BERLIN Tyson plant
Technical Report Prepared by Ellen Silbergeld, Jim Hulbert, Jane Kreiter, and Jennifer Nyland

This project was undertaken to assist the Town of Berlin in assuring the safety of repurposing the former Tyson Poultry slaughter and processing plant. The site is now owned by the town of Berlin Maryland, which plans to redevelop the site for community recreational purposes. The issue under investigation by us related to the potential presence of pathogenic bacteria at this site related to its former use.

BACKGROUND INFORMATION (FROM JANE KREITER)

The site was formerly occupied by a Tyson poultry slaughter and processing plant. There is extensive information of the presence of bacteria in these operations, including pathogenic organisms capable of causing diseases in humans. There is no indication that steps were taken by Tyson during plant activity [such steps are not required by state or federal regulation]. Since the plant closing, no remediation or cleanup was conducted inside the buildings or at the site.

Reason for concern: Of greatest concern, the site includes several ponds into which slaughter house wastes were disposed over the course of operation. Because the ponds have remained filled, they are likely to contain bacteria representing past uses. We focused on those pathogens carried by poultry that are capable of causing disease in humans. Moreover, because of the use of antibiotics in poultry feed, many studies, including research conducted in MD by the University of MD and our group have reported that antibiotic resistant pathogens are present on poultry at farms and on broiler chickens transported from farms to slaughter, The organisms of greatest concern, all of which have been reported with a high prevalence in poultry production are listed below. For cost reasons, as well as knowledge of the likelihood of persistence, we focused on *E coli.*

Campylobacter jejeuni
Enterococcus species
Staphylococci aureus
E. coli
Klebsiella

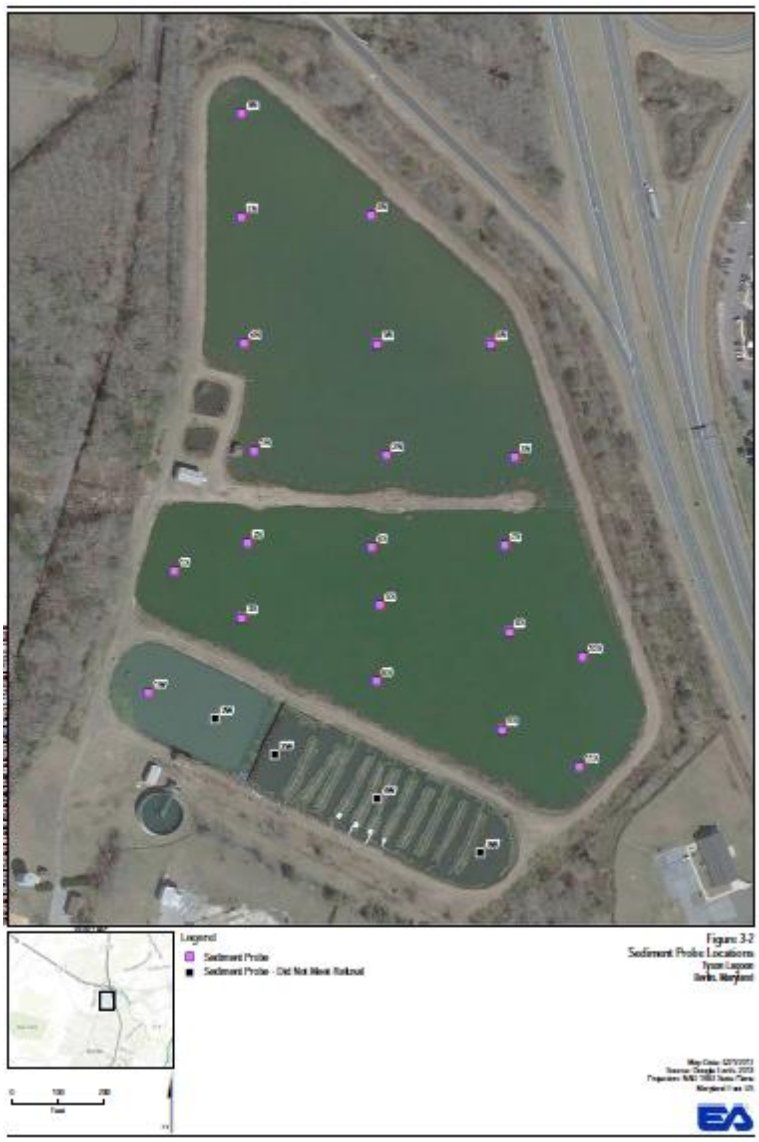
The flow from the chicken processing plant went through a pretreatment facility that was located inside the existing building. The flow then went to the round clarifier that is located South of the South Lagoon. From there it entered the South Lagoon on the East side where it was aerated. Then it flowed to the other side of south Lagoon where the floating vegetated barges are. From the Southern lagoon the flow went to the middle lagoon and then to the North lagoon. Prior to discharge into Kitts Branch the effluent went through a filter which was located in the building on the land located

between the middle and north lagoons. Chlorination and dechlorination occurred in the small cells adjacent to the building

These ponds are shown below (map from EA). Reading from top to bottom of this figure, the slaughter house waste was first discharged into the round holding reservoir shown at the bottom. From there, liquids were pumped into the small pond with plant flotation devices. This pond drained into the larger pond at the top of the figure and eventually runoff was discharged into a natural stream on the right on the ponds.

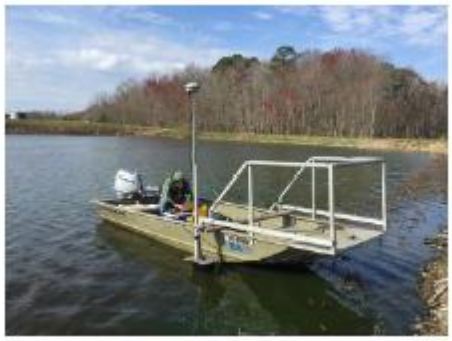
STUDY DESIGN (ELLEN SILBERGELD)
We proposed a limited study of sediments in the ponds currently on.the site since no analysis for pathogenic strains have been conducted. Owing to funding constraints, we focused on *E coli* a famiy of microorganisms that includes highly pathogenic strains.

Using information provided by EA and the Town of Berlin, we proposed to take sediment samples at three points within the first discharge pond on the map below. We did not sample from the holding reservoir.



These samples were collected by EA as described below, using standard methods prior to any drainage of water, removal of sediments or plants, or other disturbance of the bottom sediments. The cores were handled by scientists at Salisbury University, following protocols developed in the Brush laboratory at JHU and utilized by us in sampling river sediments in the Pocomoke River watershed. The cores were prepared for sectioning and storage using the same protocols.

SEDIMENT PROBE SAMPLING INVESTIGATION (conducted by Jim Hulbert, EA)
EA conducted a series of sediment probes at 25 pre-determined locations within the three wastewater management lagoons between 30 March and 31 March 2017. The probe data were used to identify the elevation of the water and sediment surfaces, as well as the elevation of the firm subgrade material at each location.



The Trimble R8S GNSS unit was mounted to the top of a fiberglass probe to provide horizontal and vertical positioning in the Maryland State Plane coordinate system (Figure 3-3). At each location, the probe was pushed into the sediment until refusal was met, or until the probe reached its full extent of 13.1ft. Three positional fixes were logged at each location: (1) water surface, (2) top of sediment, and (3) bottom of sediment. By obtaining three positional fixes, the water depth and overall thickness of the fine-grained sediment overburden was calculated for each location based on the difference of the various elevation values.

SEDIMENT CORE SAMPLING INVESTIGATION (EA)
The objective of the sediment coring effort was the collection of intact, cross-sectional samples in order to examine the sediment strata within the wastewater management lagoons. Twelve (12) locations established in a previous phase of the Site characterization were re-occupied for the collection of sediment core samples in order to sample the fine-grained material of concern (Figure 3-4). On 30 March and 31 March 2017, a 2.75-inch diameter piston corer was utilized by EA to collect 12 core samples and two duplicate core samples throughout the lagoons to a maximum depth of 5 ft below the sediment surface (Figure 3-5). Sediment core samples included:

- Four samples located within the north lagoon (SC-1N; SC-3N; SC-5N; SC-9N)
- Four samples located within the middle lagoon (SC-2S; SC-5S; SC-8S; SC-9S)

- Two samples located within the western half of the south lagoon (WWP-1; WWP-2)
- Two samples located within the eastern half of the south lagoon (WWP-3; WWP-4)

Additionally, two duplicate core samples were collected from the western half of the south wastewater lagoon (WWP-1.1; WWP-2.1) for the purpose of microbiological analyses. Sampling locations were located via GNSS by EA prior to sampling and are presented in Figure 2 above.

CORE PROCESSING (Salisbury University)

Dr Nyland received two core samples collected at the locations designated (Samples #WWP1 and WWP2) between 10:15 and 10:50am on 03/31/2017. The core samples were stored on ice and transported immediately to Salisbury University for subsampling and DNA isolation. The cores were opened under sterile conditions and subsamples (50ml volume) collected from the top (within the first 5 inches of the top) and bottom (within the first 3 inches of the bottom) of each core. DNA was isolated from these subsamples using Qiagen DNeasy PowerSoil isolation kits according to the manufacturer's instructions. Isolated DNA was stored at -80ºC until transport to Johns Hopkins for microbial genetic analyses. DNA samples were sent to Johns Hopkins on dry ice via FedEx.

DNA ANALYSIS (Johns Hopkins Bloomberg School of Public Health)

The frozen DNA samples were thawed using standard methods at Johns Hopkins. The identification of E coli was performed by polymerase chain reaction analysis of the DNA samples. The reactions were carried out on a StepOne Real-Time PCR system. The primers and probe were published in "Development of two real-time multiplex PCR assays for the detection and quantification of eight key bacterial pathogens in lower respiratory tract infections," detailing two real-time multiplex PCR assays for detection of bacterial pathogens (hyperlink here). The total volume of each reaction was 20 µl -- 10 µl 2X Veriquest USB Probe Master Mix; 1 µl of each primer (10 µM); 0.5 µl probe (10 µM); 2.5 µl ultrapure water; 5 µl DNA template. The DNA samples were tested neat (5 µl of bacterial DNA) and dilute (5 µl of 1:10 dilution of bacterial DNA). The published protocol we use to test for E. coli DNA in samples is actually a real time PCR assay and the results are expressed as cycle thresholds (CTs) for each of the samples. The CT is deinged as the number of cycles (or amplifications) required to detect a fluorescent signal about background. Positive controls were run for each assay.

RESULTS

The two positive control samples had CTs of 18.3 and 21.7. "Unknown" means that after 40 cycles there was no fluorescent signal indicating a negative result. Only Sample 2 (neat, that is, no dilution) was positive with a CT of 37.5. The maximum

number of cycles in this real-time assay is 40. While the CT is high (as expected for a nondiluted sample), it is not outside the range of the assay.

Block Type: 96well
Chemistry: TAQMAN
Experimen: F:\2017-06-13 Silbergeld samples EC.eds
Experimen: 2017-06-13 12:20:50 PM EDT
Instrument: steponeplus
Passive R: ROX

Well	Sample Name	Target Nar	Task	Reporter	Quencher	Ct	Ct Mean	Ct SD	Quantity	Quantity M	Quantity S	Automatic	Ct Threshc	Automatic Baseline	Baseline Start	Baseline End	Comments	HIGHSD	NOAMP	EXPFAIL
A2		ecoli	NTC	FAM	NFQ-MGB	Undetermined						FALSE	0.05	TRUE	3	39		N	N	N
B2		ecoli	NTC	FAM	NFQ-MGB	Undetermined						FALSE	0.05	TRUE	3	39		N	N	N
A1	Sample 1 (neat)	ecoli	UNKNOWI	FAM	NFQ-MGB	Undetermined						FALSE	0.05	TRUE	3	39		N	N	Y
B1	Sample 1 (1:10)	ecoli	UNKNOWI	FAM	NFQ-MGB	Undetermined						FALSE	0.05	TRUE	3	39		N	N	Y
F1	Sample 2 (neat)	ecoli	UNKNOWI	FAM	NFQ-MGB	37.5	37.5					FALSE	0.05	TRUE	3	34		N	N	N
G1	Sample 2 (1:10)	ecoli	UNKNOWI	FAM	NFQ-MGB	Undetermined	37.5					FALSE	0.05	TRUE	3	39		N	Y	Y
A3	Positive Ctrl 1:100	ecoli	UNKNOWI	FAM	NFQ-MGB	18.3	20.0					FALSE	0.05	TRUE	3	14		Y	N	N
B3	Positive Ctrl 1:1000	ecoli	UNKNOWI	FAM	NFQ-MGB	21.7	20.0					FALSE	0.05	TRUE	3	19		Y	N	N

CONCLUSIONS

Based on this analysis, we conclude that there is no evidence for the presence of bacteria of health concern at the site sampled.