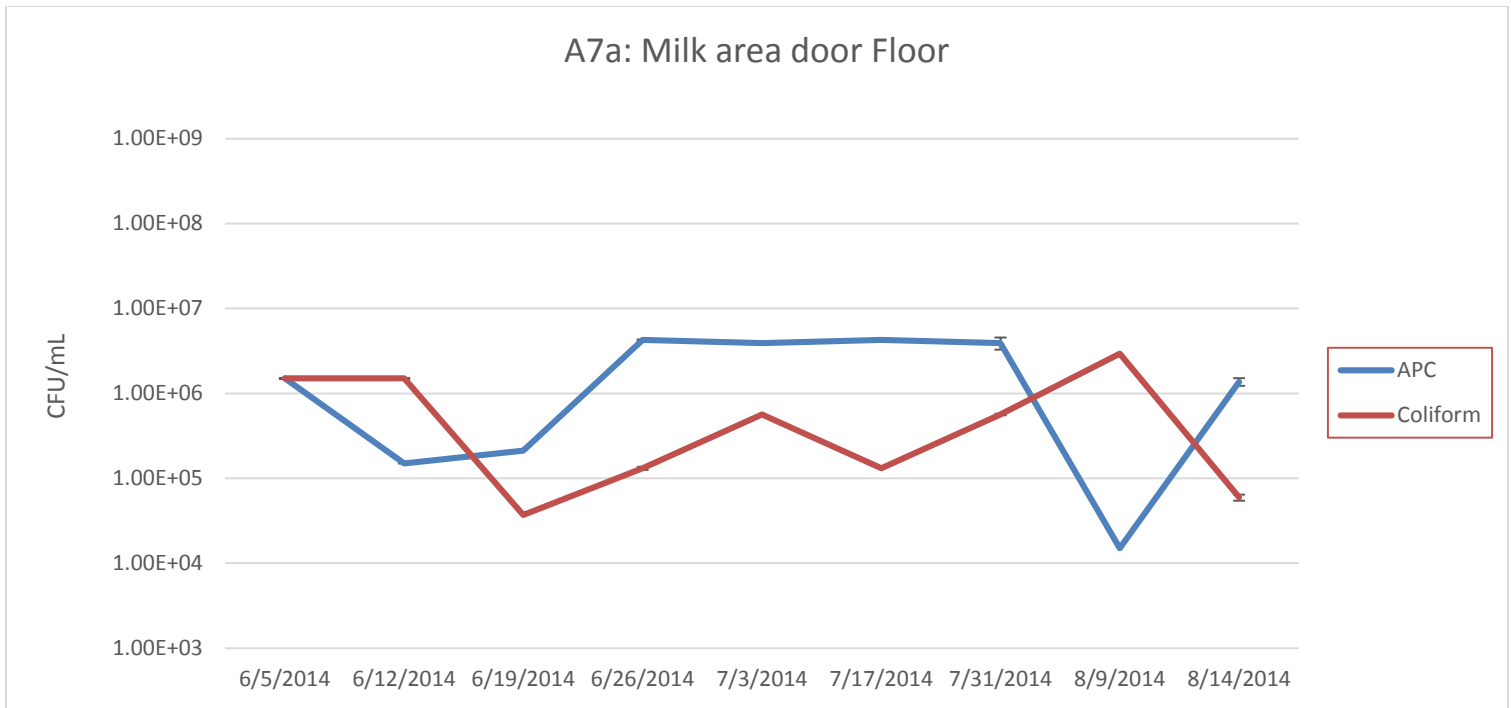




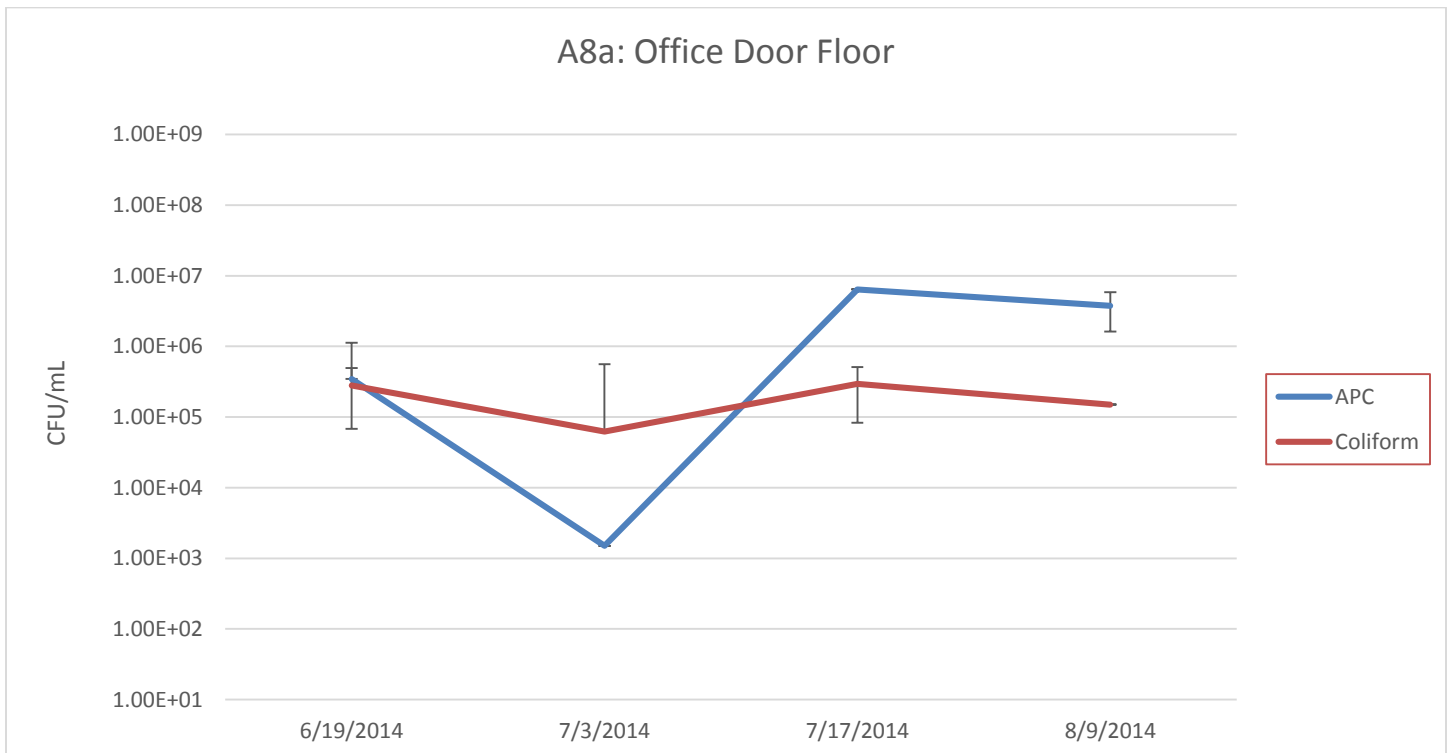
The initial high numbers on the first day of sampling this site are due to too high of a dilution being done which was not reflective of the counts found after that initial first day. In general, counts for the gravel were significantly lower than the soil especially with coliforms. APC counts began to rise in the gravel samples around the midpoint of sampling while staying relatively constant for the soil samples. A small mammal was observed once in the area by the gravel but was never seen again. There was also evidence of bird nests above, however no birds were ever observed.

Other Sites

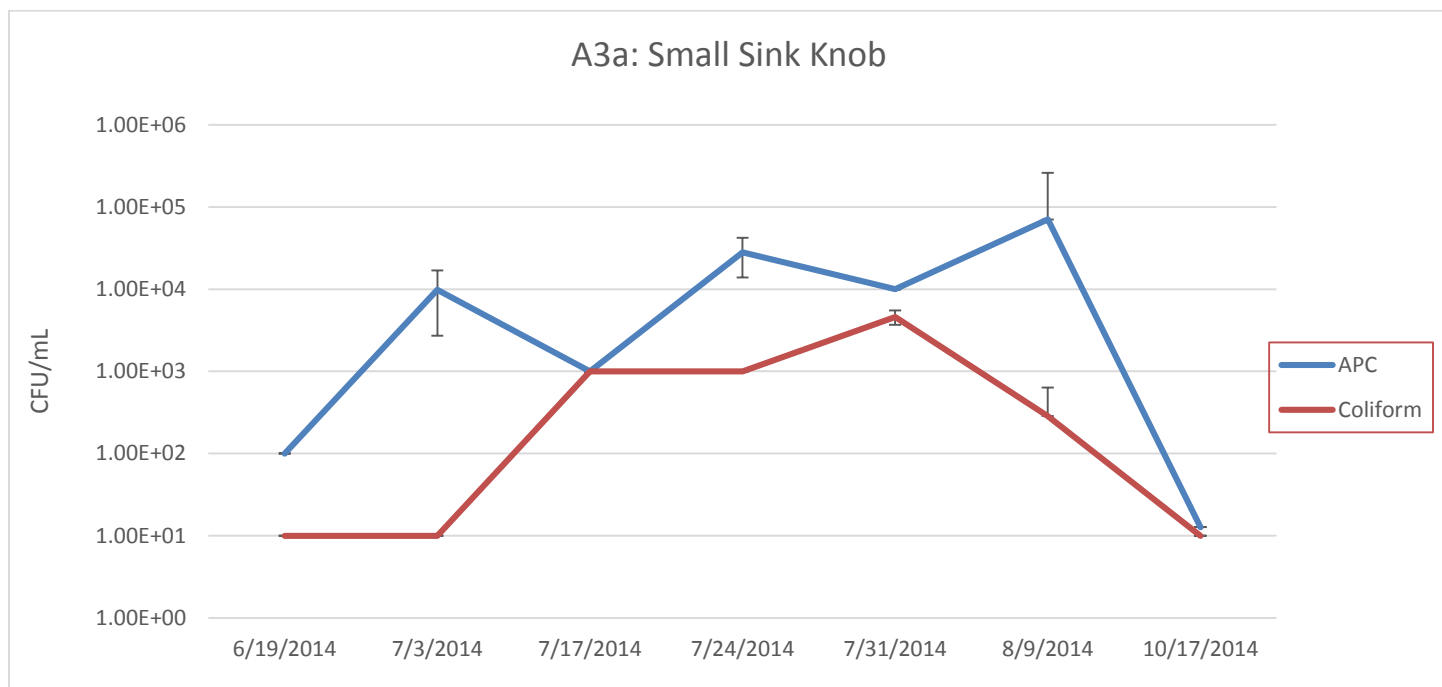
Other sites on the facility were sampled including sink drains, door knobs and floor areas.



The floor leading into the old milk area in the back of the packing area did not seem to show significant change throughout the season. Careful movement around the facility could prevent contamination from the feet of individuals working with the animals outside the milking area. Signs encouraging the door be kept closed were observed, however the door was left open on several occasions. Being mindful of movement through the processing area is an important step in minimizing outside contamination.



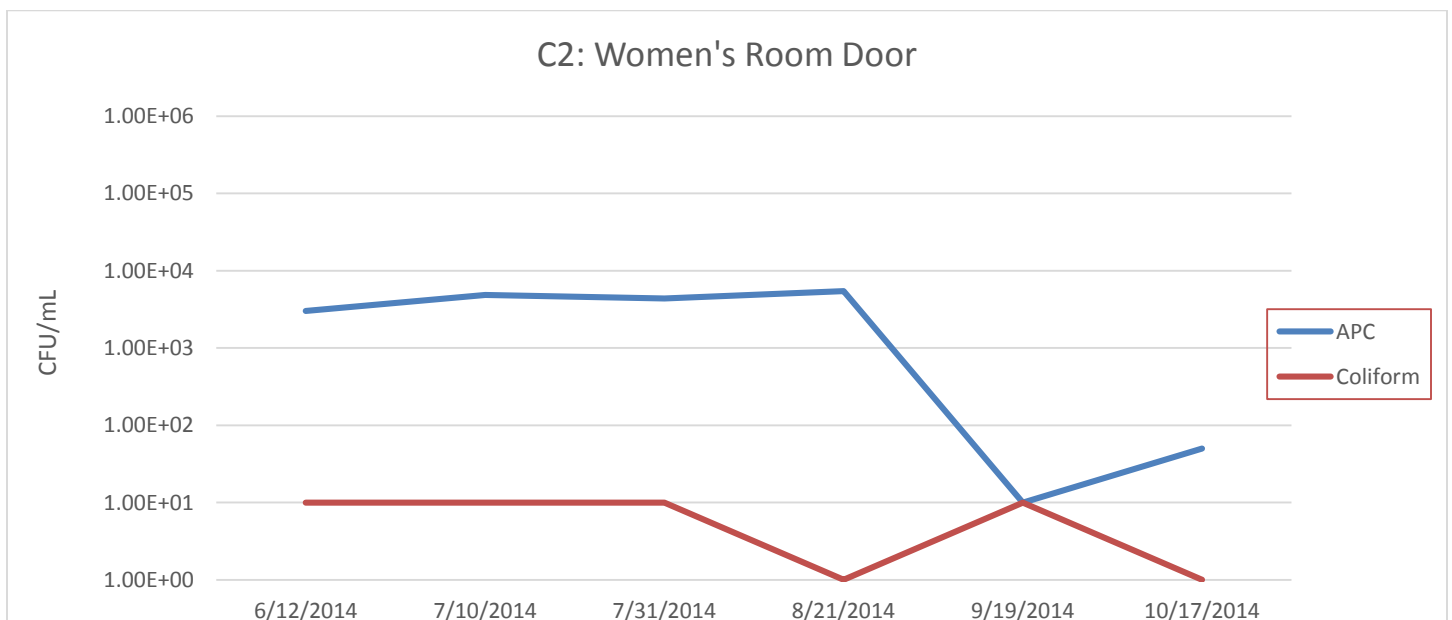
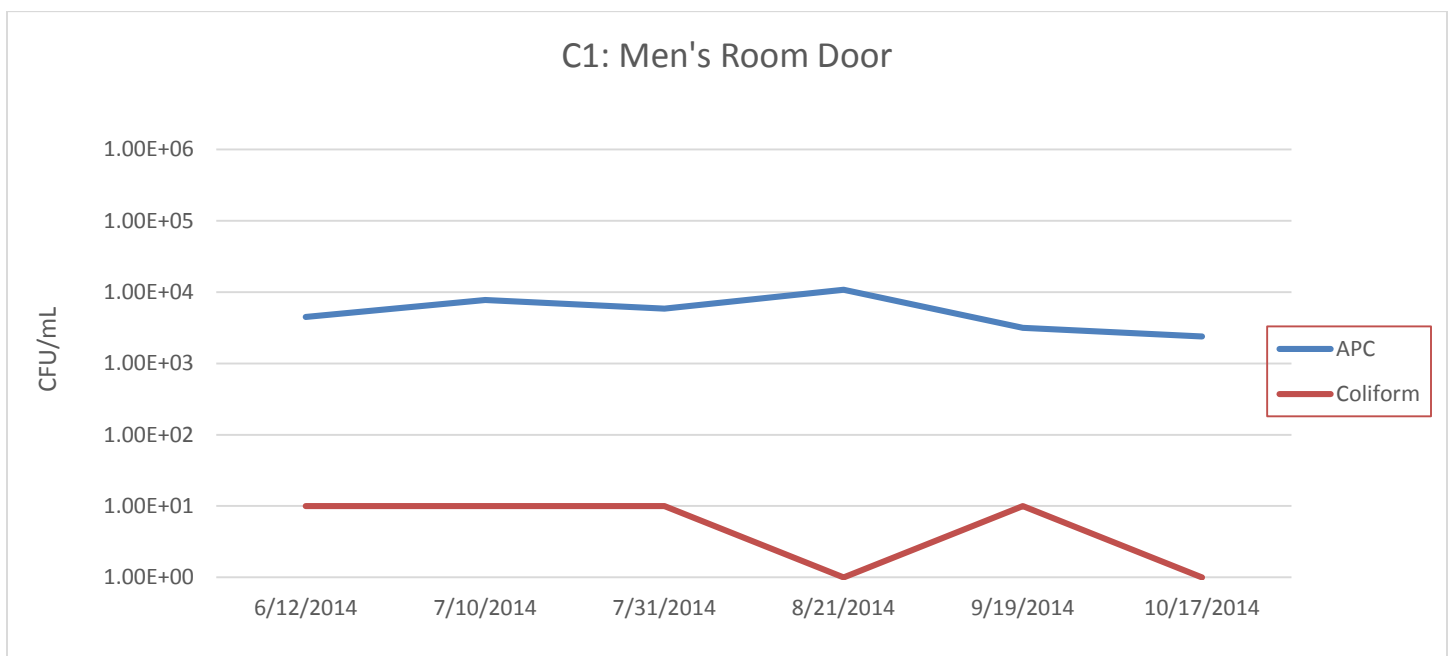
Coliform numbers stayed relatively consistent on the office door floor, but were still of some concern due to the proximity to the produce washer and potentially recently harvested produce. Regular floor cleaning may help to reduce these numbers as well as focusing on movement throughout the crossing area. Boots contaminated with manure should not be washed in the packing house and should be wiped thoroughly before entering.



The knob on the small sink in the packing area had somewhat variable counts likely correlated to frequency of use. A cleaning protocol should be developed for the sink areas. Transfer of organisms to knobs was likely a result of contamination by the hands. As hand samples were shown to potentially contain E. coli, sink knobs could potential spread organisms to other individuals who could in turn contaminate produce. Knobs should be cleaned following processing and produce washing.

Bathroom Areas

Very little change was seen in the counts for areas in the bathroom next to the packing area. The two main sites sampled were the doors which could potentially contaminate already washed hands or be contaminated by unwashed hands. The data for the two doors are expressed graphically below. Other sites from the bathroom such as the drains and sink knobs were not graphed due to insufficient data. However, numbers varied very little much like the doors showing that the bathroom is not a risk site to be concerned with.



Several other sites were sampled, but not represented graphically due to a lack of adequate data points for a cohesive graph. These sites include other areas of the floor in the packing area, soil from the hoop house as well as the beams and the area underneath the wooden root washer. Floor samples in the packing area all tended to be the same as the floor to the office represented above and carried minimal risk unless produce contacts the floor without it being washed. Soil from the hoop house showed coliform counts of $1.0E+05$ or slightly higher indicating a potential risk of contamination with the soil. If this is a result of using the compost it is possible it could be corrected by proper aging and temperature logs. The root washer had coliform counts only slightly higher than $1.0E+3$. While there is potential risk, especially because wood can retain microorganisms, a simple washing procedure following use should help to remove soil which is likely the source of the coliforms. If possible the root washer could have the wooden beams replaced with a different material to discourage potentially harmful organisms from growing.

Discussion

From the data presented above, several prominent risk sites can be identified. The produce washer, especially the brushes, show high potential to cross contaminate other produce items which have been run through after a contaminated piece of produce. A formalized standard operating procedure for cleaning the produce washer should be devised through future testing of microbial transfer to produce with this specific machine.

Compost samples showed a steady rise in the amount of coliforms later on in the season in the oldest compost which should in theory be ready to apply to fields. Temperature logs as well as proper turning of compost should be done on a regular basis to ensure that adequate temperatures are reached within the compost pile to kill off potentially harmful organisms. A standard operating procedure for this should also be developed and implemented next season.

While during the majority of visits to the facility the processing and packing area was relatively clean, it was sometimes observed to be very messy and left as it was during processing. Risks with this included the hose being left on the floor, dirt and mud around dunk washers and vegetable residues left in the drains. Often times

an order must be delivered before the area can be cleaned thoroughly. A standard procedure for cleaning this area or for communication when it can be cleaned should be established.

Average Counts for All Samples

Both coliform and APC average counts for the entire duration of sampling of each sample are recorded below.

Counts with plates that were too numerous or below 25 CFUS were rounded to provide a numerical value.

Sample Code	Description	Type	Average Count
A1	Grate a	APC	4.27E+08
		Coliform	1.20E+08
A2	Grate b	APC	8.85E+08
		Coliform	2.01E+08
A3a	Small Sink Knob	APC	1.82E+04
		Coliform	9.85E+02
A3b	Small Sink Drain	APC	2.08E+05
		Coliform	1.82E+05
A6a	Lab Door Knob	APC	5.63E+02
		Coliform	9.90E+00
A7a	Milk Area Floor	APC	2.96E+06
		Coliform	3.36E+06
A7b	Milk area Knob	APC	3.01E+03
		Coliform	1.45E+03
A8a	Office Door Floor	APC	2.63E+06
		Coliform	1.84E+05
A8b	Office Door Knob	APC	1.36E+03
		Coliform	5.45E+01
A9a	Brush Washer Output	APC	9.53E+04
		Coliform	1.42E+06
A9b	Brush Washer Brushes	APC	9.07E+03
		Coliform	9.40E+04
A9c	Brush Washer Input	APC	3.13E+06
		Coliform	1.39E+06
A9d	Brush Washer Drain	APC	7.35E+06
		Coliform	2.81E+06
A11a	Front Door Floor	APC	8.79E+06
		Coliform	5.70E+06
A11b	Front Door Knob	APC	3.44E+03
		Coliform	3.96E+01
A12a	Large Sink Drain	APC	1.40E+06
		Coliform	7.14E+05

A12b	Large Sink Knob	APC	8.78E+02
		Coliform	3.54E+01
B1	Back Door Knob/Lock	APC	5.00E+02
		Coliform	9.90E+00
B2	Tobacco Gravel Floor	APC	3.55E+04
		Coliform	1.67E+04
B3	Dunk Washer	APC	5.76E+04
		Coliform	5.00E+02
B4	Tobacco Barn Dirt Floor	APC	2.08E+06
		Coliform	1.65E+06
B5	Tobacco Barn Front Floor/Lock	APC	9.90E+00
		Coliform	9.90E+00
C1	Mens Room Door	APC	5.77E+03
		Coliform	2.59E+02
C2	Womens Room Door	APC	2.97E+03
		Coliform	2.99E+02
C3a	Mens Sink Drain	APC	2.56E+04
		Coliform	9.90E+00
C3b	Mens Sink Knob	APC	3.92E+05
		Coliform	5.00E+02
C5a	Mens Inside Knob	APC	1.32E+03
		Coliform	N/D
D1	Oldest Compost	APC	9.93E+06
		Coliform	2.99E+07
D2	Medium Compost	APC	9.91E+06
		Coliform	3.59E+05
D3	Newest Compost	APC	4.97E+07
		Coliform	9.51E+05
E2	Cooler a	APC	9.90E+05
		Coliform	9.90E+06
E3	Cooler b	APC	9.90E+05
		Coliform	9.90E+06
E10a	Door to Lab Floor	APC	9.90E+05
		Coliform	4.95E+06
E10b	Door to Lab Knob	APC	5.11E+05
		Coliform	9.90E+02
E11a	Root Washer Beam	APC	2.48E+05
		Coliform	4.42E+03
E11b	Root Washer Underneath	APC	1.63E+05
		Coliform	6.93E+02
E12	Floor Mat	APC	5.95E+04
		Coliform	1.38E+04
SHA	Hoop House Soil A	APC	7.75E+05
		Coliform	5.00E+04
SHB	Hoop House Soil B	APC	3.62E+05
		Coliform	5.00E+04

Future

Considering that the most variable sites on the facility in terms of microbial counts came from the produce washer, further studies should be conducted to determine a standard operating procedure for cleaning the machine. Inoculation studies using peppers and tomatoes should be conducted in combination with the same methods used to sample the washer to quantify transfer of organisms to the sites of the washer as well as determine an adequate method for cleaning the machine to prevent contamination. Other potential corrective actions could involve more specific procedures for floor and surface washing, hand washing and compost temperature logs. River data should be taken into account to formulate a corrective action procedure when E. coli levels go above the standards described above. Similar samples will be taken in the coming year to collect more data and potentially quantify the effectiveness of different corrective actions implemented as a result of this data.

Cited

Sheth M, Patel J, Sharma S, Seshadri S. Hazard analysis and critical control points of weaning foods. *Indian J Pediatr.* 2000;67:405–410.