# Endocrine Disruptors in the Sediment of the Quinnipiac River

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## INTRODUCTION

Anthropogenic sources, such as chemical spills, sewage, and poor wastewater treatment, have brought a significant amount of estrogenic compounds into the environment. As a result, these estrogenic compounds are known to produce developmental, reproductive, neurological and immune effects (NIEHS 2016). Estrogenic compounds may be naturally produced hormones or may take the form of synthetic chemicals that interact with the human endocrine system (xenoestrogen) (EMXE 2004), both of which may produce negative effects.

In Connecticut, the Quinnipiac River has a long history of contamination from industry use, which has resulted in more foreign substances, like plasticizers, making their way into the sediment (The Quinnipiac River Fund 2015). These plasticizers are additives that give plastics flexibility but are suspected to interfere with the endocrine system (Plasticizers 2016). Given that high exposure to estrogen has led to reproductive cancers in humans (Gao et al. 2015), as well as reproductive damages in fish (Colborn et al. 1993), it is important to determine if these substances are present in local waterways. Many cancers, like breast cancer, are caused by an estrogen dependent malignancy. The increasing rate of breast cancer reports has a direct correlation with those who are highly exposed to Bisphenol A (BPA) (Fernandez 2004). Studies have shown demonstrated that the exposure to BPA activates estrogen receptors, which stimulate breast cancer cell growth (Fernandez 2004). Steroid hormones, like estradiol, have a hydrophobic (Oren 2004) thus creating the potential that they may be drawn down into the sediment and persist there rather than moving through the water column/flow.

The yeast *Saccharomyces cerevisiae* BLYES has been genetically modified to respond to estrogenic compounds by producing bioluminescence. It does so in a quantitative manner; producing more light in response to more estrogen such that total estrogenic potential in a sample can be calculated. When the strain is exposed to samples, a correlation is made between standard concentrations of estrogen and the potential estrogens present (Sanseverino et al. 2005).

## MATERIALS & METHODS

**Glassware**- Glassware was rinsed with methanol and acetone then baked in a muffle oven for four hours at 500°C. This allowed us to sterilize the glassware and remove any organic residue.

**Solid-Phase Extraction-** Samples were extracted according to EPA Method 1694. Briefly, the samples were collected with a glass jar, then no more than 5g of sediment was measured . Samples were treated with three cycles of a phosphate buffer (pH 2), acetonitrile , 30 minutes of sonication, and 5 minutes of centrifugation at 3000 rpm. After each centrifuge, the aqueous layer is extracted and stored. The extracted sample is dried down to 20-30 mL under nitrogen while in a warm bath , then EDTA and DI water are added. A Hydrophilic-Lipophilic-Balance Oasis disk at a rate of 5-10ml/minute. The disk was treated with methanol, DI water and DI water with pH of 2 then completely dried. The sample was extracted through the Oasis disk and eluted with methanol. The sample was then dried down with nitrogen while in a warm bath until dry and then stored in a freezer until processed in yeast assays.

**Yeast Assay and Dilutions-** Samples were serially diluted and then 50 microliters of each dilution was placed into a 96 well-plate. Once the samples were dry, 100 microliters of water and 100 microliters of yeast, that had been grown over night, were added to the 96 well-plate. Then the plate was incubated for four hours at 30°C.

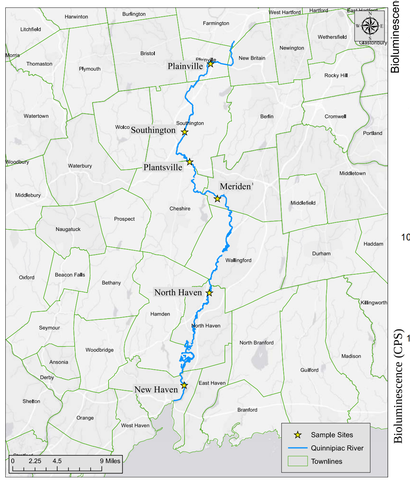


**Figure 1. Quinnipiac River watershed.**

**Figure 2. Sample collecting.**

## RESULTS

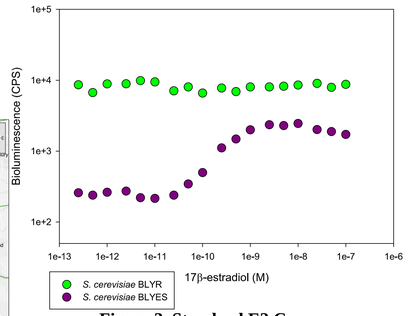


**Figure 3. Standard E2 Curve**

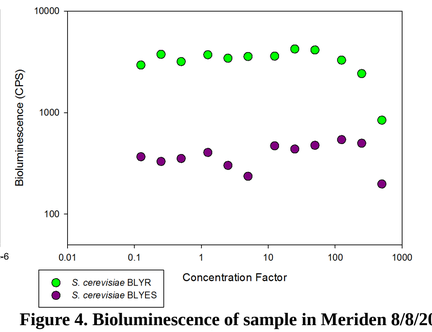


**Figure 4. Bioluminescence of sample in Meriden 8/8/2016**

**Figure 10. Solid-phase extraction.**

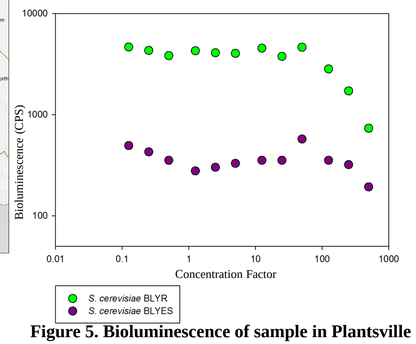


**Figure 5. Bioluminescence of sample in Plantsville 8/8/2016**

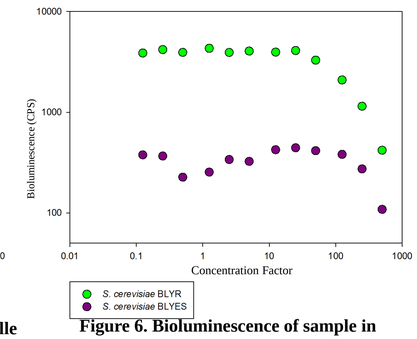


**Figure 6. Bioluminescence of sample in New Haven 8/8/2016**

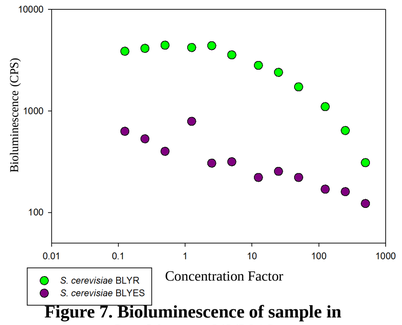


**Figure 7. Bioluminescence of sample in Southington 8/8/2016**

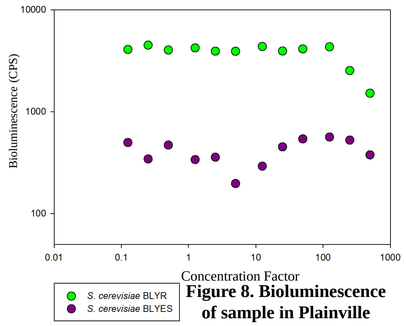


**Figure 8. Bioluminescence of sample in Plainville 8/8/2016**

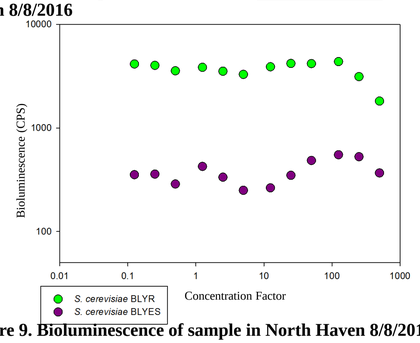


**Figure 9. Bioluminescence of sample in North Haven 8/8/2016**

## CONCLUSION

This data allows us to further understand where and how much estrogen is present in the sediment of the Quinnipiac River. Figures 4-9 show the amount of bioluminescence the *S. cerevisiae* produce when exposed to these samples of sediment. The amount of bioluminescence allows us to see how much of an impact the estrogen has on the yeast. In each graph there is a common trend occurring where the bioluminescence of the *S. cerevisiae* decreases significantly. This indicates there is a high amount of toxicity in the sediment. Currently, we are still analyzing samples with yeast assays. Our further research of analyzing macrobenthos and water samples will allow us to find the true source of the estrogen.

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## ACKNOWLEDGEMENTS

I would like to thank the SURF program, Carol Withers and Kimmie Johnson for all the help throughout the duration of the program. Lastly, I would like to thank the Quinnipiac River Fund for funding this research.