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Introduction

In Florida, water is a commodity that is actively preserved and protected for both human and animal needs. Little Lake Worth should be no exception. Over the years leaching from septic tanks buried beneath housing developments surrounding the lake has left the lower water anaerobic, killing the wildlife residing within. In my project I will be using fecal sterols as markers to confirm the contamination.¹

BACKGROUND

The pollution can further be proven by various compounds that are synthesized in the intestines of omnivores and carnivores with the compounds coprostanol, coprostane and cholestanol being the most identifiable. These compounds are synthesized in the liver of higher mammals. Starting as cholesterol, and finishing at coprostanol or Coprostane as seen in Figure 1.

To prove the contamination of Little Lake Worth, multiple samples will be taken and analyzed using gas chromatography – mass spectrometry (GC-MS). The samples will be retrieved by gravity corer and controlled derivatization of the samples with N,O-bis-(trimethylsilyl)-trifluoroacetamide (BSTFA) containing 1% of Trimethylchlorosilane (TMCS) and pyridine will confirm that the compounds are present in the water and sediment samples collected.² TMCS acts as a catalyst that leads to the trimethylsilyl (TMS) The internal standard used for this experiment is androstane, with Hexanes as the solvent. Little Lake Worth can be seen in Figure 2.

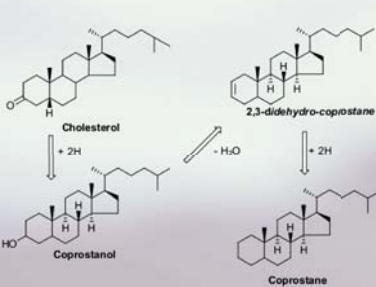


Figure 1.³



Figure 2.¹

SUPPORTING DATA

In Figure 3, coprostan-3-ol TMS derivative (MW = 460.85 g/mol) is shown. The peaks of interest are at 215 m/z at $\approx 45\%$ intensity. This represents common fragmentation in fecal sterols. This happens between the C13 and C17 and between the C14 and C15 bonds leading to the elimination of the D-ring. Depending on the D-rings specificities, it will affect the fragment ions characteristic.⁴ The other main peak is at 370 m/z as the base peak, confirming the compounds presence and derivatization.⁴ This peak represents the departure of the TMS group leaving the main component of coprostan-3-ol (Figure 4).

Figure 5 below is the MS of coprostan-3-ol with androstane being used as an internal standard to correct for the loss of analyte during sample preparation. Androstane is shown on the bottom with the signal peaks at 245 m/z and 260 m/z.⁵ Coprostan-3-ol is shown on the top with the same peaks of interest shown in Figure 3.

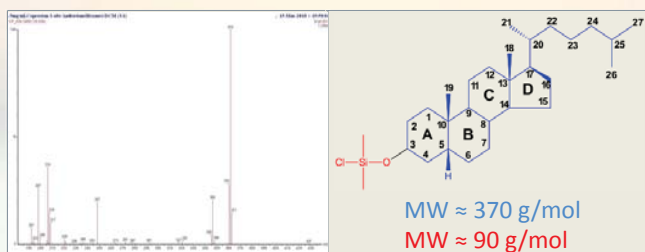


Figure 3: MS of Coprostan-3-ol

Figure 4: Coprostan-3-ol TMS derivative³

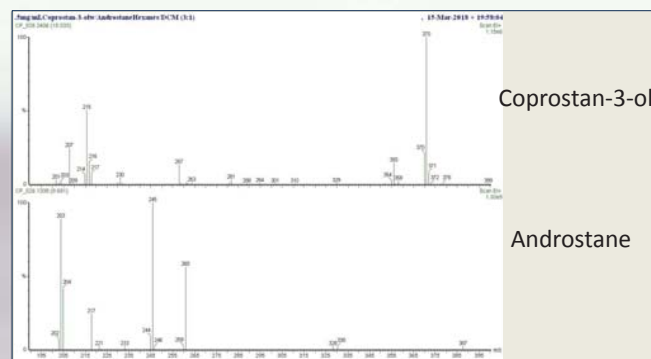


Figure 5: MS of both Androstane & Coprostan-3-ol.



Figure 6: GC-MS

CONCLUSION

Based on current knowledge, the presence of coprostan-3-ol, coprostane, and cholestanol in the sample will prove the pollution of Little Lake Worth. With the derivatization method using BSTFA w/ 1% TMCS, I will be able to continually prove that the septic tanks are leaching. Further testing must be done to confirm the actual concentrations.

References & Acknowledgement

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6. Androstane <https://pubchem.ncbi.nlm.nih.gov/compound/5alpha-Androstane#section=Mass-Spectrometry> (accessed Mar 15, 2018).

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