

Florida Department of Environmental Protection



Division of Environmental Assessment and Restoration

An Update on FDEP's Microbial Source Tracking Efforts

by

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FDEP MST Tools

- Geographic Information Systems (GIS)
- Provides information about surrounding land use, hydrology, municipal wastewater and stormwater infrastructure, previous sampling efforts, etc...
- Fecal Indicator Bacteria
- Determines compliance with water quality criteria



FDEP MST Tools

- Quantitative Polymerase Chain Reaction (q-PCR)
- Bacterial or viral genetic markers strongly or uniquely associated with particular groups of animals (e.g., human, cattle, dog, pig, chicken...)
- Analytical Chemistry
- Chemical compounds unique to human wastewater sources (e.g., sucralose, galaxolide, tonalide, gadolinium...)



FDEP MST Tools

- Walk the WBID
- Pull all of the stakeholders together, review maps of the area of interest, and physically walk the water segment and look for potential sources of fecal pollution



New Bacteria Criteria

- FDEP adopted new fecal indicator bacteria criteria in December, 2015
- New criteria have not been approved by EPA yet
- Hope to have EPA approval by early 2017
- New criteria include a monthly geometric mean (MGM) and upper value not to be exceeded in 10% or more of the samples during any 30-day period (Ten Percent Threshold Value or TPTV), with units of cfu/100 mL
 - For E. coli, MGM is 126 and TPTV is 410
 - For Enterococci, MGM is 35 and TPTV is 130
 - MGM based on a minimum of either 5 samples (Class I) or 10 samples (Class III) taken over a 30-day period
 - No minimum sample size proposed for TPTV
- Keeping fecal coliforms for Class II (shellfish) waters
 - Median value of 14 with not more than 10% exceeding 43 (MPN) or 31 (MF), nor 800 on any one day.



FDEP MST Markers

- MST markers being used by the FDEP lab
 - Human
 - Bacteroidales HF183
 - Bird
 - Gull2 (Sinigalliano, 2010)
 - *Catelicoccus marimammalium*
 - GFD (Green, 2012)
 - Unclassified *Helicobacter sp.*
 - Propidium Monoazide (PMA)
 - q-PCR treatment to determine if MST marker DNA is from live or dead bacteria



FDEP MST Markers

- Possible Future MST tools to be used by the FDEP lab
 - Human
 - HumM2 citation (Shanks, 2009)
 - Species *Bacteroides*
 - EPA Coliphage Method EPA 1601 and 1602 (plating method, with incubation)
 - Ruminant
 - BacR citation (Reischer, 2006)
 - Species *Bacteroides*
 - CowM2 citation (Shanks, 2008)
 - Species *Bacteroides*
 - Dog
 - GFE (Green, 2014)
 - *Bacteroides* DG3, DG37, DG72



Analytical Chemistry

Wastewater markers currently used by FDEP

- ❖ sucralose (Splenda®)
- ❖ acetaminophen (Tylenol®)
- carbamazepine (mood stabilizer)
- primadone (anticonvulsant)
- triclosan methyl (antimicrobial)
- caffeine (stimulant and food product)
- 1,4-dioxane (stabilizer/byproduct)
- gadolinium (NMR imaging agent)
- galaxolide (synthetic musk)
- tonalide (synthetic musk)
- iohexal (X-ray contrast agent)
- hydrocodone (semi-synthetic opioid)
- metformin (Type-2 diabetes)
- duloxetine (anxiety disorder)
- simvastatin (statin cholesterol)
- fluridone (aquatic herbicide)



Human Wastewater Tracers

Sucralose

- Artificial sweetener
- Basically a chlorinated sucrose molecule
- Is not broken down through digestion or wastewater treatment processes
- In 2012, D/EAR conducted a survey of Florida wastewater treatment plants detected sucralose concentrations between 10 and 40 parts per billion in final effluent
- Detection limit was approximately 10 parts per trillion



Human Wastewater Tracers

- Sucralose is found in reuse/reclaimed water at concentrations similar to WWTP effluent
- The detection of sucralose does not indicate the presence of untreated wastewater, only that there has been some mixing of wastewater (treated or untreated) and the surface water
- Additional markers that are not so recalcitrant to degradation by common wastewater treatment processes are needed to help discriminate treated from untreated wastewater sources



Human Wastewater Tracers

Acetaminophen (Tylenol®)

- Is present in WWTP influents in relatively high concentrations similar to sucralose
- Unlike sucralose, acetaminophen is readily removed by common wastewater treatment processes and is therefore often absent from, or significantly reduced in, final effluents

Human Wastewater Tracers

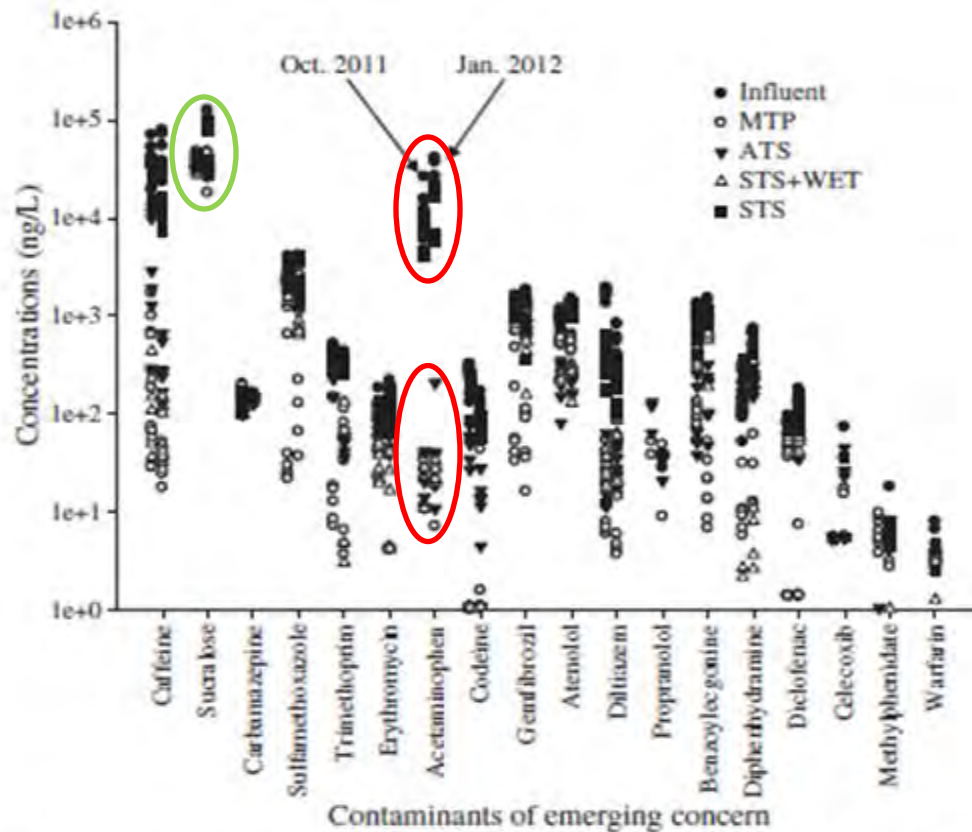


Fig. 2. Detected target contaminants of emerging concern in influent and various effluent discharges by season. MTP = municipal treatment plant; ATS = aerobic treatment system; STS = septic treatment system; WET = wetland.

Bowen, Du et al. 2014. Comparison of contaminants of emerging concern removal, discharge, and water quality hazards among centralized and on-site wastewater treatment system effluent receiving common wastewater influent. *Sci. of Total Environ.* 466-467, pp. 976-984.



Human Wastewater Tracers

Table 1
Concentrations across all 50 effluent samples.

Analyte	CasNumber	Method ^a	RL ^b (ng/L)	Number of measurements	Number of detections	PEC ^c (ng/L)	Mean ^d (ng/L)	Max ^d (ng/L)
10-Hydroxy-amitriptyline	64520-05-4	1	5	50	6	5029	<RL	<RL
Acetaminophen	103-90-2	1	5	50	7	306,955	79 (300)	1500 (4500)
Albuterol	18559-94-9	1	9.7	50	27	471	14	35
Alprazolam	28981-97-7	1	9.1	50	15	103	10	31
Amitriptyline	549-18-8	1	5	50	20	5029	11	110
Amlodipine	111470-99-6	1	5	50	11	94	6.9	18
Amphetamine	51-63-8	1	1.6	50	5	387	3.5	40
Atenolol	29122-68-7	1	6	50	48	4137	940	3000
Atorvastatin	134523-00-5	1	38	48	4	2906	<RL	<RL
Benzotropine	86-13-5	1	10	50	0	33	ND	ND
Carbamazepine	298-46-4	1	4.4	50	48	5607	97 (140)	240 (460)
Ciprofloxacin	85721-33-1	2	10	49	30	NA	67 (72)	260 (320)
Clonidine	4205-91-8	1	35	50	0	43	ND	ND
Desmethylsertraline	79902-63-9	1	9.4	50	9	615	9.9 (10)	24
Diltiazem	33286-22-5	1	2.8	49	41	3343	85	340
Diltiazem-desmethyl	130606-60-9	1	1.6	50	34	3343	24	100
Enalapril	76095-16-4	1	1	50	9	369	4.6	38

Table abbreviated

M.S. Kostich et al. / Environmental Pollution 184 (2014) 354–359



FDEP Wastewater Study

- FDEP has begun a study to quantify the levels of MST markers and tracers in the influent and effluent of approximately two dozen wastewater treatment facilities
- This information may provide us with additional tools to differentiate treated from untreated wastewater sources
- A variety of plant and treatment types are included
 - chlorine, UV, ozone disinfection
 - large and small discharge volumes



Implementing MST Studies

- MST studies are initiated on high priority waters (e.g., those perceived as being of greatest risk to human health)
 - Urban watersheds with suspected human waste contamination
 - cattle waste contamination
 - high potential for exposure through recreational use



Implementing MST Studies

- Conditions may need to be assessed over multiple seasons because site conditions are not static
 - Temperature
 - Rainfall
 - Flow
 - Ground water level
 - Light intensity
 - Land use
 - Agriculture, snow birds, vacationers
 - Wildlife patterns



Implementing MST Studies

- MST studies use a toolbox approach since no one tool is 100% reliable
- Relative source contributions are estimated using a weight of evidence approach
- Results of MST studies can be used to direct further Basin Management Action Plan (BMAP) activities



Questions?

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