



Chemical markers of human waste contamination in source waters: A simplified analytical approach

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Office of Research & Development

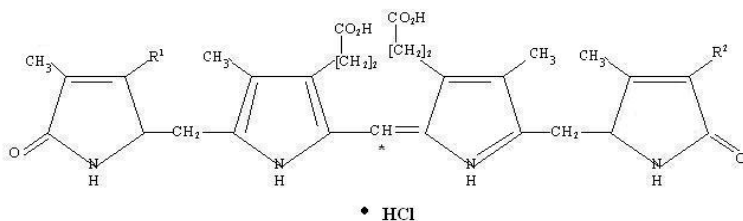
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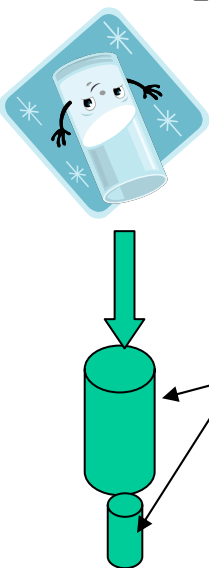
Introduction

- Giving public water authorities a tool to monitor and measure levels of human waste contamination of waters simply and rapidly would enhance public protection.
 - Chemicals shed in feces and urine might be used to detect human waste contamination of environmental waters.
 - Sterols
 - Bile acids
 - Urobilin
 - Finding human-use drugs affiliated with urobilin can help define the waste as human.
 - Azithromycin (antibiotic) and methamphetamine (substance of abuse) were detected
- 



Experimental

- Samples
 - Grab samples collected, 1L or less, keep cool, above freezing but $< 4^{\circ}\text{C}$ until extraction w/in 24-hrs
 - pH adjust to < 3.0 with 12N HCl
- Solid phase extraction
 - OASIS HLB cartridges [Waters Corporation (Milford, MA)]
 - 6-mL capacity, 0.2 g, 30- μm
 - Prep cartridges 5 mL methanol followed by 5 mL DI water at a rate of 1 mL/min
 - Load samples, 500 mL, into 60-mL reservoirs (60 mLs at a time); start extractions w/ pump at a rate of 4 mL/min
 - Dry samples, via pump, for $< 5\text{min}$.
 - Extract with 40 mL methanol:1%acetic acid at a rate of 1 mL/min.
 - Nitrogen blow-down extract to 0.5 mL – ready for LC/MS analysis.

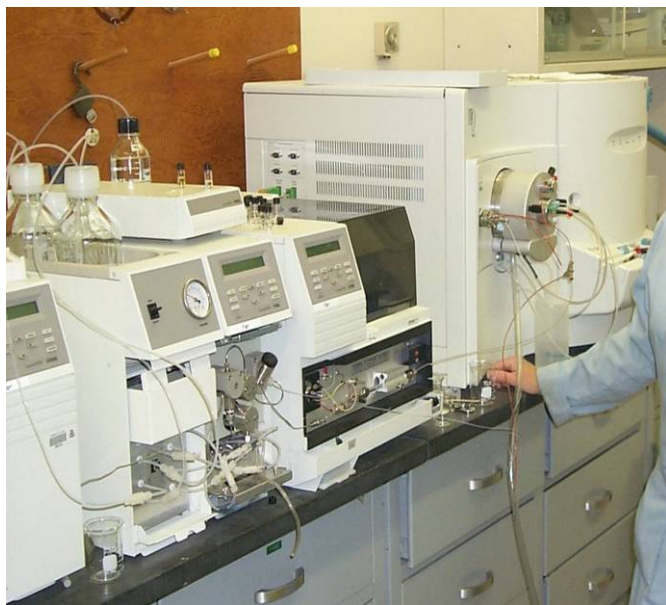


Experimental con't

- μ LC-electrospray-ion trap mass spectrometer (ThermoQuest Finnigan LCQ™)

HPLC

- C18 RP, 5 μ m particle size, 150 \times 3.2 mm liquid chromatography column
 - flow rate of 0.40 mL min⁻¹, and a 40:60 split after the column, such that 40% of the flow (160 μ L min⁻¹) goes to the ES-ITMS
- Mobile phase: A: 99% water/1 mM ammonium acetate/0.1% acetic acid/1% methanol;
B: 98% methanol/1 mM ammonium acetate/0.1% acetic acid/2% water.
100% mobile phase A (hold for 1 min) to 100% mobile phase B (hold for 5 min)
over a 20-min gradient, with a 5-min equilibrium between runs.



ES-ITMS

- positive ionization mode
- Screening - scanned from 120 to 830 amu (full-scan mode)
- Heated capillary - 215°C
- For quantifying and confirming - Two other modes, selected ion monitoring (SIM) and collision-induced dissociation (CID), were used.

Sampling Sites

- Southwest – 1 site in Southern Nevada
- Great Lakes – 2 sites on Lake Michigan



- New England – 18 sites
 - 9 in Maine
 - 9 in Connecticut

Southern Nevada – 1 site



Great Lakes – 2 sites

Lake Michigan beach site 1 (Silver Beach)

Lake Michigan beach site 2 (Washington Beach)

| Sample | Urobilin ng/L | Azithromycin ng/L | n |
|---|------------------|----------------------|---|
| Lake Michigan beach site 1 (Silver Beach) | | | |
| June 29, 2004 | nd | nd | 3 |
| July 13, 2004 | nd | nd | 2 |
| July 27, 2004 | nd | nd | 2 |
| August 17, 2004 | nd | nd | 2 |
| September 8, 2004 | no sample | | 0 |
| Lake Michigan beach site 2 (Washington Beach) | | | |
| June 29, 2004 | no sample | | 0 |
| July 13, 2004 | nd | nd | 2 |
| July 27, 2004 | nd | nd | 2 |
| August 17, 2004 | nd | nd | 2 |
| September 8, 2004 | nd | nd | 2 |

New England – Region 1 Maine

9 sites

| Sample | Urobilin ng/L | Azithromycin ng/L | n |
|---|------------------|----------------------|---|
| AA26900a – Sanford WWTP | † | 77 | 1 |
| AA26900b laboratory duplicate | † | 75 | 1 |
| AA26901 400 ft downstream Sanford WWTP | 33 | 47 | 1 |
| AA26902a² Yarmouth POTW | 11 | nd | 1 |
| AA26902b laboratory duplicate ² | 15 | nd | 1 |
| AA26903 Royal River Landing | 316 | † | 1 |
| AA26904 Lewiston WWTP | 11 | nd | 1 |
| AA26905 Androscoggin River 0.8 mi downstream Lewiston WWTP | 21 | † | 1 |
| AA26906 South Portland WWTP | 295 | 41 | 1 |
| AA26907 Fore River - marina | 16 | † | 1 |
| AA26909 Hampden boat landing | 52 | 4 | 1 |

nd = non-detect; † positive MS/MS identification, but below LOQ; ² Methamphetamine detected: 5 ng/L.

S. Portland Maine WWTP & Fore River Marina

AA26906

AA26907



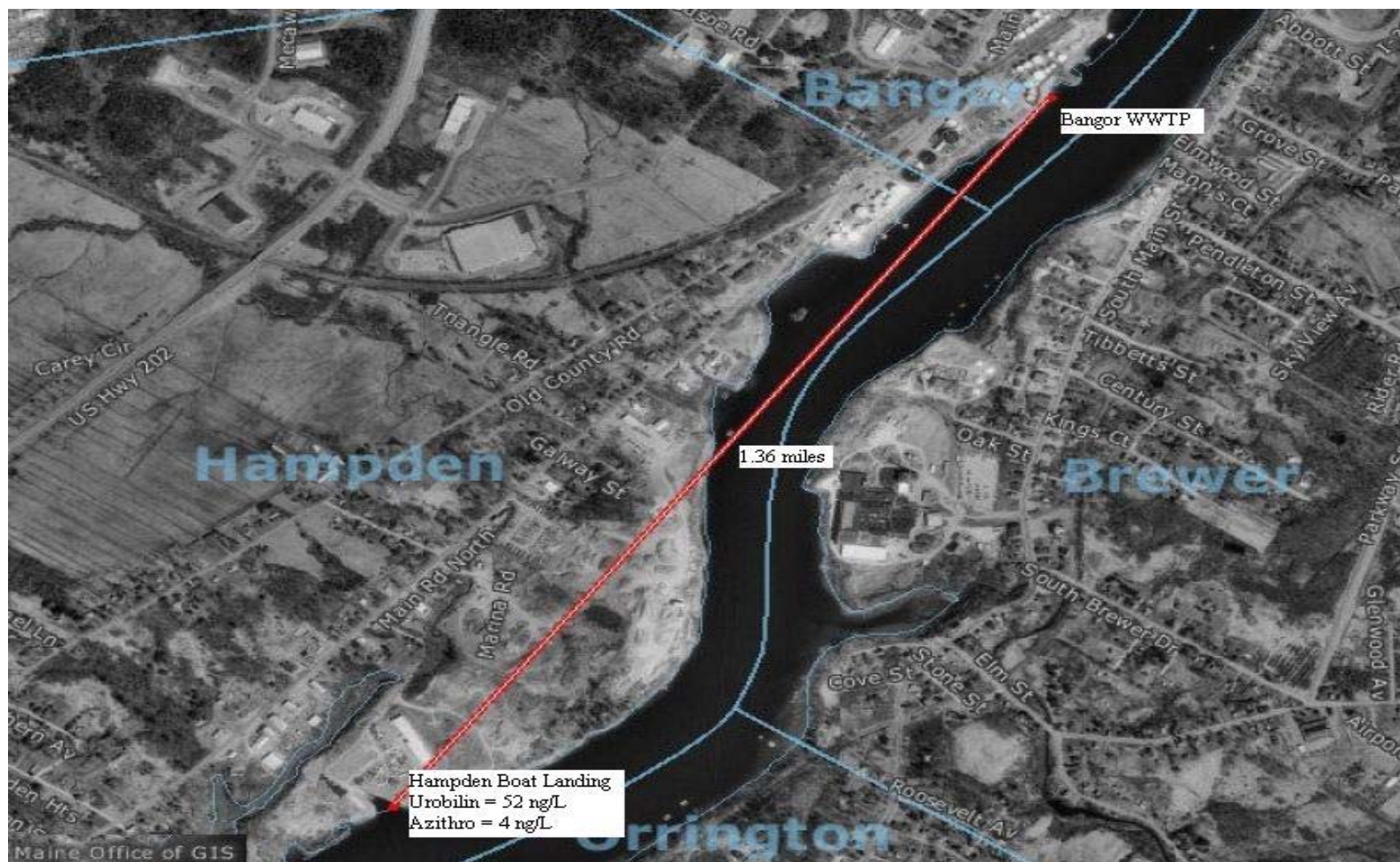
Yarmouth Boat Landing AA26903

& Yarmouth POTW AA26902



NOTE: Yarmouth water supply tested positive for coliform Aug and Oct 2002.
Samples were collected Nov 2002. Royal River Yarmouth Town Landing same area.

Hampden Boat Landing



New England – Region 1 Connecticut

9 sites

| Sample | Urobilin ng/L | Azithromycin ng/L | n |
|---|------------------|----------------------|---|
| AA29823 – 1.64 miles downstream Manchester WWTP | † | 13 | 1 |
| AA29824 – rural stream no housing in the immediate area | nd | nd | 1 |
| AA29825 – densely populated 60% stream flow wastewater 1.25mi upstream | 16 | 5 | 1 |
| AA29826 – stream flow 70% wastewater | nd | 39 | 1 |
| AA29827 -- stream flow 90% wastewater | † | 34 | 1 |
| AA29828 -- collected 0.8 km downstream from a senior housing condominium complex, which has its own small WWTP | nd | 2 | 1 |
| AA29829 0.65 mi from WWTP | nd | 15 | 1 |
| AA29830 field duplicate of 29829 but collected 15 min apart | 42 | 23 | 1 |
| AA29831 – 2mi downstream WWTP – failed septic systems 90% wastewater | 17 | nd | 1 |
| AA29832 – 2mi downstream WWTP – stream flow 40 % wastewater | 22 | nd | 1 |
| Control blank | nd | nd | 1 |

nd = non-detect; † positive MS/MS identification, but below LOQ

•None of the Connecticut samples were collected directly from WWTPs. Most were located 1 km or greater from WWTP sewage outfalls, yet both urobilin and azithromycin were detected in some samples.

Conclusions

- Detection of urobilin, along w/ a human-use drugs can equivocally show that human waste is entering a water source.
- Tertiary/secondary treatment does not seem to efficiently remove azithromycin, as evidenced by its detection at both the Sanford and Portland WWTPs, as they both have similar environmental loadings, 0.5 kg/yr and 0.6 kg/yr

Future Research

- Investigate correlations between urobilin, nitrate, and coliform levels using principal component analysis

Acknowledgments

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NOTICE

- Although this work was reviewed by EPA and approved for publication, it may not necessarily reflect official Agency policy. *Mention of trade names or commercial products does not constitute endorsement or recommendation for use.*