Estrogenic Compounds in the Quinnipiac River

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Introduction

Endocrine disruptors are chemicals that adversely affect an organism's endocrine system, which in turn can cause developmental, reproductive, and neurological damage for living creatures (National Institute, 2015). Estrogenic chemicals are a class of endocrine disruptor that has gained national attention in the last 15 years, resulting in changes to the composition of nearly all plastic water bottles. Estrogen is a chemical that is normally found in the human body and is needed for normal development of females. Increased exposure to estrogenic chemicals has been linked to breast cancer because of the chemical's part in inducing breast cell division and their effect on other chemicals that induce breast cell division (Clark, 2015). People can be exposed to estrogenic compounds in the environment through water sources. These types of chemicals can be introduced into water by leaking sewage, wastewater treatment plant effluents, and chemical spills. Sewage is filled with human waste that contains many forms of estrogen as well as numerous other types of chemicals. Sewage goes through wastewater treatment plants to remove the chemicals but not everything is cleaned before the sewage is discharged into local waterways.

The Quinnipiac River is one of the many waterways that is polluted by trash, storm water, industrial waste, fertilizers, pesticides, erosions, and sewage (River Pollution and Solutions, 2015). Since the Quinnipiac River has many sources of potential inputs of endocrine disruptors and drinking water influent is collected there, it is important to determine if estrogenic substances are found in this waterbody. The Quinnipiac River has multiple wastewater treatment plants that release treated sewage water back into the river. Therefore there is a high possibility that estrogenic chemicals will be found in wastewater treatment plant effluents. In order to test whether there are estrogenic chemicals in the water, samples are collected and then bioassays can be used to detect the amount of bioavailable estrogenic substances present in the water.

The yeast *Saccharomyces cerevisiae* BLYES has been genetically modified such that estrogenic compounds bind to the human estrogen receptor protein (contained in the yeast), which initiates a series of reactions resulting in the production of bioluminescence. The light given off by the yeast can be measured and then used to determine the concentration of estrogens (Sanseverino *et al.*, 2005).

Materials and Methods

Glassware- All the glassware was rinsed with methanol and acetone then baked in a muffler over for four hours at 500ºC. This ensures that all organic compounds in the glassware were destroyed and prevents contamination between samples.

Filtration and Extractions- One liter of sample was collected in baked glassware and then transported back to lab on ice. It was then filtered in order to remove any solids. Samples were passed through a Hydrophilic-Lipophilic-Balance Oasis disk at a rate of 5-10ml/minute, according to EPA Method 1694. Afterwards, methanol was passed through to elute chemicals that were collected in the filter. The samples were dried down with a gentle stream of nitrogen and then kept in the freezer until they were ready to be further processed.

Yeast Assays and Dilutions- Samples were serially diluted and then 50 microliters of each dilution was placed into a 96 well-plate. Once the samples were allowed to 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 30ºC.

Results

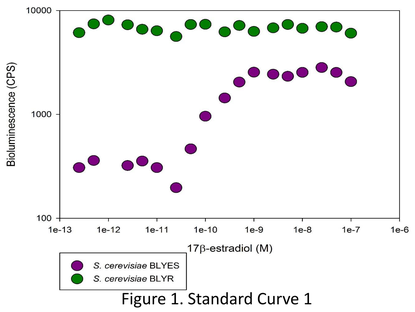

Figure 1. Standard Curve 1

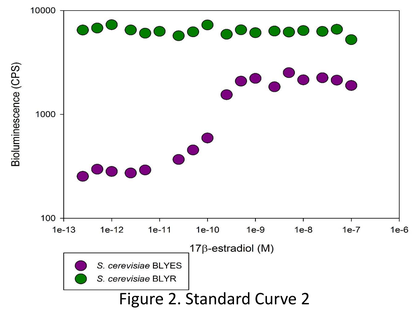

Figure 2. Standard Curve 2

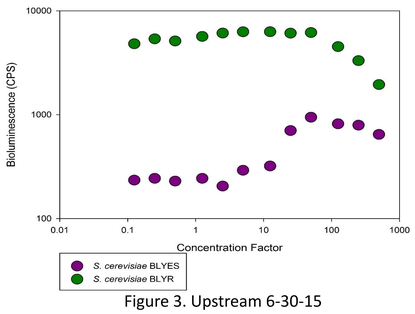

Figure 3. Upstream 6-30-15

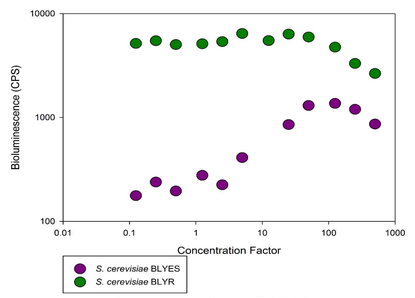

Figure 4. Upstream 7-16-15

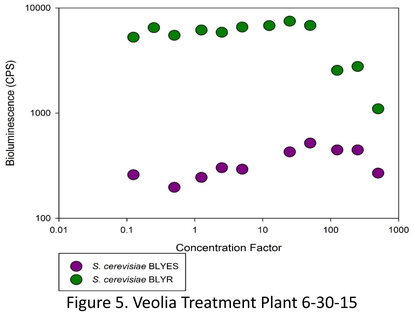

Figure 5. Veolia Treatment Plant 6-30-15

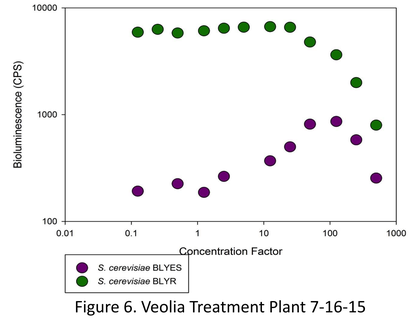

Figure 6. Veolia Treatment Plant 7-16-15

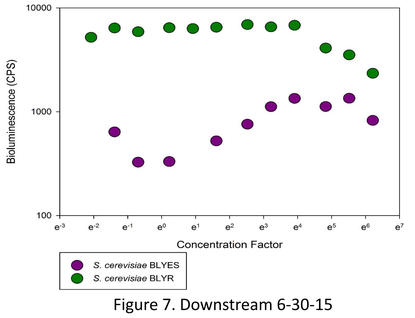

Figure 7. Downstream 6-30-15

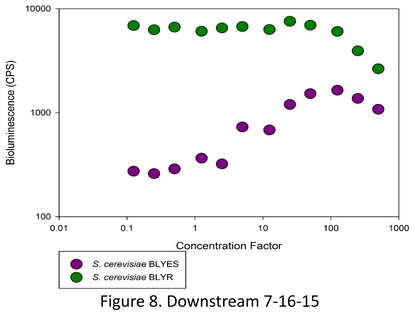

Figure 8. Downstream 7-16-15

Figure 9. (Above) Sample Collection.

Figure 10. (Above) Extraction set up.

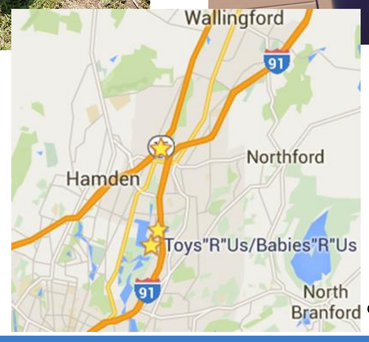

Figure 11. (Left) Map of sample collection locations.

Conclusions

The results show that there are estrogens present in our local waterways: upstream, downstream, and in wastewater treatment plant effluent. The next step, while we continue to collect and process more samples, is to quantify the concentration of estrogen present in each of the samples. This will allow us to help trace where the source of estrogenic input is into the watershed.

References

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