Progress Report

Differing Effects of Onsite Wastewater Treatment on Bacterial Populations

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Project Information

- Center for Reservoir & Aquatic Science Research (CRASR)
  - Baylor Wastewater Research Program (BWRP)

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Why Study Microorganisms from Onsite Systems?

- CBOD and TSS are indirect measures of the biological content of wastewater.
- What is actually being discharged into soils?
- How many bacteria, viruses, and protozoans survive onsite treatment and are released in the environment?

Bacteria

- Single-celled organisms with no nucleus
- Are found in virtually every environment on earth.
- Replicate by binary fission

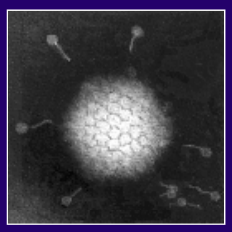
- Responsible for many essential ecosystem functions
- Many are commensal – live in mammalian intestinal tracts and improve digestion

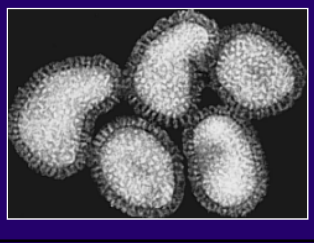
Bacteria

- Some are pathogenic
  - Respiratory diseases
  - Wound infections
  - Urinary tract infections
  - Gastrointestinal illness
  - Food poisoning
- Most enteric bacteria are nonpathogenic
- Some cause disease
  - Fecal oral transmission
  - Usually gastrointestinal symptoms

Viruses

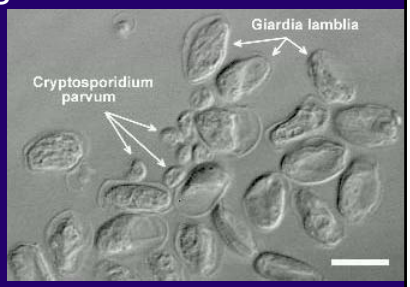
- Cannot replicate without a host cell to infect
- Some can cause disease in humans – most common
  - Respiratory infections
  - Gastrointestinal diseases





Protozoa

- Single-celled organisms with a nucleus
- Great diversity in the kingdom Protista
  - Plant-like: Protophyta
  - Animal-like: Protozoa
- Some protozoa are pathogenic
  - Gastrointestinal disease
    - *Giardia lamblia*
    - *Cryptosporidium spp.*
    - Amoebic dysentary



This Study

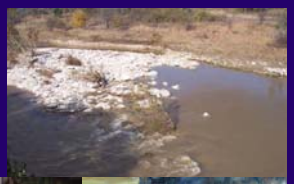
- Fecal Indicator Bacteria
  - Used to monitor environmental fecal contamination
  - *Escherichia coli, Enterococcus spp.,* fecal coliforms
  - The presence of these organisms indicate the potential for other, more harmful, microorganisms to be present

This Study

- Monitor levels of *E. coli* and total coliforms in treated effluent from onsite systems versus untreated influent
  - Indication of the effect of treatment on the microbial community
  - Use of EPA standard protocols
  - Measure in log reduction

Water Quality Monitoring

- Fecal indicator bacteria (*E. coli*, coliforms, or *Enterococcus spp.*) are commonly found in surface water
  - Origins of contaminants include animal feces, both wild and domestic, as well as human sewage

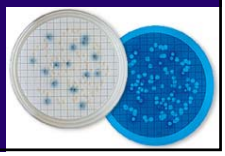


Water Quality Monitoring

- Drinking Water Standards
  - Maximum contaminant level for total coliforms and *E. coli* acceptable in drinking water is **zero** (EPA)
  - Max level for recreational water use is 394 bacteria/ 100 ml in a single sample

Determination of *E. coli* Concentrations

- Membrane Filtration Method (USEPA Method 1604)
  - Water samples filtered through 0.45um pore size filter
  - Filters placed on MI agar
  - Incubated overnight at 35°C
- *E. coli* enumerated based on presence of blue colonies on MI agar
- *E. coli* concentration per 100mL calculated



Log Reduction

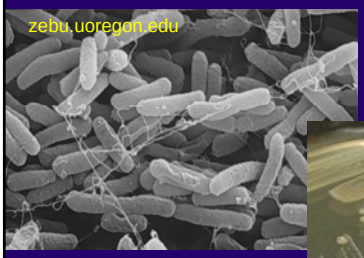
- Calculated with log-transformed *E. coli* concentrations
  - Log(input)-Log(output)

Percent Reduction	Log Reduction
90	1
99	2
99.9	3
99.99	4
99.999	5
99.9999	6

Log Reduction

- Even with greater than 99% reduction of *E. coli*, significant numbers of bacteria may still be in the treated effluent
  - High concentrations of *E. coli* in inflow

Escherichia coli



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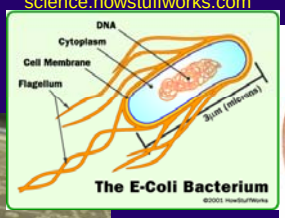
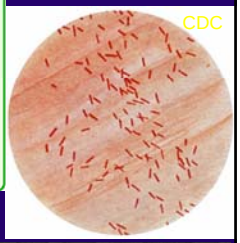
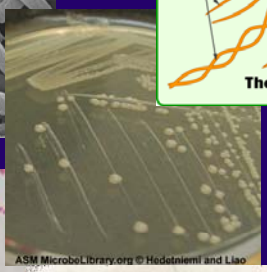
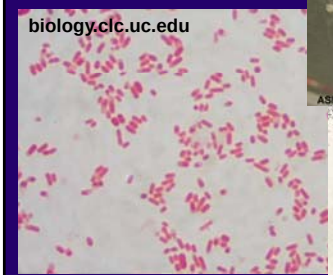
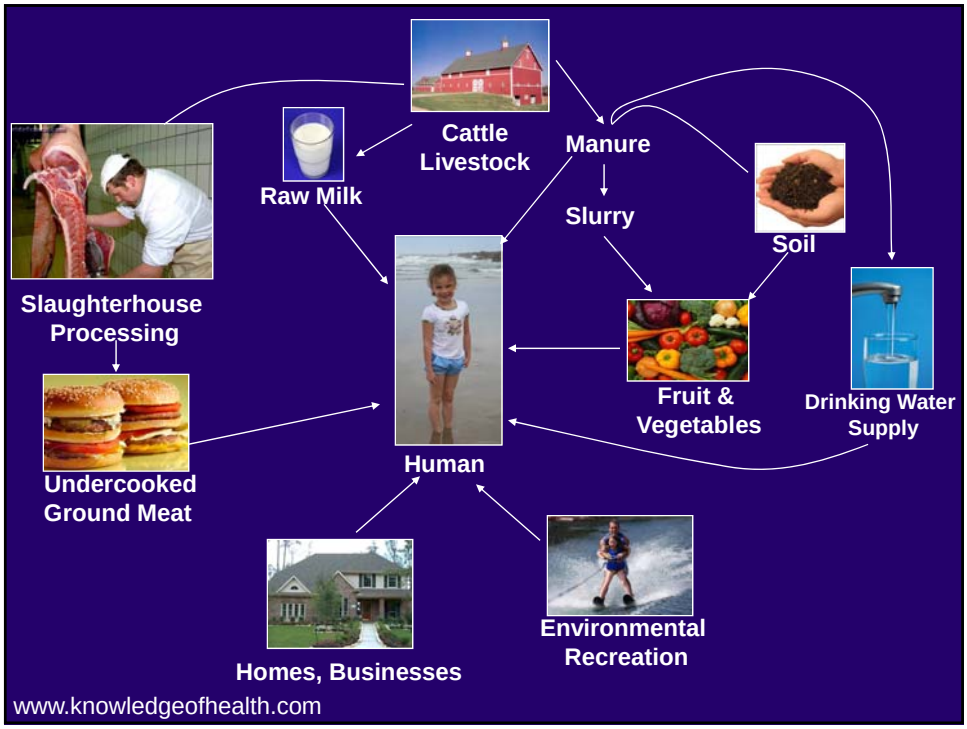









NY State Dept. of Health



So what's the connection here?

Onsite Wastewater Treatment

- Used in areas where municipal sewage treatment is unavailable
- Traditional system
  - Septic tank and leach field
  - Discharges treated wastewater into the soil
    - Vertical and horizontal movement
    - Potential contamination of ground and surface water
    - Fate and transport of microorganisms in the wastewater is largely unknown

Onsite Wastewater Systems

- Traditional: Septic Tank with Leach Field
  - Leach field mimicked using soil columns
- Alternative: Addition of Submerged-bed Wetland
  - Between septic tank and soil columns
- Alternative: Aerobic Treatment of Wastewater
  - Can be used in place of a traditional septic tank

Relevance

- Onsite wastewater systems are a potential source of contaminants in soil, groundwater and surface water supplies.
- What is the potential impact of onsite systems on the environment?
- How can this impact be minimized?
- Primary Hypothesis:
  - Some onsite systems will better reduce bacterial concentrations in effluent than others.

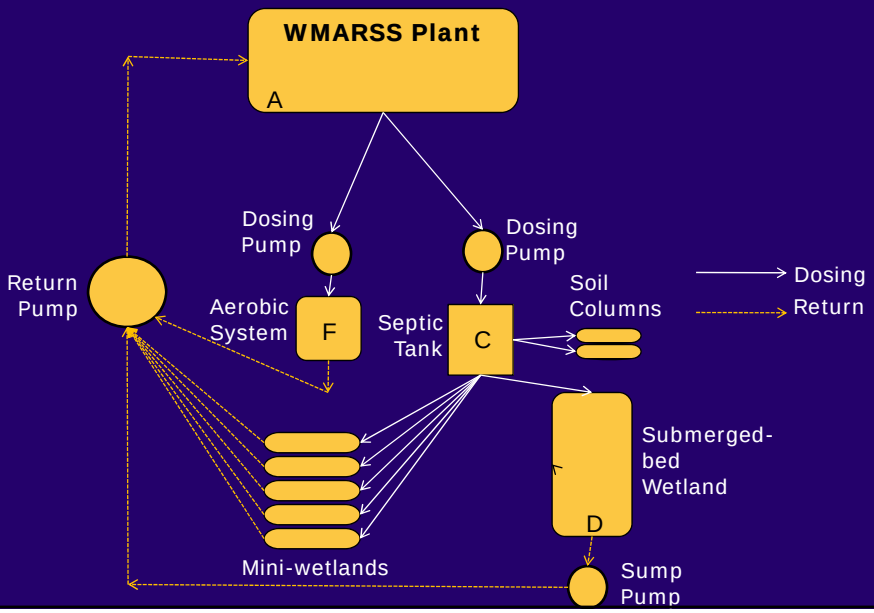
Study Questions

- How do different types of WWTS compare on bacterial removal?
  - Septic + Leach Field
  - Septic + Wetland
  - Aeration System
- How does soil type in the leach field affect bacterial removal?
- How much does UV or chlorination improve the bacterial removal of these systems?

Study Questions

- Are there differences in bacterial antibiotic resistance between treatment systems?
  - Antibiotic resistance analysis
- Are there differences in the presence of pathogens between the different treatment systems?
  - Genetic analysis

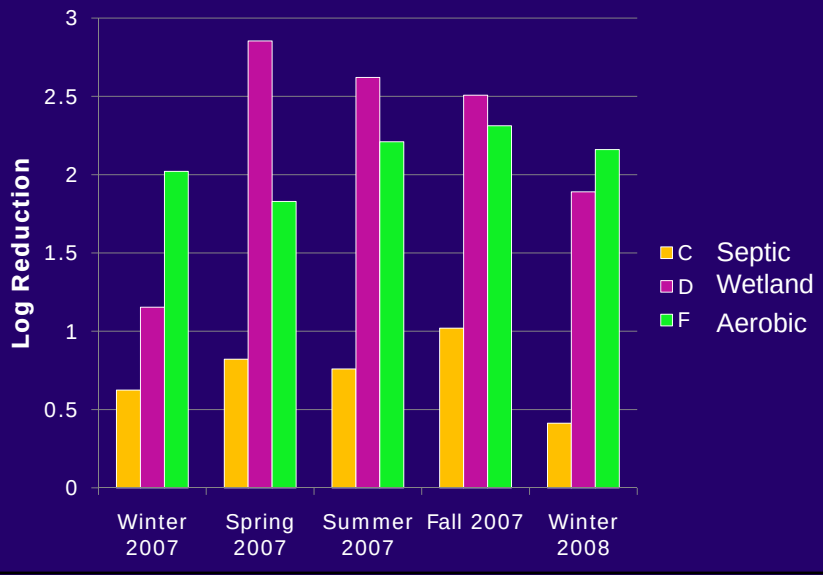
Schematic of Study Site



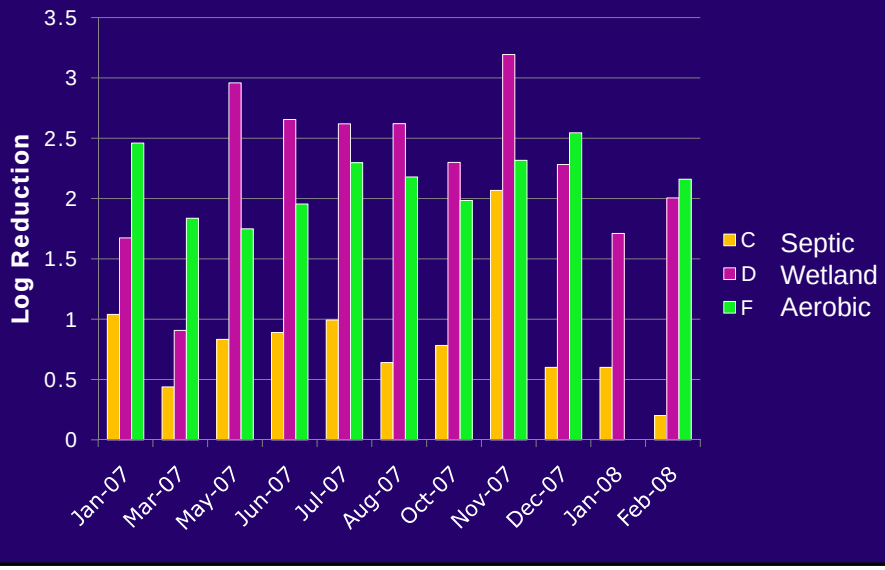
Treatment Conditions

Treatment	Disinfection	Soil Columns
Septic tank (alone) "C"	+/- chlorine, UV	Type Ib, III
Septic tank + subsurface-flow wetland #1		
Septic tank + subsurface-flow wetland #2		
Septic tank + subsurface-flow wetland #3		
Septic tank + subsurface-flow wetland #4		
Septic tank + subsurface-flow wetland #5		
Septic tank + subsurface-flow wetland (Big Wetland) (D)	+/- chlorine, UV	Type Ib, III (E1, E3)
Aeration (F)	+/- chlorine, UV	Type Ib, III
Control (Raw sewage into dosing shed) (H)		
Control (WWTP Effluent site) (G)		
WWTP Raw Sewage		

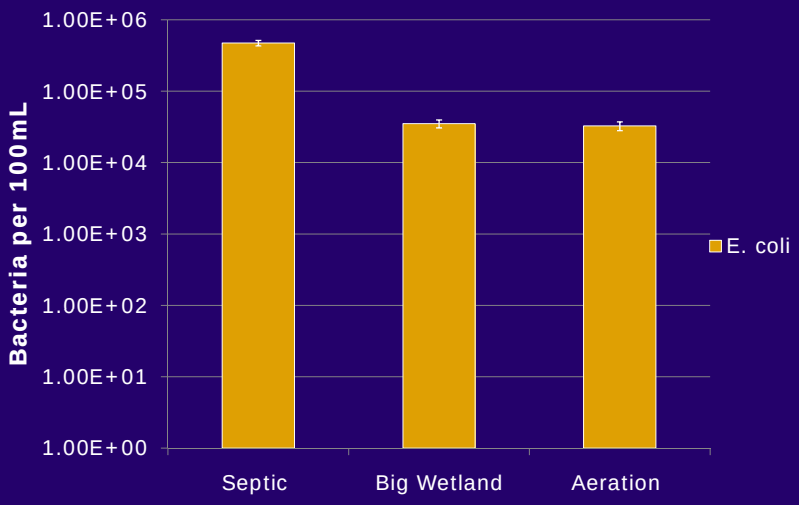
Quarterly Log Reduction in *E. coli* from Raw Sewage



Monthly Log Reduction in *E. coli* from Raw Influent



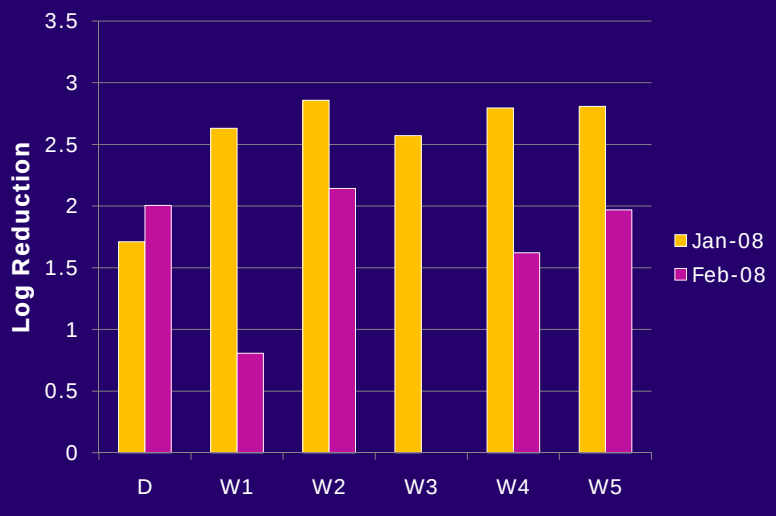
Mean Concentrations of *E. coli*



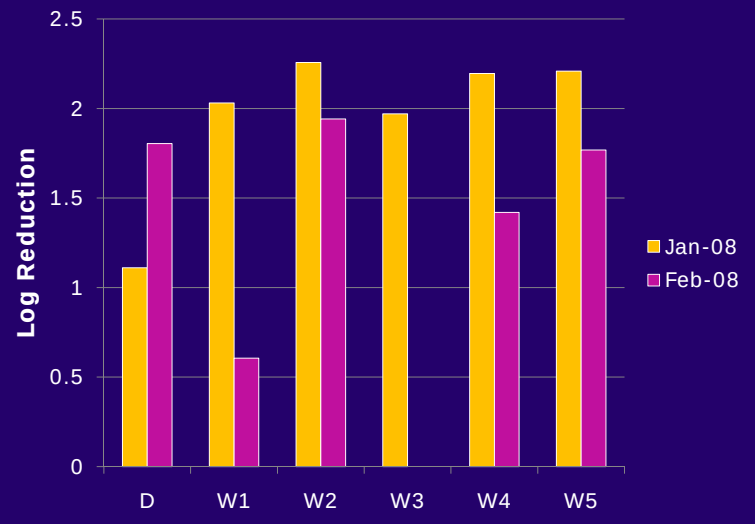
Wetland Characteristics

Wetland	Comments
Wetland 1	Plants, expanded shale, continuous loading
Wetland 2	No plants, expanded shale, continuous loading
Wetland 3	Plants, expanded shale, intermittent loading
Wetland 4	Plants, no expanded shale, continuous loading
Wetland 5	Plants, expanded shale, continuous loading, screened elevation drops
Big Wetland	Plants, expanded shale, continuous loading

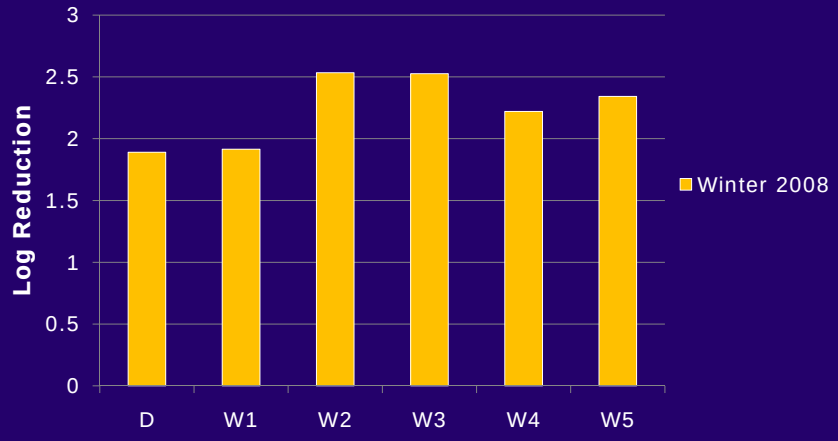
Monthly Log Reduction in *E. coli* from Raw Influent



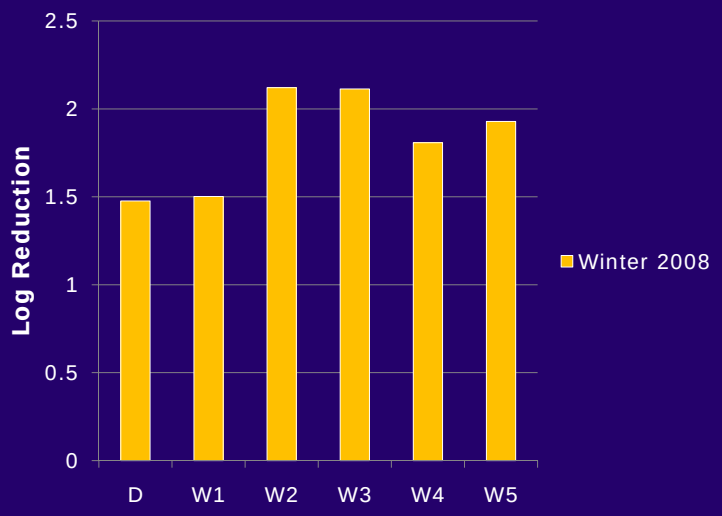
Monthly Log Reduction in *E. coli* from Septic Effluent



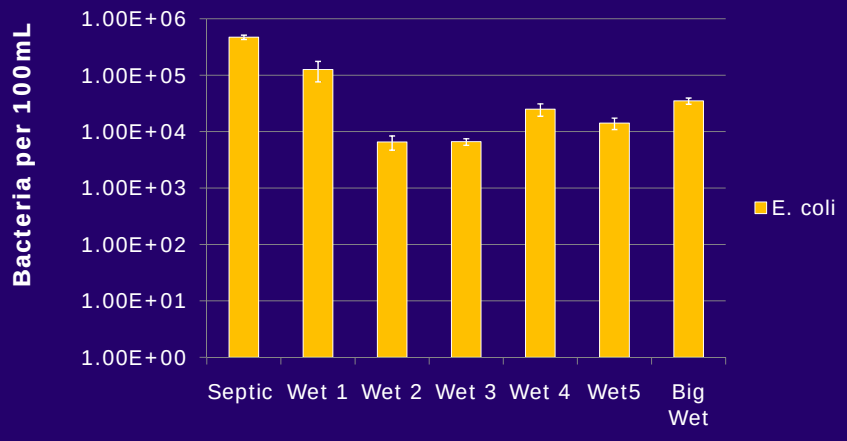
Quarterly Log Reduction in *E. coli* from Raw Influent



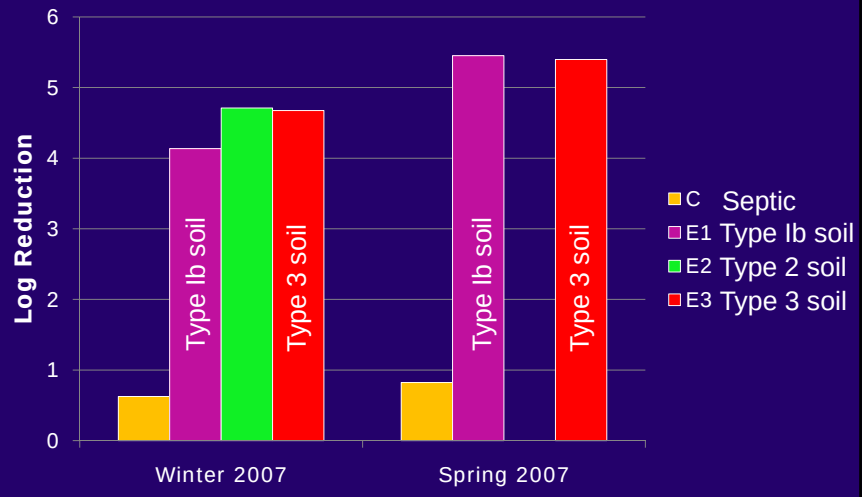
Quarterly Log Reduction in *E. coli* from Septic Effluent



Mean Concentrations of *E. coli* from Wetlands



Quarterly Log Reduction in *E. coli* from Raw Influent



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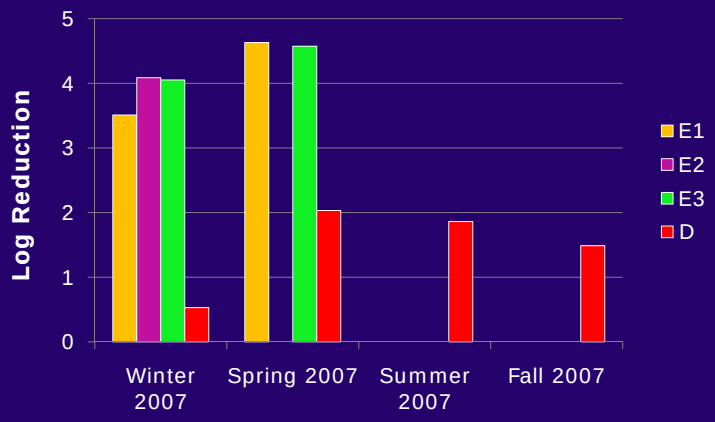
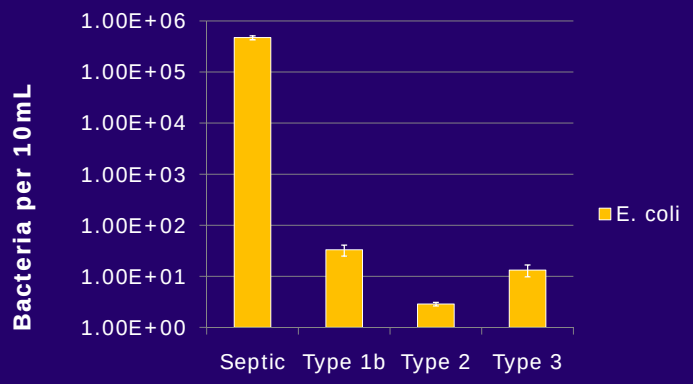


Mean Concentrations of *E. coli* from Soil Columns



Analysis of Antibiotic Resistance

- Growth overnight at 35°C in Mueller-Hinton Agar
- Replicate plated onto Mueller-Hinton agar containing antibiotics at concentrations ranging as follows:
  - Ampicillin: 25, 50, 100 µg/mL
  - Erythromycin: 5, 10, 20, 40 µg/mL
  - Streptomycin: 15, 30, 60, 120 µg/mL
  - Tetracycline : 6, 12, 24, 48 µg/mL
- Grown at 35°C overnight
- Scored for growth (less than 50% of control scored as no growth)

Percentage of Antibiotic Resistant Bacteria by Site

Antibiotic	Septic Inflow	Septic	Wet-land	Type 1 Soil	Type 3 Soil	Aerobic Inflow	Aerobic
Ampicillin 100 µg/mL	39.2	46.8	52.6	37.5	43.2	32.3	46.1
Erythromycin 40 µg/mL	16.4	14.4	23.2	18.2	12.0	13.9	15.4
Streptomycin 120 µg/mL	5.6	13.4	12.8	3.3	11.3	2.0	6.6
Tetracycline 48 µg/mL	38.1	52.8	50.5	40.0	27.1	33.4	36.9

Conclusions

- Comparative Wastewater Treatment – average over entire time period
  - All treatments significantly reduced *E. coli* and total coliforms relative to influent
  - Septic tank alone significantly reduced *E. coli* and total coliforms
  - Septic + Wetland and Aerobic Unit = statistically similar
    - Greater reduction over septic tank alone
    - *E. coli* and total coliforms

Conclusions

- Antibiotic Resistance in Treated Effluents
  - Can be found in effluent from all onsite wastewater systems.
  - The relative proportion of antibiotic-resistant bacteria tends to increase during treatment (influent vs. effluent).
  - Effluents from the septic tank and submerged-bed wetland tended to have higher amounts of antibiotic-resistant *E. coli*

Future Directions

- Compare virulence factors in *E. coli* from treated effluents
  - Gene-specific PCR analysis
- Complete antibiotic resistance analysis (over entire study period)
- Analysis of chlorination and UV disinfection

Acknowledgments

Texas Onsite Wastewater Treatment Research Council

Dr. Joe Yelderman and Crew

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