Hazen Technical Memorandum

Technical Memorandum: Assessing the Impact of Anthropogenic Discharges of Endocrine Disruption in the Potomac River

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Summary of Key Findings

This technical memorandum summarizes a research project designed to address the impact of anthropogenic discharges of endocrine disruptors in the Potomac River which was developed and performed in accordance with the requirements of the Water Quality Assurance Amendment Act of 2012. The project was designed to provide holistic monitoring and identification of emerging and unregulated contaminants, in the form of estrogenic endocrine disrupting compounds (eEDCs), in the District's drinking water source (i.e. the Potomac River) and the Blue Plains Advanced Wastewater Treatment Plant (Blue Plains AWTP) Effluent. The two primary objectives of the project are summarized below:

- Evaluate the upstream and downstream impacts from 'best-in-class' nutrient control, agriculture management, stormwater management and wastewater treatment strategies on concomitant eEDCs (estrogens and estrogenic activity were the template eEDCs evaluated) mitigation from the Potomac River, and
- Assess the relative contribution of eEDCs from wastewater treatment plants (WWTPs) performing biological nutrient removal.

To address these objectives, sampling campaigns in the Potomac Watershed were performed on a bimonthly schedule (exceeding the quarterly mandate), with an emphasis on identifying relative source contributions from point sources (including Blue Plains) and non-point sources to the watershed. Sampled locations were chosen to represent a "paired watershed" approach for studying non-point inputs and impact of BMPs. In addition, the project aimed to evaluate the impact of advanced wastewater treatment (Blue Plains AWTP), along with several other best management practices for improving wastewater quality and drinking water source quality throughout the Potomac through co-management of nutrients and eEDCs. As summarized below, major conclusions from the study indicated that:

- Implementation of BMPs focused on nutrient management showed great potential for co-managing inputs of estrogenic compounds to the Potomac Watershed (Objective 1).
- Greater than 95% reduction in estrogens and estrogenic activity were observed at the Blue Plains advanced nutrient control process (Objective 1).
- Input of Blue Plains effluent may dilute estrogenic activity and correlated with observed reductions in estrogen concentrations in the Potomac between Hains Point and National Harbor, the location of the Blue Plains outfall (Objective 2).

- An annual load calculation indicated non-point sources (agriculture and urban) accounted for over 90% of estrogen load to the Potomac, with Blue plains contributing less than 7% (Objective 2).

Objective 1 Results

Because of the environmental sensitivities associated with the Chesapeake Bay ecosystem, examples of point- and non-point source nutrient management Best Management Practices (BMPs) have been implemented throughout the Potomac Watershed. To date, most emphasis has been on implementing advanced nutrient control at point sources like the Blue Plains WWTP. BMPs for non-point sources (i.e. agriculture and urban runoff) have been implemented less frequently throughout the watershed. Examples of Nutrient management BMPs are shown in Figure 1 and described in greater detail in the memo and accompanying detailed report (Appendix).

Figure 1: Examples of Nutrient Management BMPs for Potomac Watershed Urban and Agriculture non-point sources and the Blue Plains Advanced Nutrient Control Facility

Results from the paired watershed evaluation indicated statistically significant differences in estrogen concentrations associated with implementation of BMPs across all sectors, summarized below:

- Measured estrogenic activity was observed to be 74% less with agriculture BMP implementation, and 87% less with urban stormwater BMP implementation.
- Measured estrone concentration was observed to be 68% less with agriculture BMP implementation, and 44% less with urban stormwater BMP implementation.
- Measurements indicated an average of 99% reduction in estrogenic activity and 96% reduction in estrone across the Blue Plains advanced nutrient removal facility.

Objective 2 Results

Using the results from the sampling plan, combined with available land use data, observed precipitation, and assumed BMP implementation rates, a load analysis was performed to estimate annual contributions from the various contributing sources of estrogenic contaminants into the Potomac watershed. Summarized in Figure 2, the analysis indicated that inputs from point sources implementing nutrient management strategies similar to Blue Plains contributed less than 7% of the observed estrogenic load to the Potomac system, with agriculture and urban runoff sources representing significantly larger proportions of the total estrogen load (>65% and >25%, respectively).

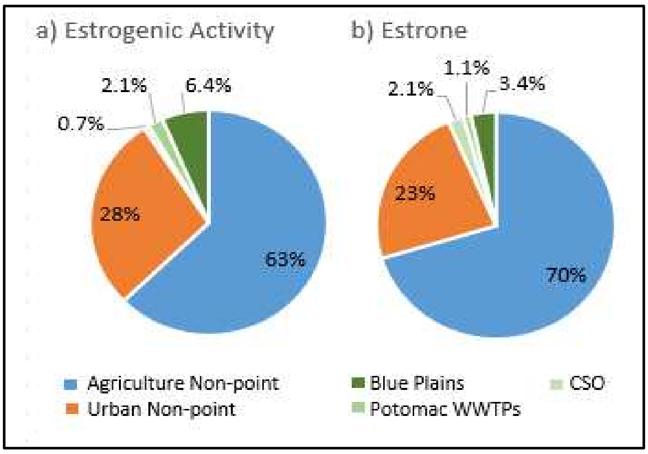

Figure 2: Summary of Estrogen Loads to the Potomac Watershed as a function of sector

Recommendations

In order to best balance management of water quality for human, economic, and ecological benefit in a resource limited environment, the consideration of holistic approaches for watershed protection are beneficial. This research project, while limited in scope and geographic focus, provided evidence detailing relative contributions of various sources of estrogenic compounds in the Potomac (focusing on point versus non-point contribution), and simultaneously showed potential for co-managing estrogenic compounds and nutrients through implementation of agriculture and urban stormwater BMPs.

In order to more fully understand holistic benefits of co-management BMP strategies, it is recommended that the District evaluate and fund additional studies to investigate the use of BMPs more holistically for the protection of the Potomac watershed. This evaluation should consider the use of BMPs to control multiple pollutants; nutrients, estrogens, and pathogens for the protection of the watershed, and utilize a risk assessment approach to ensure maximum watershed quality benefit returns on investments. Greater detail of several potential projects for developing this improved understanding are provided in the attached technical memorandum.

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1.0 Introduction

This technical memorandum summarizes a research study performed to address the impact of anthropogenic discharges of estrogenic endocrine disruptors (eEDCs) in the Potomac River which was performed as a result of the Water Quality Assurance Amendment Act of 2012. The Act required the following:

- To establish a Water Quality Assurance Panel to monitor and identify emerging and unregulated contaminants in the District's drinking water and wastewater discharge;
- To mandate quarterly testing for unregulated contaminants in the District's drinking water and wastewater effluent;
- To recommend to the Mayor an appropriate course of action for improving the reduction of unregulated contaminants and endocrine disruptor compounds at their source.

There are two locations along the Potomac River that are the drinking water source for the District and are upstream of Blue Plains Advanced Wastewater Treatment plant. A quarterly sampling event required by the Environmental Protection Agency's Safe Drinking Water Act Unregulated Contaminant Monitoring Rule 3 (UCMR3) was performed throughout 2014 as a separate monitoring program from this study. Those results are not discussed in this report but can be found on DC Water's website https://www.dcwater.com/emerging-compounds-testing.

This research study was developed in accordance with the requirements of the Act and designed to provide holistic monitoring and identification of emerging and unregulated contaminants, in the form of estrogenic endocrine disrupting compounds (eEDCs), in the District's drinking water "source" (i.e. the Potomac River) and the Blue Plains Wastewater Treatment Plant Effluent.

Sampling was performed on a bi-monthly schedule (exceeding the quarterly mandate), with an emphasis on identifying relative source contributions from point sources (including Blue Plains) and non-point sources to the watershed. In addition, the project aimed to evaluate the impact of advanced wastewater treatment (Blue Plains AWTP), along with several other best management practices for improving wastewater quality and drinking water source quality throughout the Potomac through co-management of nutrients and eEDCs.

This memorandum represents the documentation of the research project, to be presented to a Water Quality Assurance Panel within 30 days of study completion. The Panel shall convene a public meeting to discuss the results of the study with respect to issues listed in Act within 90 days. The Panel shall issue a report to the Mayor and GM within 120 days from the Panel convention, summarizing discussion and recommendations. Upon receipt of the Panel report, DC Water shall create and implement a Plan considering remediation to submit to Mayor and Council.

2.0 Project Background

The Chesapeake Bay has long been considered one of the more ecologically sensitive water environments in the United States, heavily impacted by eutrophication and hypoxia related to agriculture and urbanization of the watershed. Recognizing its importance to the health of the coastal marine system, the contributing freshwater tributaries to the Bay have been heavily scrutinized over the years and significant measures have been taken to improve the quality of the water entering the Bay, particularly with regard to nutrient load. The Potomac River is a major contributor of freshwater to the Bay system, with the watershed also serving as an important spawning and nursery ground for migratory and resident fish species, and as a drinking water source for more than 4 million people in the Maryland/DC/Virginia corridor. The multiple and diverse needs of the Potomac system, as well as the focus on this sensitive waterway for examining human and ecological health impacts of emerging contaminants and nutrients, makes it an ideal study site for examining management of multiple water quality goals.

In the Potomac River watershed, both nutrients and eEDCs have been identified as major issues that will continue to negatively impact the health of the river's aquatic fauna. Municipal wastewater treatment facilities (WWTPs) have been implicated as major contributors of nutrients and eEDCs in this and other watersheds. However, it is known that eEDCs also originate from non-point sources such as agricultural runoff (e.g., confined animal feed operations (CAFOs) and manure and pesticides used in crop-based farming) or urban/suburban runoff. Previous eEDC research in the Potomac River watershed has shown linkages between fish health and point sources such as WWTP effluents or non-point sources such as animal feeding operations, and similar sources for nitrogen and eEDCs from the watershed.

Currently, possible nutrient management strategies in the Potomac River include upgrades of wastewater treatment plants (WWTP), upgrades of stormwater management facilities and implementation of various agricultural best management practices (BMP's). Billions have been spent at hundreds of reclaimed water treatment plant facilities throughout the Chesapeake Bay watershed to upgrade with advanced technology aimed to reduce the amount of nutrients that are discharged into the Bay's tributaries. Additionally, relatively simple and inexpensive BMPs also exist for reducing nutrient loads from urban stormwater and agricultural runoff. Since WWTPs are not the sole contributor of eEDCs in waterways, there is a critical need to accurately quantify the relative input of each discharge on the overall endocrine disruption in the Potomac River.

In cases where eEDC load reductions may be required, it will also be necessary to recommend specific technologies for this purpose. A practical approach is to use or improve upon existing infrastructure that provides the capability for enhanced nutrient removal. Herein, the Potomac watershed is unique. The major WWTPs in this watershed provide high levels of nutrient removal (TN < 3 mg/L and TP <0.18 mg/L), and research has indicated the potential of advanced nutrient removal strategies for excellent eEDC reduction as well. Despite this evidence, a thorough elucidation of eEDC fate through WWTPs performing nitrogen and phosphorus removal is lacking. Clearly, a further understanding of how nutrient removal configurations impact eEDC toxicological fate 'in the watershed' is necessary, especially if the desired goal is to minimize eEDC discharge.

2.1 Project Objectives

To address these needs, two project objectives were identified. The first objective is to evaluate the upstream and downstream impacts from 'best-in-class' nutrient control, agriculture management, stormwater management and wastewater treatment strategies. The second objective is to assess the relative contribution of eEDCs from WWTPs performing biological nutrient removal.

3.0 Project Approach

3.1 Description of Sampling Efforts

In order to address the project objectives, two sampling campaigns were undertaken. From January 2015 to January 2016, bimonthly samples were collected at 15 locations throughout the Potomac Watershed, selected to monitor inputs to the Potomac Watershed from agricultural and urban non-point sources, as well as two wastewater treatment plants and a combined sewer overflow point sources. Monitoring locations are shown on Figure 1, and represent a "paired watershed" approach to non-point source monitoring, where geographically similar watersheds with varying degrees of BMP implementation were selected, along with an "impact assessment" approach to point-source monitoring, where effluent samples were collected in conjunction with upstream and downstream samples.

Collected samples from point and non-point sources using different degrees of nutrient control strategies were collected, processed, and characterized using estrogen sensitive bioassays, liquid chromatography with tandem mass spectrometry (LC-MS/MS) analyses, conventional water quality, nutrient isotope tracking, and excitation emission matrix fluorescence spectroscopy (EEMs).

In addition, passive sampling was performed at six (6) locations in the Potomac River (shown in Figure ES-1 Inset), focusing on locations in the DC metro area, to parse eEDC and nutrient contributions of upstream rural and suburban sources, urban sources, and Blue Plains effluent impacts on the Potomac water quality.

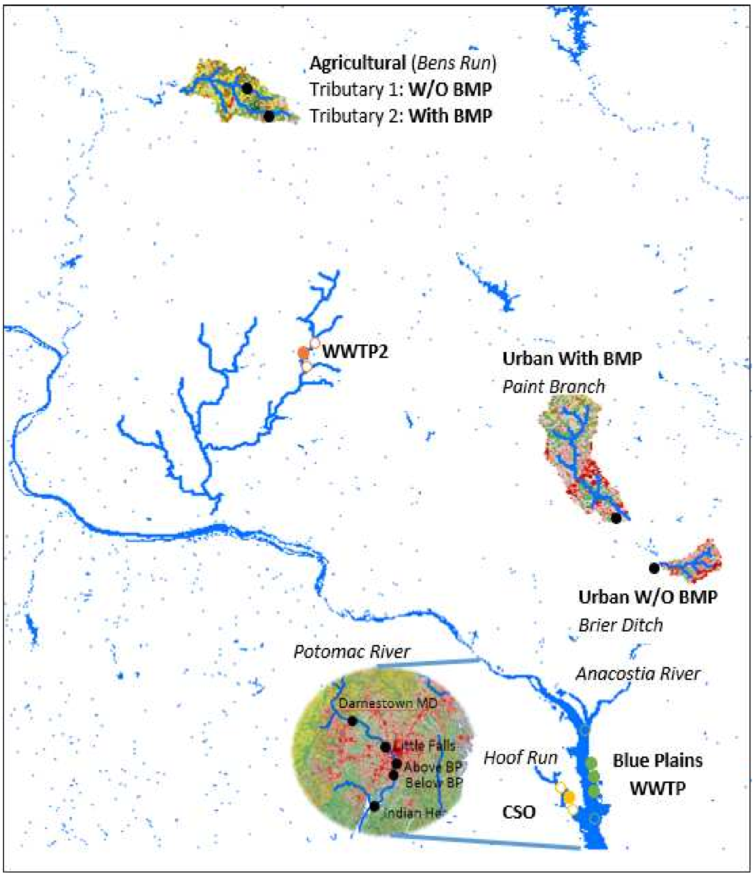

Figure 1: Locations of 15 bi-monthly sample collection locations within the Potomac watershed. (Inset image displaying passive sampling deployments in DC Metro area.

Passive sampling devices (polar organic chemical integrative samplers, POCIS) were deployed for 30 day intervals on two occasions during the study, in November 2015 and April 2016. These stainless steel canisters are capable of holding three Oasis HLB sorbent membrane and facilitating flow past the cartridges. These samples were analyzed with biological eEDC assays, LC-MS/MS characterization and EEMs analyses. Additionally, load analysis was used to estimate contributions of eEDCs from various sources.

3.2 Description of BMPs Evaluated

Currently, possible nutrient management strategies in the Potomac River include upgrades of wastewater treatment plants (WWTP), upgrades of stormwater management facilities and implementation of various agricultural best management practices (BMPs). Urban stormwater management upgrades include 1) converting and retrofitting current facilities into BMP's that employ more effective treatment mechanism(s) such as wetlands or reuse options 2) increasing stormwater BMP treatment volume and/or increasing harvesting capacity and hydraulic retention time, and 3) repairing stormwater BMPs to restore performance through major sediment cleanouts, vegetative harvesting, filter media upgrades, or full-scale replacement. Common agricultural BMPs, another type of nutrient management strategy for non-point sources, include fencing for grazing livestock, cover crops, forest buffers, manure storage areas and rotational grazing. Figures 2 and 3 display examples of urban and agriculture management strategies take from studied watersheds implementing BMPs, including the Paint Branch sub-watershed (urban) and the Ben’s Run watershed (agriculture).

Figure 2: Example of Stream Restoration Project in the Paint Branch Sub-watershed

Figure 3: Example of Fencing, Cattle Watering Relocation, Stream Crossings, and planting BMPs in the Ben’s Run Watershed in 2006 (Before) and 207 (After)

The Blue Plains AWTP serves over two million customers with a collection area of Washington DC and surrounding suburbs of Maryland and Virginia. It is the largest treatment plant in the Potomac River watershed and the largest treatment facility of its kind in the US, with a rated capacity of 384 million gallons per day. The treatment process utilizes preliminary and primary treatment, secondary treatment, along with advanced wastewater treatment processes including nitrification/denitrification, effluent filtration, chlorination- dechlorination and post aeration. Nitrification /denitrification processes upgrades were implemented at the Blue Plains Wastewater Treatment Plant to constitute the enhanced nutrient removal process. The upgrade project, completed at a cost of $950 million, reduces nitrogen to less than 4 mg/L, approaching the limit of conventional treatment technology. Figure 4 details a portion of the nutrient removal technology implemented as part of the Blue Plains Advanced Wastewater Treatment Process.

Figure 4: Images from Blue Plains Advanced Wastewater Treatment Plant and Enhanced Nutrient Removal Process

3.3 Analytical Methods

Methods of detection are discussed further in the report, however it is important to note that both biological and chemical analyses were performed to evaluate concentration of eEDCs in the samples. A bioassay analysis known as the bioluminescent yeast estrogen screen (BLYES) was used to quantitatively assess estrogenic activity relative to 17β-estradiol. This technique provides an opportunity to assess net estrogenic activity in a sample. In addition, LC/MS/MS technology was utilized to evaluate samples for a suite of estrogen compounds, including a variety of natural and synthetic estrogens, along with common human and ecological estrogen metabolites. The eEDC analytes evaluated with this method is shown in Table 1.

Table 1: Analyzed estrogens and their metabolites.

Targeted analytes	abbreviation	Chemical Description
Estrone	E1	natural; metabolite of estradiol
17α-estradiol	E2α	natural hormone from human & animals
17β-estradiol	E2β	natural hormone from human & animals
17β-Estriol	E3	natural; metabolite of estradiol
17α-Ethinylestradiol	EE2	synthetic , active ingredient of birth control pills
estrone-3-sulfate	E1-3S	natural; metabolite of estrone
estrone-3-glucuronide	E1-3G	natural; metabolite of estrone
17β-estradiol -17-sulfate	E2-17S	natural; metabolite of estradiol
17α-estradiol -3-sulfate	E2α-3S	natural; metabolite of estradiol
17β-estradiol -3-sulfate	E2β-3S	natural; metabolite of estradiol
17β-estradiol-3-glucuronide	E2-3G	natural; metabolite of estradiol
17α-ethinylestradiol-3-glucuronide	EE2-3G	natural; metabolite of estradiol
17α-Ethinylestradiol-d4	EE2-d4 (ISTD)	USED AS STANDARD
17β-estradiol-d3	E2-d3 (ISTD)	USED AS STANDARD
Estrone-d4	E1-d4 (ISTD)	USED AS STANDARD
17β-estradiol-3-sulfate-d4	E2-3S-d4 (ISTD)	USED AS STANDARD
17β-estadiol-3-glucuronide-d3	E2-3G-d3 (ISTD)	USED AS STANDARD
Estrone-3-sulfate-d4	E1-3S-d4 (ISTD)	USED AS STANDARD

In addition to the eEDC measurements, a full suite of conventional and advanced water quality parameters were analyzed at the University of Maryland laboratory. The list of water quality parameters included:

Conventional Water Quality Metrics

- Dissolved Organic Carbon (DOC)
- Total Dissolved Nitrogen (TDN)
- Nitrate (NO3-)
- Nitrite (NO2-)
- Soluble reactive phosphorus (SRP)
- Dissolved organic nitrogen
- Ammonia/ammonium
- UV-Vis absorbance

Advanced Water Quality Metrics

- Advanced Nutrient Fingerprinting
  - δ^{15}N-NO_3
  - δ^{18}O-NO_3
- Fluorescence Excitation Emission Matrices
  - Fluorescence Index (FI)
  - Humification Index (HIX)
  - Biological Freshness Index (BIX)

4.0 Summary of Results

Figure 5 provides a summary of key eEDC and nutrient results from the study, for point and non-point sources throughout the Potomac Watershed. The data are presented as mean values and standard errors (SE) for bulk estrogenic activity (measured by BLYES), estrone (which was the only estrogen detected consistently by LC-MS/MS), total dissolved nitrogen (TDN), and soluble reactive phosphorus (SRP). The values for each parameter are provided relative to background Potomac River levels to aid in comparison.

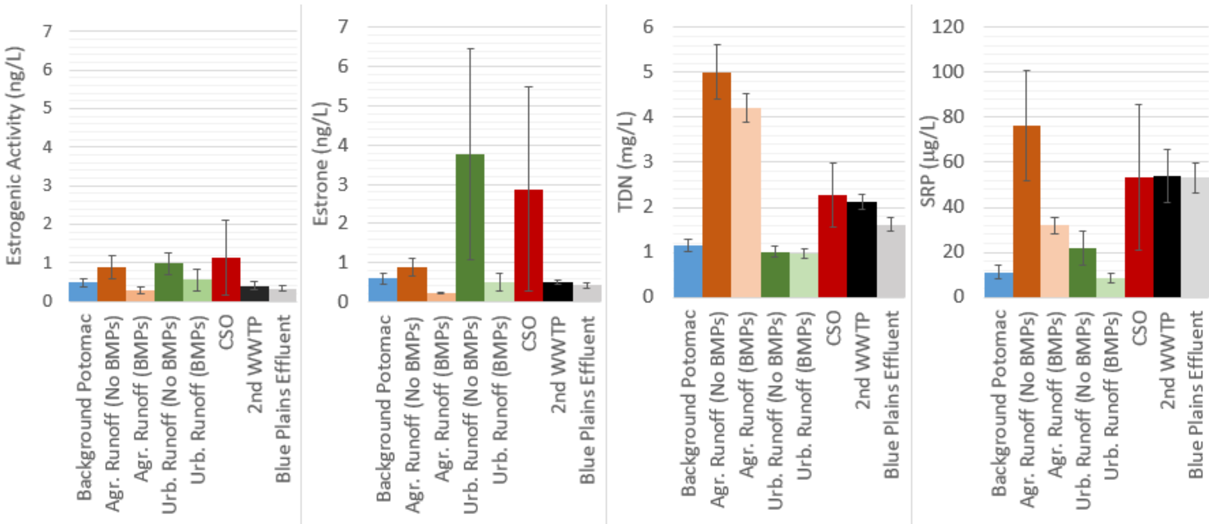

Figure 5: EDCs and nutrients for various sources in the Potomac Watershed

Levels of eEDCs measured were generally low in the background Potomac, as well as in all sources. To provide some context for what a 1 ng/L (or part per trillion) detection of these compounds means, 1ppt can be described by analogy, being equivalent to 1 minute in 2 million years, or approximately $11 of the February, 2017 US National debt of $19.9 trillion. Major sources of eEDCs showing levels above background Potomac levels included the “untreated” sources, including agriculture and urban without implementation of BMPs, along with CSOs. CSOs, while intermittent in flow contributions, provided relatively high levels of eEDCs to the system when discharging significantly. Implementing treatment however, whether as simple as fencing to keep livestock from watering in streams to urban stream restoration to costly advanced wastewater treatment upgrades, were all effective and resulted in levels comparable to the background Potomac.

For nutrients, the story was a bit more complicated. Agriculture, CSO, and WWTPs all showed significantly higher levels of nutrients than the background Potomac. Implementation of BMPs provided some reduction in levels, particularly in phosphorus for the non-point sources and nitrogen for the point sources. Interestingly, levels of nutrients in urban runoff were similar to background Potomac levels, with implementation of BMPs showing modest improvements in nutrient levels.

In order to meet the objectives of the project, a subset of the data has been further analyzed to develop correlations aimed at deconvoluting the complex set of eEDC inputs into the Potomac River. Presentation and discussion of additional results of the study are organized to address the following objectives:

- Objective 1: Assess the Performance of BMPs for emerging contaminants and nutrients
- Objective 2: Assess the relative contribution of eEDCs from WWTPs performing biological nutrient removal

4.1 Objective 1: Assess the Performance of BMPs for co-managing emerging contaminants and nutrients

Table 2 summarizes performance of BMPs for controlling levels of eEDCs and nutrients, from agriculture and urban non-point sources, and from wastewater treatment plant point sources. Large reductions in EDCs were observed with implementation of BMPs. These reductions in eEDC levels with BMP implementation associated well with reductions in SRP for the non-point sources (agriculture and urban) and associated poorly with reductions in nitrogen, suggesting that BMPs designed to minimize soluble reactive phosphorous may be more effective in co-managing eEDCs than those designed to achieve total nitrogen reductions for non-point sources. Conversely, while eEDC reductions in the point source (WWTP) were associated well with large nitrogen reductions, SRP levels actually increased, suggesting advanced wastewater treatment targeting nitrogen control is more effective for co-managing eEDCs.

Table 2: Performance of BMPs for emerging contaminant and nutrient reduction

	% Less with Implementation of Best Management Practices		
PARAMETER	**AGRICULTURE**	**URBAN**	**WWTP***
Estrogenic Activity (ng/L)	74%	87%	99%
Estrone (ng/L)	68%	44%	96%
Total Dissolved Nitrogen (mg/L)	16%	4%	94%
Soluble Reactive Phosphorus (μg/L)	62%	64%	-305%**
Dissolved Organic Carbon (mg/L)	58%	27%	44%

Notes:
*BMPs for WWTP are advanced tertiary treatment at Blue Plains. Comparison is between concentrations in secondary treated and tertiary treated water (advanced nutrient removal).
** SRP increases across tertiary treatment from an average of 0.012 mg/L to 0.05mg/L, which is still well below the TP limit of 0.18 mg/L

Figure 6 displays results from seasonal profile sampling events which occurred at the Blue Plains Advanced Wastewater Treatment Plant. The results show that effluent levels of nitrogen, and eEDCs are extremely low, with the bulk of the reductions of both parameters occurring in the advanced nitrogen removal process. On average, more than 99% of net estrogenic activity was removed between secondary effluent and post-advanced nitrogen removal at Blue Plains. While reduction was significantly higher in warmer months than cooler, this corresponded with significantly higher levels of eEDCs in the post-secondary effluent during these months. After nitrogen treatment, levels never exceeded 0.57 ng/L (part per trillion) as E1, even with post-secondary levels exceeding 70 ng/L on several occasions. Even in colder weather, when nitrogen treatment can be impacted, observed levels of eEDCs did not increase over background after the advanced treatment step.

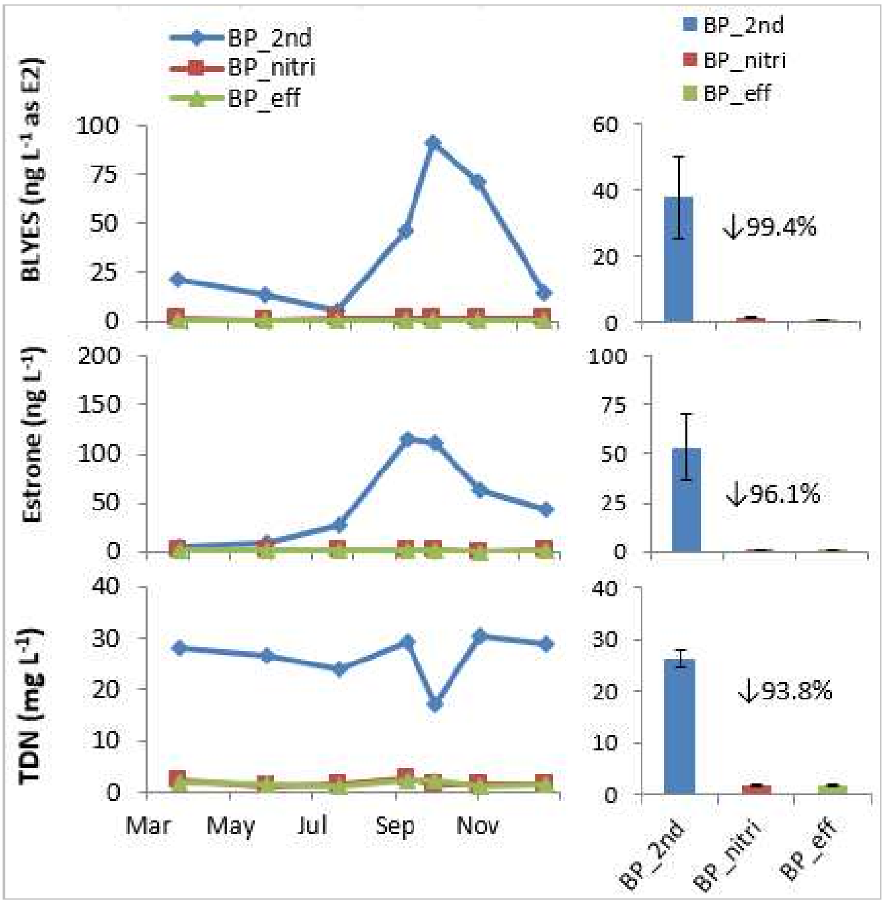

Figure 6: Seasonal variability and mean values for estrogenic activity, estrone, and nitrogen treatment profiles at Blue Plains. Note: BP = Blue Plains, 2nd = Post Secondary treatment, nitri = post advanced nutrient control, eff = plant effluent.

4.2 Objective 2: Relative Contribution of WWTPs to eEDC Activity in the Potomac

To address the question "What is the contribution of eEDCs of point vs. non-point sources to receiving waters?" three approaches to data analysis were utilized to compare the relative contributions from Point and Nonpoint sources. To provide the most robust analysis possible, two quantitative measures, along with a qualitative "fingerprinting" technique, were employed to characterize the relative source contributions to the Potomac system, as follows:

- Estimate and compare eEDC "loads" from point- and non-point sources into the Potomac.
- Passive, 30-day, sampling (POCIS) of eEDCs in urban Washington DC, to evaluate impact of Blue Plains effluent in the Potomac
- Preliminary Evaluation of Nutrient and DOC "Fingerprinting" to characterize relative source contributions to the Potomac System

4.2.1 eEDC and Nutrient "Loads" to the Potomac River.

To estimate annual contributions from the various sources of contaminants into the Potomac watershed, a load analysis was performed. By multiplying flow-average concentration with mean annual flow, annual loads of eEDCs, TDN, SRP and DOC from non-point and point sources to the Potomac River were estimated. Precipitation hydrographs were used to accurately quantify runoff potential from non-point sources, and generally acceptable and reasonable assumptions were made with respect to input flows from each source category. Assumptions were made for implementation of BMPs, included 30% for agriculture and 50% for urban, and advanced nutrient control on all WWTPs in the watershed. A summary of the load contributions for eEDCs, TDN, SRP, and DOC from agriculture and urban non-point sources, as well as WWTP point sources, is presented in Figure 7. Loads from Blue Plains were individually calculated to assess the relative contribution to the Potomac River for each of the parameters above.

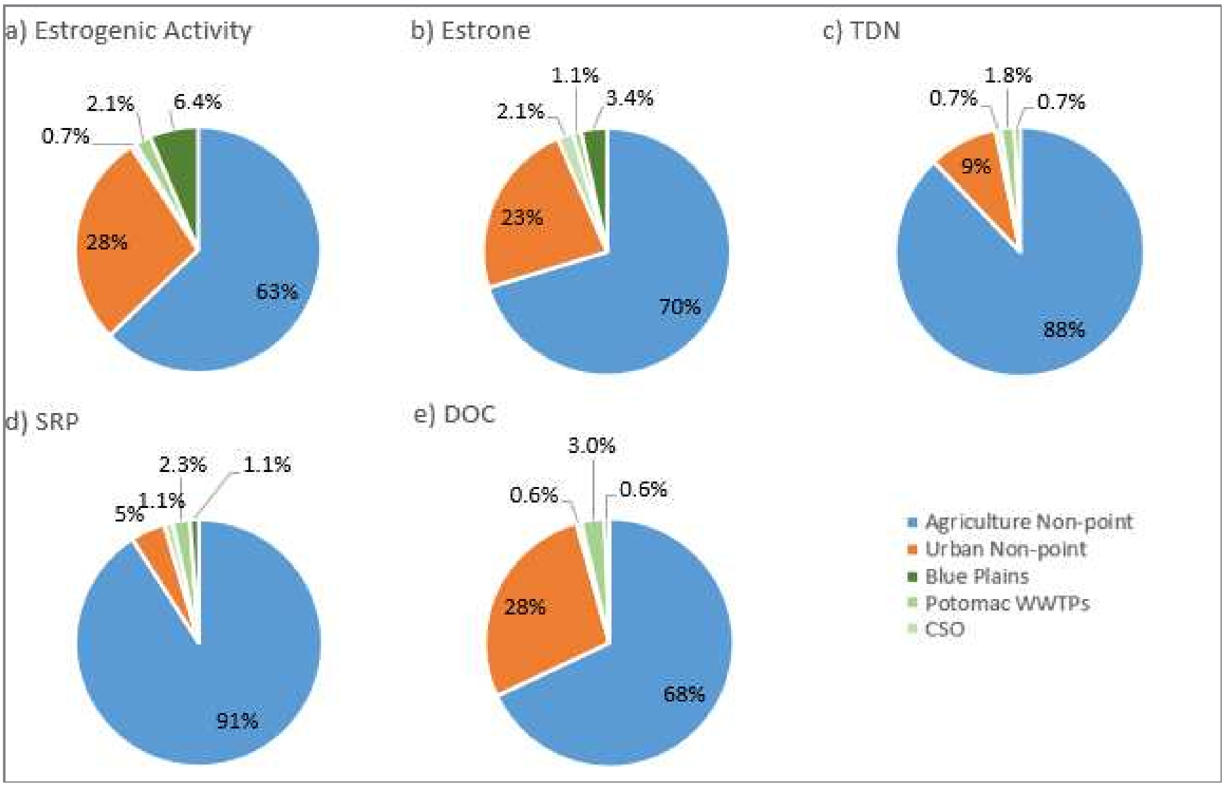

Figure 7: Percentage of Annual Load to the Potomac by Source for EDCs and Nutrients

The data clearly indicate that agriculture and urban non-point sources account for the great majority of eEDC and nutrient inputs into the Potomac River, with agriculture sources accounting for approximately 50% of the load for each parameter into the Potomac. By way of a sensitivity analysis, the assumed implementation rate of BMPs for non-point sources was increased to 80% implementation of BMPs which performed similarly for eEDC reduction as those evaluated in this study. The results of this analysis indicated significant reductions were possible with wider implementation, with up to 48% and 46% reductions possible in estrogenic activity with 80% implementation of BMPs. This would reduce the overall eEDC activity load to the Potomac from non-point sources by nearly 2.5 kg/yr (47%).

4.2.2 Passive Sampling Results in the Urban Area.

To further focus on the contributions from the Blue Plains AWTP effluent to the Potomac River, passive sampling devices (POCIS) were deployed at several locations in the Potomac, throughout the DC Metropolitan Area (Figure 8). The passive samplers were deployed for 30 day increments in the fall and spring. Hydrophobic compounds (including eEDCs) passively adsorb to the POCIS adsorbent material as the river flows past the sampler. The technique was utilized in this application to attempt to better assess the very low levels of eEDCs measured in the monthly grab samples, by providing essentially 30 days' worth of adsorbed material for detection. A summary of the estrone, the sum of all estrogens analyzed, and estrogenic activity (measured with YES) for each POCIS deployed is provided on Figure 8. Generally higher levels of measured estrogens and YES response were observed in the spring 2016 deployment. One interesting observation is the apparent reductions in measured estrogens and activity between Hains Point and National Harbor. The Blue Plains AWTP discharges into the Potomac between these two locations, contributing to this reduction, suggesting that highly treated wastewater flow can actually reduce levels of estrogens and/or estrogenic activity into the system over extended periods.

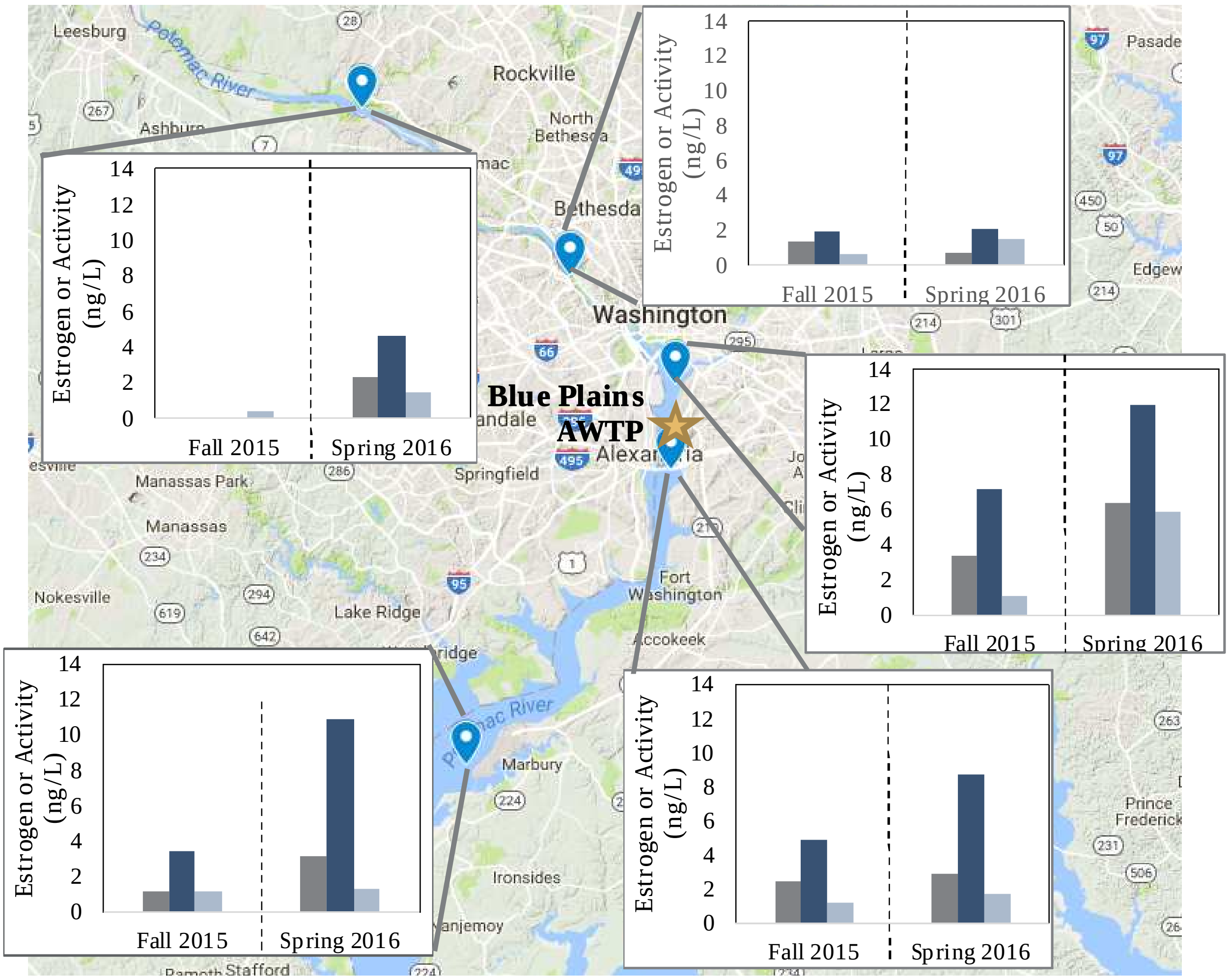

Figure 8: Results of Passive (30 day) sampling. Grey Bar represents E1, dark blue represents Total Estrogens, and light blue represents Estrogenic Activity as measured by the Bioluminescent YES Assay.

4.2.3 Advanced Metrics: Preliminary Analysis of Nutrient Isotope and DOC "fingerprinting" to correlate eEDCs with WWTP impacts.

As a third metric for understanding impact of non-point and point source inputs to the Potomac, nitrate isotope analysis and fluorescence analysis were used to evaluate changes in water quality, affected by inputs from the Blue Plains WWTP, a second analyzed WWTP (WWTP2), and non-point sources. Preliminary results from this nutrient and natural organic matter (NOM) "fingerprinting" are shown in Figure 9. For comparison, a box describing "background Potomac" ranges of each parameter is included, to provide context and compare impact of various sources.

The results of the qualitative fingerprint analysis indicated three main findings. First, even though direct measurements of the qualitative fingerprint nutrient and NOM markers indicated Blue Plains AWTP effluent to be different than observed in the Potomac background samples (boxed range in Figure 9), it was found that modest changes to the nutrient and NOM fingerprint of the Potomac occurred at the Hains Point locations above and the National Harbor location below its discharge. Further study should be performed to elucidate any potential relationships which may exist between input of flow from the Blue Plains AWTP and improved water quality in the Potomac.

Next, effluent from WWTP 2 significantly changes the nature of the organics in the receiving stream toward microbial in nature, provides minimal nutrient enrichment, and does not change levels of eEDCs in the receiving stream. Finally, it was observed that the non-point sources do not enrich the nutrients, but do provide organics that are significantly more aromatic in nature, indicating potential for anthropogenic impacts on the watershed from these sources. In addition, these sources show potential for introducing elevated levels of eEDCs to the watershed, particularly without implementation of appropriate BMPs.

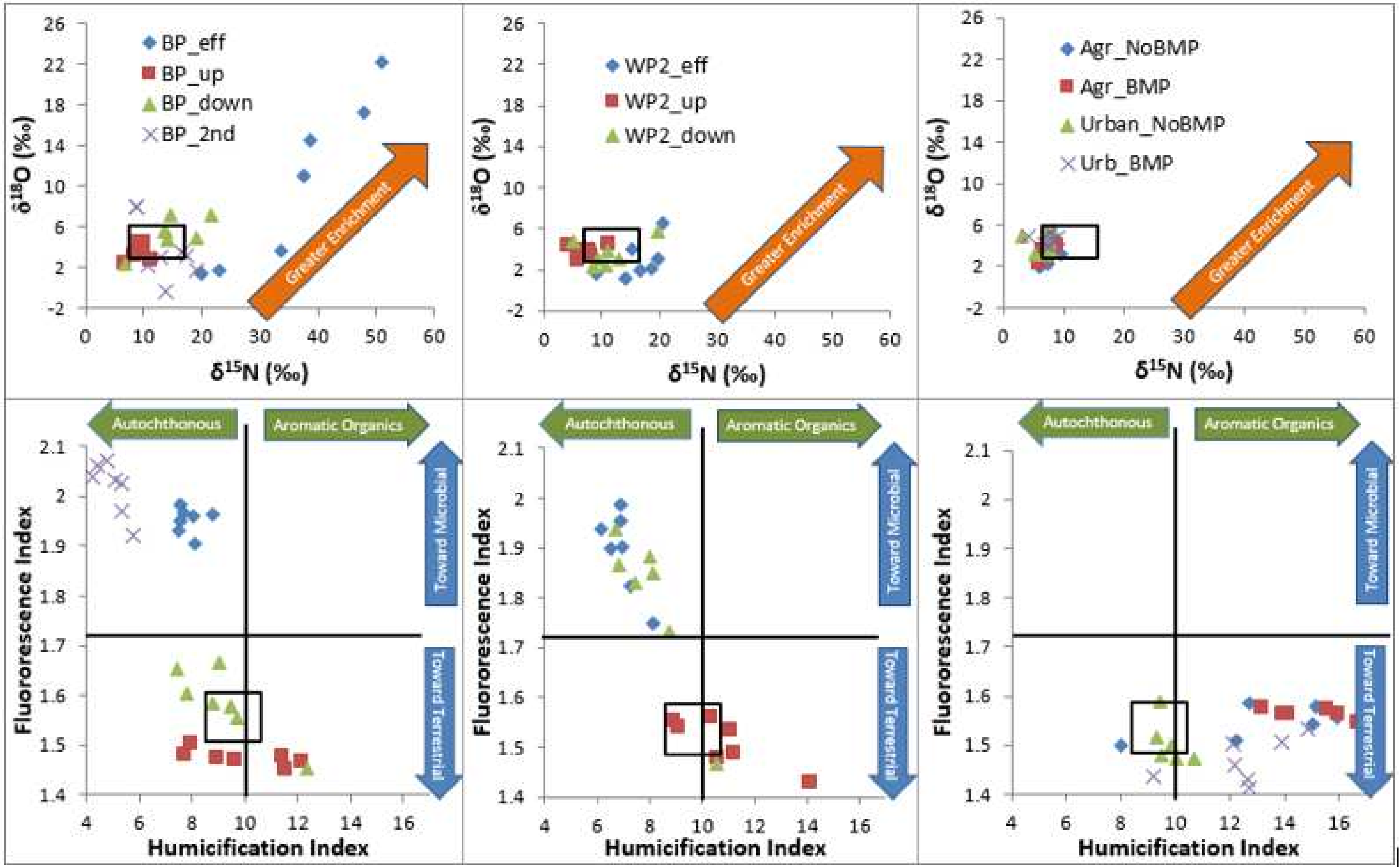

Figure 9: Nutrient and NOM fingerprinting techniques for Blue Plains, WWTP2, and the non-point sources to the Potomac. The black box on each plot summarizes ranges of eEDCs considered as "background" in the Potomac. BP = Blue Plains WWTP, WP 2 = Second tested WWTP, Agr = Agricultural Site, Urb = Urban Site. Eff = effluent, up = Upstream, down = Downstream, BMP = Best Management Practices, No BMP = Lacking Best Management Practices.

5.0 Conclusions

The following conclusions can be drawn from this research.

Addressing Objective 1: Upstream and Downstream Impacts on eEDCs from "best-in-class" nutrient control, agriculture management, stormwater management, and wastewater treatment strategies:

- In general, implementation of BMPs showed significant reductions in eEDC inputs to the Potomac Watershed from agriculture and urban runoff. BMPs studied included:
    - Agriculture: restricting livestock access to streams, planting grasses for stream shading and improving streambank stability.
    - Urban: maintaining shaded habitat, reducing impervious area, restoring stream habitat and riparian areas, and creating wetlands.
- Reductions in eEDCs with implementation of BMPs for non-point sources suggested effective co-management of eEDCs with phosphorous control methods for non-point sources.
- Blue Plains profile sampling revealed large reductions in eEDCs with advanced nitrogen control, suggesting effective co-management of eEDCs and nitrogen from WWTP point-sources.

Addressing Objective 2: Assess relative contribution of eEDCs from WWTPs performing biological nutrient removal:

- An annual load analysis indicated non-point sources (agriculture and urban sources) account for over 80% of eEDC load to the Potomac, with Blue Plains contributing less than 3%.
- Implementation of non-point source BMPs could effectively reduce non-point source loads of eEDCs to the Potomac.
- Results from two, 30-day, passive sampling campaigns indicated:
    - Higher levels of eEDCs were observed at 5 locations in the Potomac during the spring of 2016 deployment when compared with the fall of 2015.
    - Reductions in observed eEDC mass between Hains Point and National Harbor in both fall 2015 and spring 2016 deployments, possibly associated with the input of Blue Plains Effluent.
- Nutrient and NOM fingerprinting analysis qualitatively suggests that:
    - Both WWTPs analyzed affected the fingerprint of the receiving stream, while non-point sources significantly affected NOM but did not affect nutrient enrichment.
    - Changes in nutrient fingerprint associated with Blue Plains Effluent correlated with a reduction in eEDC concentration in the Potomac River below the outfall, suggesting the high level of nutrient management employed at Blue Plains is effectively co-managing eEDC and nitrogen inputs to the Potomac

6.0 Recommendations

The research project was designed specifically to provide an evaluation of inputs of estrogens to the Potomac Watershed associated with the Blue Plains Advanced Wastewater Treatment Plant, using this facility as a model of the impact of co-managing nutrients and estrogens. An additional focus of the work was to provide context for Blue Plains inputs compared to other sources (point and non-point) within the watershed, and to provide a glimpse at potential reductions associated with implementation of non-point source BMPs. The results of the study indicate the Blue Plains process is providing significant reductions of estrogens with advanced nutrient control, and preliminary correlations developed in this study have provided seeds for critical "next steps" for and evaluation considering holistic approaches for watershed protection. This evaluation should consider the use of BMPs to control multiple pollutants; nutrients, eEDCs and pathogens for the protection of the watershed. A risk assessment approach should be considered to better understand the impacts of BMPs on watershed protection. A summary of several potential follow up projects are provided below:

- A broader view of the impacts of nutrient and water quality and quantity management strategies on inputs of chemical contaminants is warranted. The project team assembled to perform this work is currently performing some of this work, with funding secured through the USEPA Science to Achieve Results (STAR) program call "Human and Ecological Health Impacts Associated with Water Reuse and Conservation Practices". The project aims to facilitate prioritization of reuse and management strategies and actions for the Potomac and beyond, by building a framework for informing federal agencies, local governments, water utilities and other stakeholders as they shape future management approaches in large human-impacted watersheds. More details on the project can be found at: https://cfpub.epa.gov/ncer_abstracts/index.cfm/fuseaction/display.abstractDetail/abstract/10501/report/0.
- The project focused on nutrients, water quality, and emerging chemical contaminants inputs into the Potomac. Another concern, human pathogenic organisms, drives many drinking water treatment decisions for water providers throughout the region. Preliminary steps are being performed toward this objective in conjunction with (but outside of the stated scope of) the EPA STAR project. A more detailed look at sources and controls of human pathogens and the impact of nutrient BMPs on pathogen control in the watershed is suggested.
- Calculations of estrogen loads to the Potomac was informed by the latest available land use data. However, detailed land use evaluations are performed periodically for a watershed of the size of the Potomac. In addition, several assumptions regarding BMP implementation and vegetative cover were required to perform the load calculations. Remote sensing tools are available, which can be used to significantly improve land use estimations for any current year. In addition, these tools are currently being used to assess, in a predictive manner, temporal and spatial variability in water quality throughout a watershed. Implementation of remote sensing tools are suggested to improve load calculation estimates and assess temporal and special vulnerability to emerging contaminants.
- The preliminary load analysis suggest non-point sources (without implementation of BMPs), contribute the majority of estrogenic inputs to the Potomac Watershed. Advanced nutrient control provides an effective point-source barrier to watershed inputs of these contaminants. Preliminary

projections associated with more widespread implementation of BMPs in the non-point sources suggested major reductions in estrogen inputs to the system may be realized with more robust implementation of BMPs. As an example, if the assumed implementation rate of BMPs for non-point sources was increased from 30% to 80% implementation of BMPs, reductions of up to 48% estrogenic activity load and 46% estrone load to the system may be realized, representing potential to bring concentrations in the Potomac closer to the EPA suggested "trigger" of 1 ng/L estrogen equivalent concentration.[1]

[1] Conley JM, et al. (2018) "Occurrence and In Vitro Bioactivity of Estrogen, Androgen, and Glucocorticoid Compounds in a Nationwide Screen of United States Stream Waters" *Environmental Science and Technology* 51 (9), 4781 - 4791

APPENDIX

Project Report for "Assessing the Impact of Anthropogenic Discharges of Endocrine Disruption in the Potomac River"

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Assessing the Impact of Anthropogenic Discharges of Endocrine Disruption in the Potomac River

Final Project Report

March 1, 2018

Job no

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Project Introduction

This technical memorandum summarizes research study addressing the impact of anthropogenic discharges of endocrine disruptors in the Potomac River which was performed as a result of the Water Quality Assurance Amendment Act of 2012. The Act required the following:

- To establish a Water Quality Assurance Panel to monitor and identify emerging and unregulated contaminants in the District's drinking water and wastewater discharge;
- To mandate quarterly testing for unregulated contaminants in the District's drinking water and wastewater effluent;
- To recommend to the Mayor an appropriate course of action for improving the reduction of unregulated contaminants and endocrine disruptor compounds at their source.

There are two locations along the Potomac River that are the drinking water source for the District and are upstream of Blue Plains Advanced Wastewater Treatment plant (Blue Plains AWTP). A quarterly sampling event required by the Environmental Protection Agency's Safe Drinking Water Act Unregulated Contaminant Monitoring Rule 3 (UCMR3) was performed throughout 2014 as a separate monitoring program from this study. Those results are not discussed in this report but can be found on DC Water's website https://www.dcwater.com/emerging-compounds-testing.

This research study was developed in accordance with the requirements of the Act and designed to provide holistic monitoring and identification of emerging and unregulated contaminants, in the form of endocrine disrupting compounds (EDCs), in the District's drinking water "source" (i.e. the Potomac River) and the Blue Plains AWTP Effluent.

Sampling was performed on a bi-monthly schedule (exceeding the quarterly mandate), with an emphasis on identifying relative source contributions from point sources (including Blue Plains AWTP) and non-point sources to the watershed. In addition, the project aimed to evaluate the impact of advanced wastewater treatment (Blue Plains AWTP), along with several other best management practices for improving wastewater quality and drinking water source quality throughout the Potomac through co-management of nutrients and EDCs.

This memorandum represents the documentation of the research project, to be presented to a Water Quality Assurance Panel within 30 days of study completion. The Panel shall convene a public meeting to discuss the results of the study with respect to issues listed in Act within 90 days. The Panel shall issue a report to the Mayor and GM within 120 days from the Panel convention, summarizing discussion and recommendations. Upon receipt of the Panel report, DC Water shall create and implement a Plan considering remediation to submit to Mayor and Council.

1. Background

The Chesapeake Bay has long been considered one of the most ecologically sensitive water environments in the United States, heavily impacted by eutrophication and hypoxia related to agriculture and urbanization of the watershed. Recognizing its importance to the health of the coastal marine system, the contributing freshwater tributaries to the Bay have been heavily scrutinized over the years and significant measures have been taken to improve the quality of the water entering the Bay, particularly with regard to nutrient load. The Potomac River is a major contributor of freshwater to the Bay system, but the watershed also serves as an important spawning and nursery ground for migratory and resident fish species and as a drinking water source for more than 4 million people in the Maryland/DC/Virginia corridor. The multiple and diverse needs of the Potomac system, as well as the focus on this sensitive waterway for examining human and ecological health impacts of emerging contaminants and nutrients, makes it an ideal study site for examining management of multiple water quality goals.

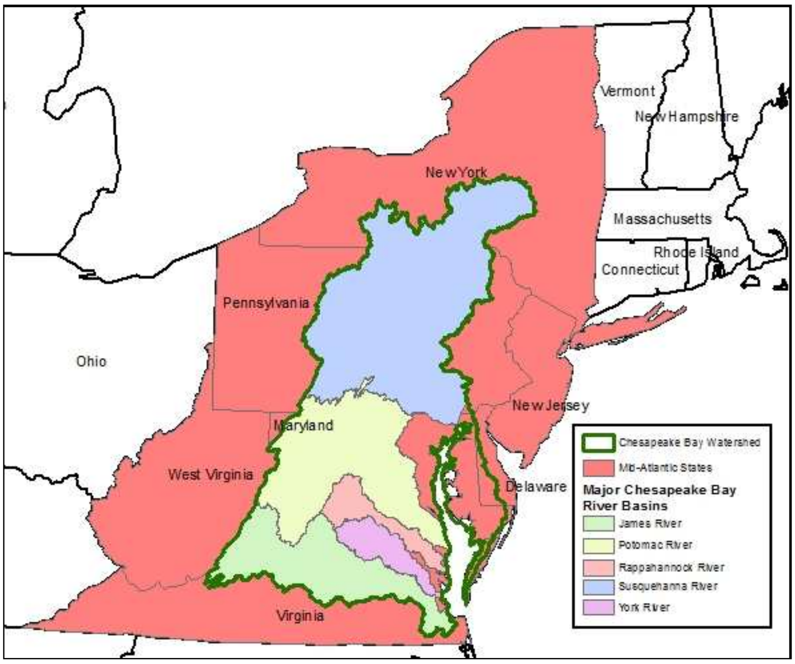


Figure 1 Major River Basins in the Chesapeake Bay Watershed

Of the freshwater sources to the Chesapeake Bay, the Potomac River contributes about 20% of the total streamflow, 28% of the total nitrogen load, and 33% of the total phosphorus load to Chesapeake Bay (Belval and Sprague 1999). Additionally, recent research has indicated intersex conditions of fish are widespread in the river and tributaries, owing to inputs of endocrine-disrupting chemicals (EDCs) from wastewater-treatment plant effluents and runoff from agricultural land, animal feeding operations and urban/suburban land (Blazer et al. 2012). These findings speak to the fact that water quality in the Potomac is falling into a declining condition, and a survey in 2002 showed that around 50% of the river segments were impaired (http://www.potomac.org).

This declining water quality is in spite of significant efforts to reverse the effects of human alterations of nitrogen and phosphorus inputs from agriculture and urbanization, which have been directly linked to eutrophication and hypoxia in the Chesapeake Bay (Kemp et al. 2005). EPA and USGS have largely addressed point sources for the Potomac River. In recent years, expenditures exceeding $15 billion (Chesapeake Bay Watershed Blue Ribbon Finance Panel, 2004), have been implemented to reduce nutrient inputs by 40% through restoration efforts and best management practices (BMPs) to reduce hypoxia (Burke and Dunn 2010). Despite these substantial public and private expenditures, reports of record-sized hypoxic zones in 2003 and 2005 (http://ian.umces.edu/ecocheck/forecast/chesapeake-bay/2012/) have raised public concerns whether progress has actually been made to reduce nonpoint sources. Additionally, EDCs are a major concern in the region, which is not even covered in previous regulatory approaches designed with the health of the Chesapeake Bay in mind.

1.1 Links between Land Use and Impacts from EDCs and Nutrients

In the Potomac River watershed, land use varies spatially sequentially transitioning across forest, agricultural, and urban land as the river flows to the Chesapeake Bay (Figure 2), and elevated concentrations of EDCs and nutrients can be related to agricultural/urban land-use and bedrock type (Ator et al. 1998). Current approaches for estimating nutrient sources from nonpoint contributions to the Potomac watershed include subunits for delineation (Blomquist et al. 1995) and a watershed model (SPARROW or Bay Model). Meanwhile, as EDCs and nutrients are transported in streams and rivers, a large fraction (>60%) can be retained or transformed in the watershed and streams (Jaworski et al. 1992), sources of the contaminants are difficult to identify as they are transported downstream to the Chesapeake Bay. Prior studies (e.g., Blomquist et al. 1996, Ator et al. 1998) indicate that elevated N and P in the Potomac River basin can result from a mixture of sources that vary temporally and spatially in the watershed including: agricultural fertilizers, manure, atmospheric deposition, and municipal wastewater-treatment plants (WWTP). Sources of nutrients are not homogeneously distributed in watersheds, but occur as hot spots that can be generally linked to land use (McClain et al. 2003).

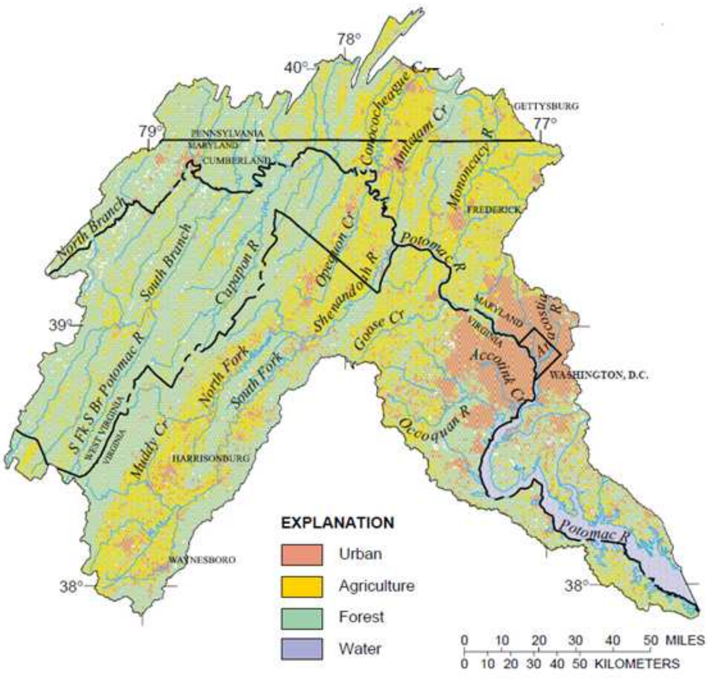


Figure 2 Map of the Potomac River Watershed and Tributaries with different land use (adapted from USGS Circular 1166).

Previous EDC research efforts in the Potomac River watershed (Figure 2) have also shown linkages between fish health and point sources such as WWTP effluents or non-point sources such as animal feeding operations, and similar sources for nitrogen and EDCs from the watershed (Iwanowicz et al. 2009, Ciparis et al. 2012). Much of this work has been instrumental in indicating relationships between ecological health indicators (intersex prevalence and severity), and point and non-point sources, as described in Table 1.

Table 1: Pearson correlation coefficients (r^2) describing the relationship between intersex fish and land-use characteristics for rivers in the Potomac watershed (modified from Blazer et al., 2011)

Land-use	Intersex prevalence		Intersex severity	
	r^2	*p*	r^2	*p*
Human population density	0.39	0.10	0.42	0.08
Number of WWTPs	0.22	0.24	0.34	0.13
WWTP flow	0.32	0.15	**0.63**	**0.02**
Percent agricultural land use	**0.63**	**0.02**	**0.50**	**0.05**
Number of animal feeding operations	0.28	0.17	**0.56**	**0.03**
Number of poultry houses	0.27	0.18	**0.50**	**0.05**
Total number of animals	0.27	0.18	0.48	0.06
Animal density	**0.49**	**0.05**	**0.58**	**0.03**

Temporally, EDC and nutrient inputs and transformations in stream/rivers generally occur during short periods of time that show disproportionately high reaction rates relative to longer intervening time periods (McClain et al. 2003). Historically, greater nutrient loads have been transported from the Potomac watershed to the Chesapeake Bay during wet years or high-flow events, causing seasonal and inter-annual variability of hypoxia (Hagy et al. 2004). During droughts and low-flow conditions, however, there can be substantial denitrification and biological assimilation of nitrogen in the Potomac River, and increased production of organic carbon by algae and bacteria.

Hydrologic variability can considerably alter sources of nonpoint nutrient pollution in Chesapeake Bay watersheds (Kaushal et al. 2011), and has been observed to impact levels of emerging contaminants in source waters. Taking nutrients as an example, non-point sources like manures may represent important sources during short time scales of high-flows but account for a substantial proportion of nitrogen and phosphorous from an annual mass balance perspective in the upper Potomac watershed (Jaworski 1992). WWTPs, however, may contribute substantial nitrogen and phosphorous sources during droughts and low flow conditions, although the importance of in-stream transformations on regulating sources is less known. As another example, concentrations of pharmaceuticals and EDCs were observed to increase significantly at a drinking water treatment plant intake on Lake Meade, NV as drought conditions increased, despite reductions in concentrations provided from significant wastewater sources (Benotti et al., 2010).

1.2 Need for Evaluating Impact of Advanced Reclaimed Water Treatment and BMPs for Nutrient and EDC Reductions

Currently, possible nutrient management strategies in the Potomac River include upgrades of WWTPs, upgrades of stormwater management facilities, and implementation of various agricultural best management practices (BMPs). Billions are being spent at hundreds of reclaimed water treatment plant facilities throughout the Chesapeake Bay watershed to upgrade with advanced technology aimed to reduce the amount of nutrients that are discharged into the Bay's tributaries. Wastewater treatment plant upgrades account for a large portion of overall estimated nutrient reductions to date, and jurisdictions in the Chesapeake Bay watershed are relying on additional reductions from wastewater to achieve 15% of total overall nutrient reduction goals (http://www.chesapeakebay.net/issues/issue/wastewater#inline). Upgrades of stormwater management facilities for non-point sources is also considered as part of an overall strategy to meet nutrient reduction targets for existing urban development under the Chesapeake Bay TMDL. The stormwater management upgrades include 1) converting and retrofitting current facilities into BMP's that employ more effective treatment mechanism(s) such as wetlands or reuse options 2) increasing stormwater BMP treatment volume and/or increasing harvesting capacity and hydraulic retention time, and 3) restoring stormwater BMP performance through major sediment cleanouts, vegetative harvesting, filter media upgrades, or full-scale replacement. Common agricultural BMPs, another type of nutrient management strategy for non-point sources, include fencing for grazing livestock, cover crops, forest buffers, manure storage areas and rotational grazing.

In the Potomac River watershed, nutrients and EDCs have been identified as major issues that will continue to negatively impact the health of the river's aquatic fauna (Iwanowicz et al., 2009, Potomac Conservancy, 2011). Municipal WWTPs have been implicated as major contributors of nutrients and EDCs in this and other watersheds. However, it is known that EDCs also originate from non-point sources such as agricultural runoff (e.g., confined animal feed operations (CAFOs) and manure and pesticides used in crop-based farming) or urban/suburban runoff. Since WWTPs are not the sole contributor of EDCs in waterways, *there is a critical need to accurately quantify the relative input of each discharge on the overall endocrine disruption in the receiving water body.*

In cases where EDC load reductions may be required, it will also be necessary to recommend specific technologies for this purpose. EDCs can be eliminated with varying efficiencies using conventional and advanced physio-chemical unit operations; however, the energy and cost requirements associated with implementing these advanced technologies, such as those related to chemically assisted clarification, filtration, membranes, advanced oxidation and activated carbon, can be prohibitive. A practical approach is to use or improve upon existing infrastructure that provides the capability for enhanced nutrient removal. Herein, the Potomac watershed is unique. The major WWTPs in this watershed provide high levels of nutrient removal (TN < 3 mg/L and TP <0.18 mg/L). This infrastructure requires both physico-chemical separation and biological treatment. Indeed, it has been noted that WWTPs with nitrogen removal appear to have enhanced remediation of steroidal estrogens (Vader et al., 2000, Khunjar et al., 2011) (one sub-class of EDCs), thereby reducing the mass input of EDCs into the environment. Furthermore tertiary clarification and filtration infrastructure associated with phosphorus removal can potentially be effective options for remediating EDCs. Despite this fact, a thorough elucidation of EDC fate through WWTPs performing nitrogen and phosphorus removal is lacking. *Clearly, a further understanding of how nutrient removal configurations impact EDC toxicological fate 'in the watershed' is necessary, especially if the desired goal is to minimize EDC discharge.*

2. Project Objectives

Considering the previously described justifications, two project objectives have been identified as follows.

Objective 1 - Evaluate the upstream and downstream impacts from 'best-in-class' nutrient control, agriculture management, stormwater management and wastewater treatment strategies. Effluent samples from point and non-point sources using different degrees of nutrient control strategies were collected, processed, and characterized using EDC bioassays, LC-MS/MS analyses, conventional water quality, nutrient isotope tracking, and excitation emission matrix fluorescence spectroscopy (EEMs). Results from this task will be used to understand the toxicological impact of the effluent, the chemical composition of the EDCs present and characterize the composition of the dissolved nutrients and organic matter present in the samples, and will help to answer the question, "To what extent is EDCs dependent on nutrient control strategies?"

Objective 2 - Assess the relative contribution of EDCs from WWTPs performing biological nutrient removal. Passive sampling was performed at 5 locations in the Potomac River, focusing on locations in the DC metro area, parsing contributions of upstream rural and suburban sources, urban sources, and Blue Plains effluent impacts on the Potomac water quality. Samples were analyzed with biological EDC assays, LC-MS/MS characterization and EEMs analyses. Additionally, load analysis was used to estimate contributions of EDCs from various sources. Result from this phase of research will help provide a fingerprint of which sources are primarily responsible for endocrine disruption at test sites and answer the question, "What is the contribution of EDCs of point vs. non-point sources to receiving waters?"

3. Project Approach

3.1 Sampling Locations

Study sites are shown in Figure 3, and labeled as follows: Solid black dots are paired agricultural and urban rivers with/without (W/O) best management practice (BMP), while solid or open colorful dots refer to point inputs (wastewater treatment plants or WWTP, and combined sewer overflow or CSO) and upstream/downstream sites. The three solid dots in Blue Plains AWTP are located in the outlet of 2nd reaction basin, nitrification/denitrification/sedimentation basin, and effluent outfall. The inset image displays the location of the POCIS (Passive Sampler) deployments in the Potomac River, focusing on the DC Metro area.

All sample sites are located within the Potomac River watershed, the second largest river flowing to the Chesapeake Bay in terms of water quantity and nutrient load (Alter et al. 1998), and the fourth largest river along the Atlantic coast of the United States. The river is divided into the Upper and Lower Potomac by the Fall Line near Washington DC. In the studied portion of the Potomac watershed immediately above Washington DC, the river flows through different land use zones, from agricultural in Fredrick (Maryland), suburban in Rockville (Maryland), and urban in Washington DC, with patches of forested land scattered among the other land use types (Interstate Commission of the Potomac River Basin 2006). Inputs from both point (WWTPs, CSOs), and nonpoint sources (agricultural and urban runoff) were sampled.

Representing point sources, a CSO in the Hoff Run watershed (Alexandria, Virginia) and the effluent of Blue Plains AWTP (in Washington DC) that flows directly to Potomac River (Figure 3) were sampled. Effluent of a second wastewater treatment plant (WWTP2) was also collected in order to make comparisons between small and large WWTPs implementing a range of nutrient control treatment. In addition to CSO and WWTP effluent samples, stream water above and below points of input were sampled to examine relative effects of these point sources on tributary water quality. In order to ensure complete mixing between WWTP effluent and stream water, the downstream sites were at least 200 m below the confluence. The upstream site of the Blue Plains was selected on the Hains Point of Washington DC, where the Potomac River and Anacostia River meet. The downstream site of the Blue Plains, was located in an outstanding point below National Harbor in Maryland, where effect of bank inputs was likely minimal. At the Blue Plains AWTP, samples were also collected throughout the plant profile, after secondary treatment, nitrification/denitrification and disinfection, to examine the changes in EDC and water quality during conventional and advanced wastewater treatment.

The Blue Plains AWTP serves over two million customers with a collection area of Washington DC and surrounding suburbs of Maryland and Virginia. It is the largest treatment plant in the Potomac River watershed and the largest treatment facility of its kind in the US, with a rated capacity of 384 million gallons per day. The treatment process utilizes preliminary and primary treatment, secondary treatment, nitrification/denitrification, effluent filtration, chlorination- dechlorination and post aeration (https://www.dcwater.com/about/facilities.cfm). In the last several years for example, the plant has dramatically upgraded and improved its liquid processing systems, and consequently improvements in water quality of the Potomac River were reported (Pennino et al. 2016).

The second wastewater treatment plant (WWTP2), was originally constructed in the late 1970s, designed to treat 5 million gallons per day (mgd), with a peak of 8 mgd. Two facility upgrades have been undertaken over the years, with the latest expansion and upgrade occurring in the early 2000s to treat an average daily flow of 20 mgd and meet biological nutrient removal (BNR) effluent limits. The existing plant includes an influent pumping station, pretreatment facility with fine screens and grit removal units, four aeration basins that use the Modified Ludzack-Ettinger (MLE) activated sludge process, three secondary clarifiers with spiral rake sludge removal systems; tertiary monomedia sand filters, UV disinfection, and cascade post-aeration. The solids handling facilities include gravity belt thickeners (GBT), dewatering centrifuges, and lime stabilization to produce a Class B biosolids.

To represent inputs from nonpoint sources, stream water from typical sub-watersheds with agricultural and urban land use was collected. In order to examine the effect of BMPs, a paired series design was used where two streams within the same sub-watershed were sampled, each with similar land use patterns, but different degrees of best management practices adoption. Best management practices are designed to mitigate the negative environmental consequences that come with conversion of natural land for agricultural or urban development. These practices are meant to reduce erosion, manage storm water runoff, control nutrient loading, and stabilize other aspects of the watershed.

The effects of agricultural BMPs were investigate by comparing two geographically proximate sites in the watershed, one where BMPs were implemented and one without BMPs. The tributary of Bens Branch implemented BMPs after it was identified by the Maryland Department of the Environment in a 1996 List of Impaired Waters. Implemented BMPs focused on restricting livestock access to the tributary and consisted of 8,800 new feet of fencing and the development of three springs to eliminate in stream cattle watering. Two stream crossings were put in place and over nine acres of grasses were planted for stream shading and improvement of streambank stability (Shanks et al. 2008).

To evaluate the effects of urban stormwater BMPs two urban sites were sampled, the Paint Branch sub-watershed which employs BMPs and The Briar Ditch sub-watershed which does not. The BMPs at the Paint Branch site were designed to minimize the impact of urban development and water runoff from a high level of impervious surfaces. The Maryland National Parks and Planning Commission (MNPPC) purchased undeveloped land along the stream to maintain shaded habitat and leaf litter inputs and implemented regulations that limited impervious area to 10% of all new development in the watershed. Additionally 75 projects to manage stormwater runoff from developed areas, restore stream habitat, create wetlands, and restore the riparian zone (eopb.org). The Briar Ditch watershed has not implemented BMPs and is of the most densely populated regions of the area with the fewest stormwater management controls. Only 13% of the stream has adequate riparian forest buffer compared to Paint Branch's 53% (Anocostia.net).

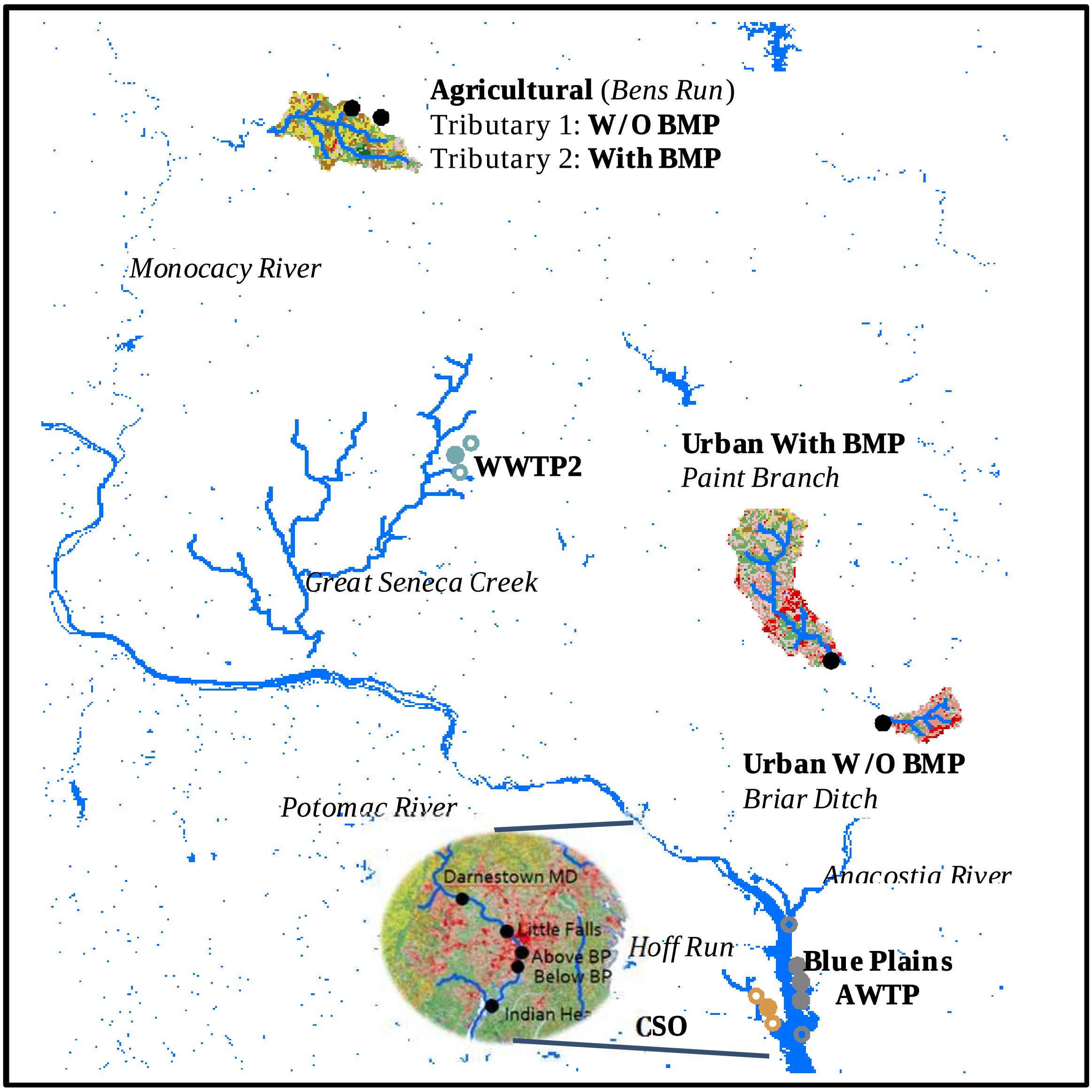


Figure 3: Locations of 15 bimonthly sampling sites in the Potomac watershed. Inset are locations of passive sampling deployments.

3.2 Sample Collection and In Situ Measurements

At each site, 1500 mL unfiltered samples were collected by immersing three 500-mL amber glass bottles under the water surface without disturbing bed sediment. The bottles were acid-cleaned, and rinsed three times with stream water before sample collections. Next, water temperature, and conductivity were measured in situ, using a WTW Oxi 1970i DO meter. Water samples were put in an iced cooler for storage during transportation. The samples were filtered the same day in the lab, using pre-weighed Whatman GF/F filters. The filtered samples were either saved in the refrigerator for analyses of dissolved inorganic carbon (DIC), or in the freezer for the other analyses. Sample collection and processing were generally conducted within two days of collection. Samples were collected on a bimonthly schedule (once every 2 months), along with one special event timed to capture the first significant flush after a significant rainfall event (Figure 4).

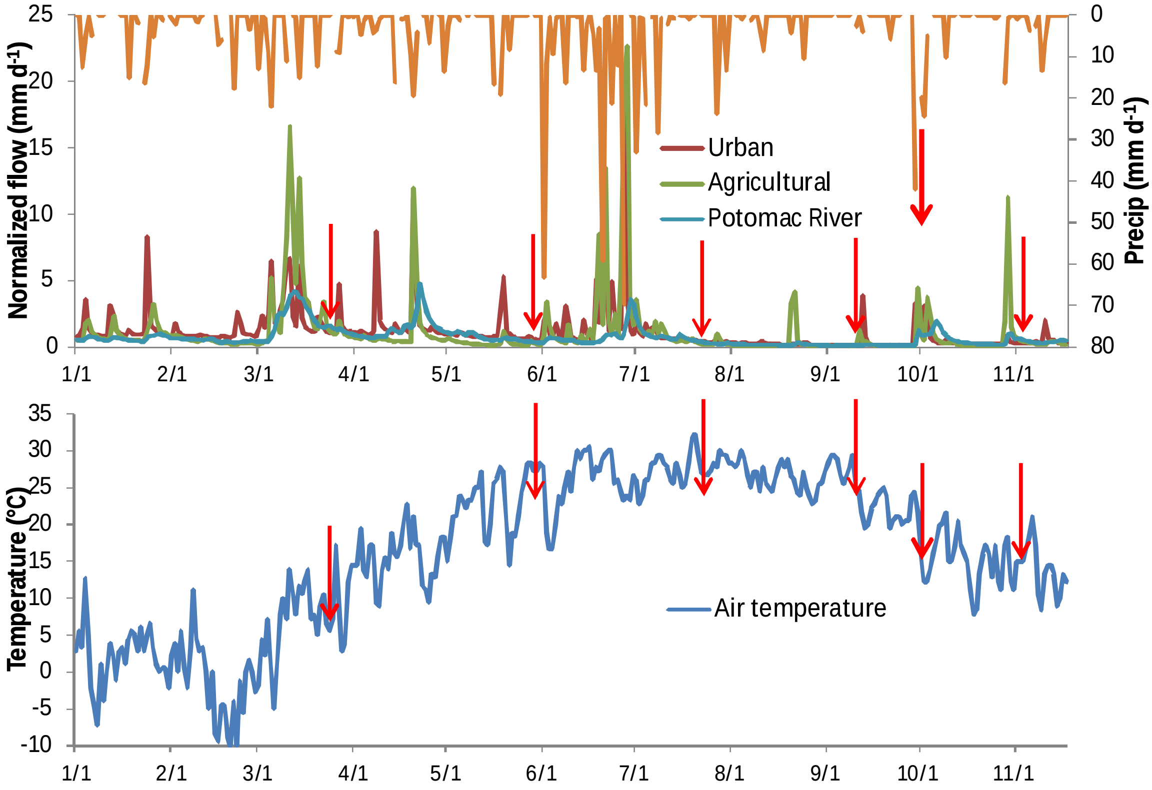


Figure 4 Daily precipitation, air temperature (at DC Reagan International Airport), and runoff from Potomac River watershed (estimated from flow at Little Falls). Red Arrows Represent Sampling events, providing precipitation and temperature data for the surrounding period.

Passive sampling devices (polar organic chemical integrative samplers, POCIS) were deployed for 30 day intervals on two occasions during the study, in November 2015 and April 2016 (Figure 5). These deployments correspond to base flow conditions in the Potomac and can be used to represent background levels in the Potomac. POCIS Samplers (shown inset in Figure 5), are stainless steel canisters, which are capable of holding three HLB Oasis filters and facilitating flow past the cartridges. Samples were deployed in the river at each of the POCIS test sites shown in Figure 3.

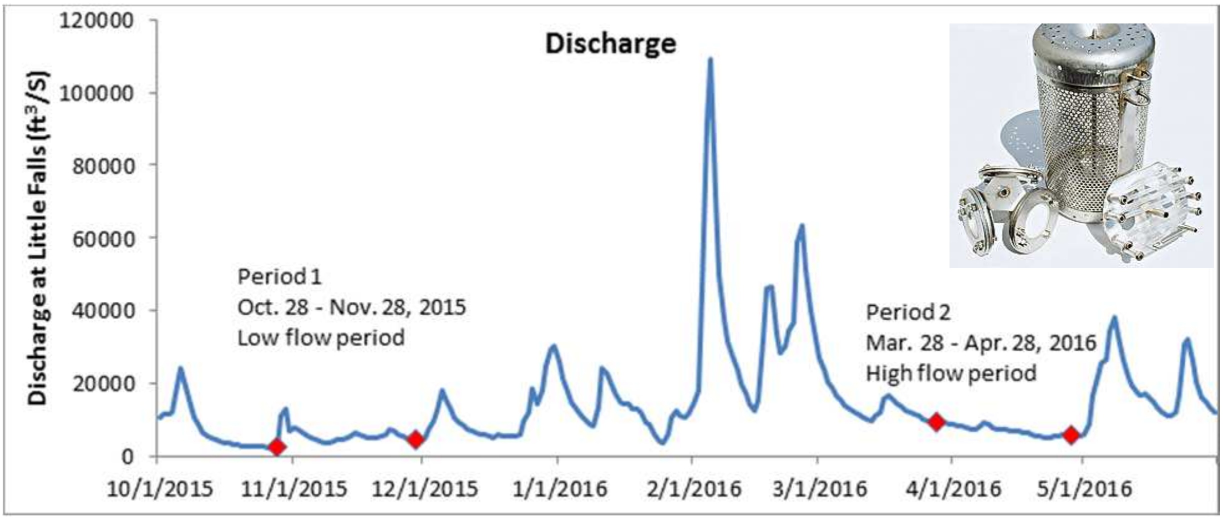


Figure 5. Discharge at Little Falls, Potomac River, and highlighting periods of POCIS Deployment. Inset image of POCIS Samplers.

3.3 Water Quality Analyses

Concentrations of DOC and total dissolved nitrogen (TDN) were measured on a Shimadzu Total Organic Carbon Analyzer (TOC-L CPH/CPN), using a high temperature catalytic oxidation method (Duan and Kaushal, 2013). Three injections (with a maximum of 5 times) were run for each sample to obtain a standard deviation of less than 0.2 mg/L. Nitrate (NO_3^-), nitrate (NO_2^-) and soluble reactive phosphorus (SRP) were measured with a QuikChem 8500 Series 2 FIA System, and the ascorbic acid-molybdate blue method (Murphy and Riley, 1962). Blank and standards were run every 15 samples to ensure accuracy of the analyses. Total Kjeldahl nitrogen (TKN), including dissolved organic nitrogen, ammonia, and ammonium, was calculated by subtraction of nitrate-N from TDN.

Frozen samples were analyzed for $\delta^{15}N-NO_3$ and $\delta^{18}O-NO_3$ using the denitrifier method at the USGS Stable Isotope Laboratory in University of California Davis. Briefly, denitrifying bacteria (Pseudomonas auroeofaciens) convert nitrate to gaseous nitrous oxide (N_2O) for isotopic analysis (Sigman et al. 2001; Casciotti et al. 2002). A minimum of 60 nmol of nitrate was required to analyze samples on a continuous flow Micromass IsoPrime isotope ratio mass spectrometer (CF-IRMS). Samples were corrected using international reference standards IAEA-N3, USGS34, and USGS35 and values are reported in parts per thousand (%) relative to atmospheric N_2 and Vienna Standard Mean Ocean Water, for $\delta^{15}N$ and $\delta^{18}O$, respectively. Sample duplicates had an average standard deviation of 0.2%for $\delta^{15}N-NO_3$ and 0.7%for $\delta^{18}O-NO_3$.

Dissolved organic matter (DOM) was characterized using UV/Vis absorbance and fluorescence excitation emission matrices (EEMs) (Coble, 1996; Cory et al., 2010; Duan and Kaushal, 2013). UV/Vis absorbance scans were collected with a Shimadzu spectrophotometer (UV 1800), and the values at 254 nm were used to calculate specific ultraviolet absorption (SUVA254) by normalization with DOC concentrations in mg/L. Previous studies have shown that $SUVA_{254}$ is strongly correlated with percent aromaticity of DOC as determined by 13C NMR (e.g., Weishaar et al., 2003), and it can be used to track terrestrial organic carbon in aquatic systems. In addition, water samples were analyzed for fluorescence emissions (300-600 nm with a 2 nm increment) on a FluoroMax-4 spectrofluorometer (Horiba Jobin Yvon, Edison NJ, USA) with an excitation range of 240 - 450 nm at a 5 nm increment. Fluorescence excitation emission matrices (EEMs) were instrument corrected, blank subtracted, and normalized by the water Raman signal following the method of Cory et al. (2010) and Duan and Kaushal (2013). To characterize DOM composition and estimate its sources, fluorescence EEMs were analyzed to obtain fluorescence index (FI, McKnight et al., 2001; Cory and McKnight, 2005), humification index (HIX, Zsolnay et al., 1999; Huguet et al., 2009), and biological freshness index (BIX, Huguet et al., 2009). FI, the ratio of the fluorescence intensity at 450 nm to that at 500 nm with excitation at 370, was used to indicate the relative abundance of terrigenous vs. microbial DOM (McKnight et al., 2001). BIX was estimated as the ratio of fluorescence intensity at emission wavelength 380 nm to that at 430 nm with excitation wavelength at 310 nm (Huguet et al., 2009). HIX was calculated as the ratio of fluorescence intensity recorded integrated from 230nm to 280nm with excitation at 255nm, is used to estimate the degree of maturation of organic matter, with increased HIX corresponding to a higher degree of aromaticity and less microbiological availability.

3.4 EDC chemical analysis

Method for determinations of estrogen and its metabolites were adapted from Tso et al. (2011). A 0.5 L aqueous sample was collected in an amber glass bottle, and filtered through 0.7 µm Whatman glass filters (Clifton, NJ). Filtered samples were each spiked with 100 µL of surrogate standards (250 ng/mL) of SMX-d4, E2-3G-d3, 17β-E2-3Sd4, E1-3S-d4, 17β-E2-d3, and E1-d4 and then immediately stored at 4°C. Prior to extraction, the pH of each aqueous sample was adjusted to 4 with either sulfuric acid or ammonium hydroxide, which was based on the study from Pailler et al.(2009) showing that pH 4 is ideal for all compounds.

Solid phase extraction (SPE) to concentrate water samples was performed at the University of Maryland (UMD) without elution, and the cartridges were sent to the University at Buffalo (UB) for LC-MS/MS analysis. During SPE, each sample was loaded onto an Oasis HLB™ SPE cartridge for the extraction of target EDC analytes. The SPE cartridges were first conditioned using 6 mL of methanol followed by 10 mL of MilliQ™ water. Each sample was loaded onto an SPE cartridge at approximately 5 to 10 mL/min. After loading, the SPE cartridge was rinsed with 10 mL of water/methanol (95/5, v/v) and allowed to dry under vacuum for approximately 30 min. These cartridges were stored at -4 °C until shipment to UB, where they were eluted.

Upon receipt of the SPE cartridges at UB, they were stored at -4 °C until ready for elution. The first elution using 10 mL of an ethyl acetate/methanol (9/1, v/v) mixture was collected in an amber vial. Then, the SPE cartridge was washed sequentially with 10 mL of acid-wash solution (5% methanol with 2% acetic acid by volume), and 10 mL of base-wash solution (5% methanol with 2% ammonium hydroxide by volume), which were discarded. The SPE cartridges were then dried by maintaining the vacuum for approximately 30 min. The remaining analytes in the SPE cartridge were eluted with a second solvent consisting of 10 mL of methanol + 2% ammonium hydroxide; this elution was collected in a separate vial. The collected extracts were evaporated to less than 1 mL under a stream of nitrogen at 30°C. The two separate extracts were combined into a graduated tube and evaporated to approximately 0.2 mL. The combined extract was then brought to a 1 mL volume with water/acetonitrile (95/5, v/v) +0.1% acetic acid solution and vortexed. An aliquot of 0.4 mL was spiked with 20 µL of spiking solution containing 500 ng/mL of each target analyte. This spiked sample was used for quantification using a single-point standard addition technique to account for matrix effects in LC-MS/MS. The samples (nonspiked and spiked) were then centrifuged at 7000g for 5 min to remove any particles from the extract. Both aliquots (nonspiked and spiked) were analyzed by LC-MS/MS. A water blank was prepared using only water as the sample and treated identically to the samples to assess any presence of cross-contamination or carry-over throughout the analytical procedure.

POCIS extracts solubilized in methanol were received from Environmental Sampling Technologies Inc. and stored immediately stored at -20°C. Prior to the analysis samples were taken to dryness and solubilized in 1mL of the LC-MS/MS mobile phase to match initial composition of LC gradient condition.

Analysis of estrogens (E1, E2, E3, and EE2) and their conjugated metabolites listed in Table 2 was performed using an Agilent 6410 triple quadrupole MS equipped with an 1100 HPLC system (Palo Alto, CA). Data collection and analysis were performed using Agilent Technologies MassHunter^{TM} Software Version B (Palo Alto, CA), following the method of Tso et al. (2011). For quality assurance parameters, blank injections were made at the beginning, at the end, and before each quality control standard to check for carry over. Quality control standards were injected after 10 samples, and percent recovery should be within 20% from the beginning of the analysis. Surrogate spikes were only used to monitor for recoveries in all samples; reported values of the analytes were not corrected using the observed surrogate recoveries.

Table 2: Analyzed estrogens and metabolites.

Targeted analytes	abbreviation
Estrone	E1
17α-estradiol	E2α
17β-estradiol	E2β
17β-Estriol	E3
17α-Ethinylestradiol	EE2
estrone-3-sulfate estrone-3-glucuronide	E1-3S E1-3G
17β-estradiol -17-sulfate	E2-17S
17α-estradiol -3-sulfate	E2α-3S
17β-estradiol -3-sulfate	E2β-3S
17β-estradiol-3-glucuronide	E2-3G
17α-ethinylestradiol-3-glucuronide	EE2-3G
17α-Ethinylestradiol-d4	EE2-d4 (ISTD)
17β-estradiol-d3	E2-d3 (ISTD)
Estrone-d4	E1-d4 (ISTD)
17β-estradiol-3-sulfate-d4	E2-3S-d4 (ISTD)
17β-estadiol-3-glucuronide-d3	E2-3G-d3 (ISTD)
Estrone-3-sulfate-d4	E1-3S-d4 (ISTD)

3.5 EDC biological activity analysis

Solid phase extraction of water samples for biological analysis was similar to the method described above, except that the water samples were not spiked with any surrogates. Upon receipt of SPE cartridges processed by UMD, the samples were stored at -20°C. Analyte was eluted from the sorbent as identically to that for the chemical analysis. Eluates were reduced to dryness and solubilized in 1mL of 100% methanol. Reconstituted samples were stored at −20°C, protected from light until screened. POCIS extracts solubilized in methanol were received from Environmental Sampling Technologies Inc. and stored immediately stored at -20°C. Prior to the assay samples were taken to dryness and solubilized in 1mL of 100% methanol.

Assays were performed blinded to the sample location. The bioluminescent yeast estrogen screen (BLYES) was used to quantitatively assess estrogenic activity relative to 17β-estradiol. Strain BLYES was purchased from 490 BioTech. The BLYES assay was run according to Ciparis et al (2012). In short, 5 µL of sample extract was added to 95 µL of yeast minimal media (YMM leu^-, ura^-) in triplicate wells of a white, solid-bottom 96-well plate (Phoenix Research Products #MPG-655207). To this 100 µL of a 48h culture of strain BLYES at ~ 0.75 (OD_{600}) was added to each well. A 17β-estradiol (E2; Sigma-Aldrich Co.) standard curve ranging from 4 pg/mL – 500 pg/mL was included on each plate. The final concentration of methanol in sample and standard wells after the addition of strain BLYES was 2.5%. A media control was included on all plates to establish background luminescence. Plates were covered and incubated in the dark at 30°C for 4 h. Luminescence was quantified using a SpectraMax M4 microplate reader (Molecular Devices), in luminescence mode (1000 ms integration time) and relative estrogenicity of each sample was interpolated using a 4-paramter curve-fit within SoftMax Pro 6.2.2 (Molecular Devices). Relative estrogenicity per liter of river water was then calculated based on sample concentration.

4. Results and Discussion

Figure 6 provides a summary of key EDC and nutrient results from the study, for point and non-point sources throughout the Potomac Watershed. The data summarize the results from the 7 sampling events (bimonthly sampling and one wet weather event), and are presented as mean values and standard errors (SE) for bulk estrogenic activity (measured by BLYES), estrone (which was the only estrogen detected consistently by LC-MS/MS), total dissolved nitrogen (TDN), and soluble reactive phosphorus (SRP). The values for each parameter are provided relative to background Potomac River levels to aid in comparison.

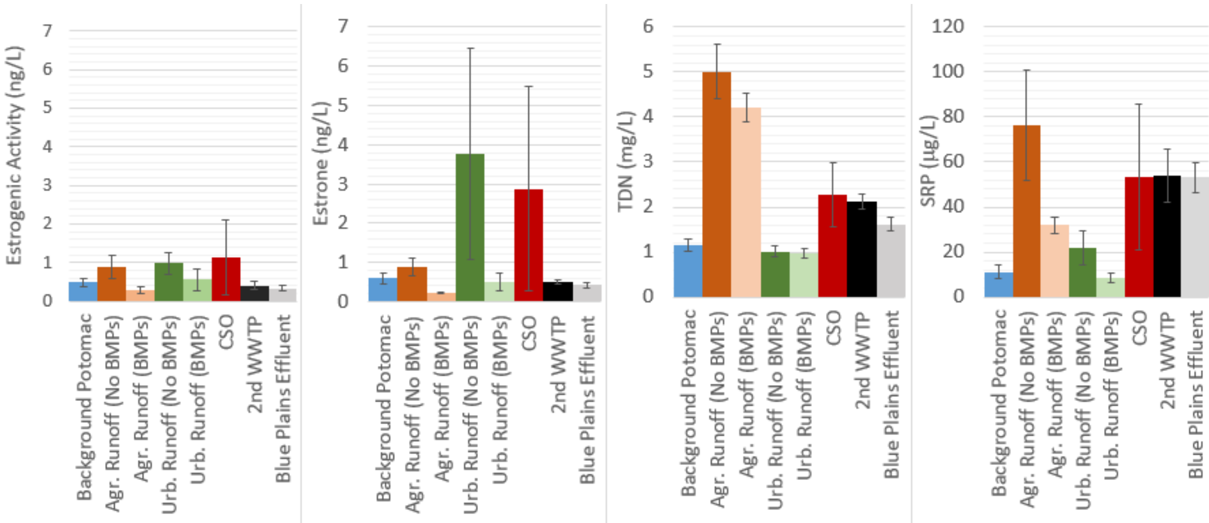


Figure 6: EDCs and nutrients for various sources in the Potomac Watershed

Levels of EDCs measured were generally low in the background Potomac, as well as in all sources. Of all the estrogens targeted for analysis, only estrone was consistently detected. While there were a few detections of very low levels of estrone sulfate (E1-3S), these conjugates are typically not persistent in the environment and are likely to de-conjugate back to E1. The levels of E2 and EE2 were below detection limits in all water samples, which is not unexpected as these compounds tend to convert to E1 and also preferentially sorb to sediments rather than dissolve in the aqueous phase. Major sources of EDCs showing levels above background Potomac levels included the "untreated" sources, including agriculture and urban without implementation of BMPs, along with CSOs. CSOs, while very intermittent in flow contributions, provided relatively high levels of EDCs to the system when discharging significantly. This was observed during the wet weather event that was sampled. Implementing best management practices for agriculture and urban non-point sources, as well as for wastewater treatment plant point sources, were effective and resulted in levels comparable to the background Potomac.

For nutrients, the story was more complex. Agriculture, CSO, and WWTPs all showed significantly higher levels of nutrients than the background Potomac. Implementation of BMPs provided some reduction in levels discharge, particularly in phosphorus for the non-point sources and nitrogen for the point sources.

Interesting, levels of nutrients in urban runoff were similar to background Potomac levels, with implementation of BMPs showing modest improvements in nutrient levels.

In order to meet the objectives of the project, a subset of the data has been further analyzed to develop correlations aimed at deconvoluting the complex set of EDC inputs into the Potomac River. Presentation and discussion of the results of the study are organized to address the following objectives:

- Evaluate the impacts on EDC discharges from 'best-in-class' nutrient control, agriculture management, stormwater management and wastewater treatment strategies
- Assess the relative contribution of EDCs from WWTPs performing biological nutrient removal

4.1 Objective 1: Impact of nutrient control measures on EDC discharges

To answer the question "To what extent are levels of EDCs impacted by nutrient control strategies", two approaches to data collection and analysis were utilized for analysis of impact from Point and Nonpoint sources. To assess impacts on EDC concentrations of agriculture and urban stormwater "non-point" nutrient control best management practices, data were compared from two paired, geographically similar sub-watersheds. Impacts of nutrient control strategies on EDC concentrations at wastewater "point" sources were evaluated by comparing levels collected from the effluent of two wastewater treatment facilities and one combined sewer overflow with levels found upstream and downstream of the source. Sample locations are displayed in Figure 3.

Presentation and data analysis are organized according to the following structure:

- Comparing agriculture and urban stormwater nutrient control strategies via paired watershed analysis
- Assessing EDC impacts on receiving water from point-source discharges, including two wastewater treatment plants (WWTPs) and one CSO
- Evaluation of Blue Plains ADWP advanced nutrient control strategies for removal of EDCs.

Values of bi-monthly measured hormones by LC-MS/MS and by BLYES, total dissolved nitrogen (TDN), soluble reactive phosphorus (SRP) and dissolved organic carbon (DOC) have been provided in Appendix A-1. It should be noted that because estrone (E1) was the only consistently detected hormone throughout the dataset, the following analysis has been restricted to E1, BLYES, along with the nutrients and other water quality parameters.

4.1.1 Impacts of BMPs on estrogen, nutrients, and DOC inputs – agriculture and urban non-point sources

Figure 7 and Table 3 display the results of the grab sample analysis performed in bimonthly sampling throughout 2015, for EDCs (measured by BLYES and E1), as well as TDN, SRP, and DOC, from the agriculture non-point sources.

In the agricultural streams, levels of EDCs (BLYES and E1), SRP, and DOC were generally higher in the summer months, correlating with low flow conditions. Conversely, nitrogen inputs were lowest during this time. In the agriculture non-point stream *without* implementation of BMPs, DOC, SRP, and estrone all peaked in July, during the beginning of low flow following spring rains. In the watershed with BMPs, levels of all parameters were not subject to the large "spikes" in concentrations of EDCs and nutrients (SRP and DOC), as observed in the samples from the stream without BMP implementation. The differences between watersheds were less notable with respect to nitrogen.

To provide a longer-term, integrated evaluation of the inputs from the watersheds throughout the year, the datasets were analyzed as a yearly composite, to evaluate if significant differences in loading is likely with implementation of BMPs. Table 3 displays the resulting mean, standard deviations, difference in means (calculated as percent lower with implementation of BMPs), as well as p-values to describe the likelihood of the means being statistically significantly different, with $p<0.05$ used to indicate statistical significance that the null hypothesis (equal means) is likely *not* true.

Table 3: Comparison of Statistical Significance between paired Agriculture watersheds with and without implementation of BMPs for stormwater management.

	Mean +/- Standard Error		Average Difference	p
	With BMPs	**W/O BMPs**	**(no BMP – BMP)/(no BMP)**	**(<0.05 = statistical significance)**
BLYES (ng E2 Eq/L)	0.23 +/- 0.02	0.89 +/- 0.23	74%	0.038 (Yes)
E1 (ng/L)	0.28 +/- 0.08	0.88 +/- 0.31	68%	0.042 (Yes)
TDN (mg/L)	4.22 +/- 0.32	5.01 +/- 0.60	16%	0.137 (No)
SRP (μg/L)	31.9 +/- 3.71	84.9 +/- 27.5	62%	0.042 (Yes)
DOC (mg/L)	2.28 +/- 0.36	5.49 +/- 2.54	58%	0.117 (No)

The analysis indicates significant differences in the mean inputs to the Potomac system for estrogens (BLYES and E1), as well as for SRP, suggesting successful co-management of EDCs with utilization of BMPs designed to minimize phosphorus, including restricting livestock access to the tributary planting of grasses for stream shading and improvement of streambank stability. Large reductions of DOC were also observed with implementation of BMPs (although not statistically significant due to high variability in DOC in the stream *without* BMPs). Interestingly however, the reductions in the levels of EDCs, SRP, and DOC did not correlate with levels of TDN, where almost no difference between the watersheds was observed. This suggests that agriculture BMPs designed to minimize reactive phosphorus (biologically available) and DOC may be more effective in co-managing EDCs than those designed to achieve total nitrogen reductions.

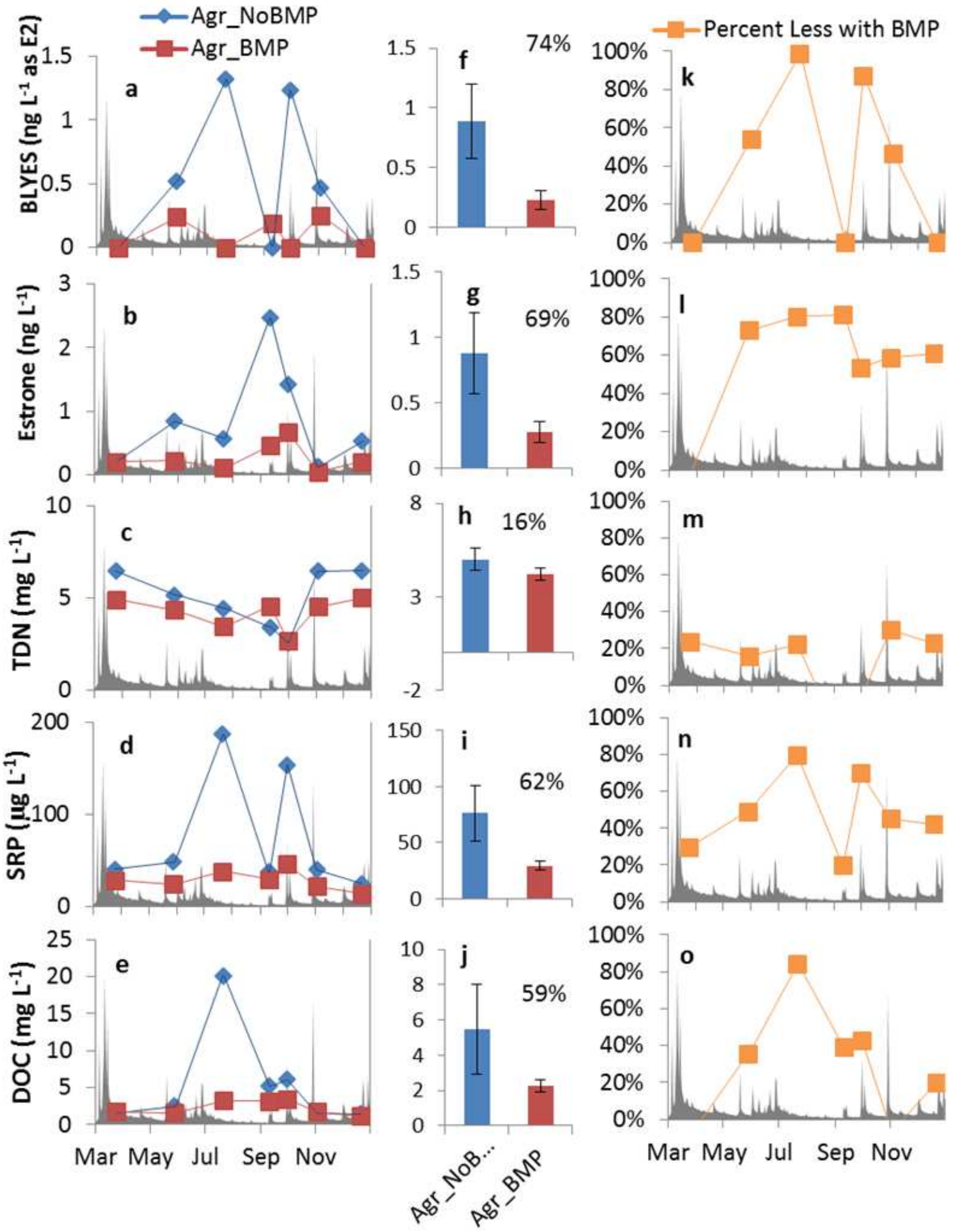


Figure 7. Concentrations and relative decreases of estrogenic activity, estrone, TDN, SRP, and DOC at agriculture (Agr) sites with and without BMPs. Potomac hydrograph in the background.

Figure 8 and Table 4 display the results of the grab sample analysis performed in bimonthly sampling throughout 2015, for EDCs (measured by BLYES and E1), as well as TDN, SRP, and DOC, from the urban non-point sources.

In the urban streams, levels of EDCs (BLYES and E1), SRP, TDN and DOC were generally higher in the late summer/fall months, corresponding with rains after dry summer conditions. In the urban non-point stream *without* implementation of BMPs, maximum levels of EDCs and SRP were greater and variability was higher than in the managed streams. Differences in variability between watersheds were less notable with respect to nitrogen and DOC.

To provide a longer-term, integrated evaluation of the inputs from the watersheds throughout the year, the datasets were analyzed as a yearly composite, to evaluate if significant differences in loading is likely with implementation of BMPs. Table 4 displays the resulting mean, standard deviations, difference in means (calculated as percent lower with implementation of BMPs), as well as p-values to describe the likelihood of the means being statistically significantly different, with $p<0.05$ used to indicate statistical significance that the null hypothesis (equal means) is likely *not* true.

Table 4: Comparison of Statistical Significance between paired urban stream watersheds with and without implementation of BMPs for stormwater management.

	Mean +/- Standard Error		**Average Difference**	**p**
	With BMPs	**W/O BMPs**	**(no BMP – BMP)/(no BMP)**	**(<0.05 = statistical significance)**
BLYES (ng E2 Eq/L)	0.49 +/- 0.23	3.76 +/- 2.69	87% (55% w/o Jan '16)	0.112 (0.03 w/o Jan '16)
E1 (ng/L)	0.55 +/- 0.28	0.98 +/- 0.28	44%	0.147
TDN (mg/L)	0.97 +/- 0.10	1.01 +/- 0.12	4%	0.403
SRP (μg/L)	8.61 +/- 2.13	23.6 +/- 8.71	64%	0.062
DOC (mg/L)	3.98 +/- 0.46	5.45 +/- 0.76	27%	0.061

The analysis indicates no significant differences in the mean inputs to the Potomac system for estrogens (BLYES and E1), TDN, SRP, or DOC, even though relatively large differences in the mean concentrations were observed with the management practices. Interestingly, when the BLYES data were analyzed considering the very large measurement from January 2016, a 55% reduction is observed in the stream with BMPs, as well as a statistically significant difference in the measured means. While not statistically significant, the large differences in the means again suggest the potential for successful co-management of EDCs with utilization of BMPs designed to minimize phosphorus and DOC, including maintaining shaded habitat, reducing impervious area, and restoring stream habitat and riparian, and creating wetlands. This again suggests that BMPs designed to minimize reactive phosphorus (biologically available) and DOC may be more effective in co-managing EDCs than those designed to achieve total nitrogen reductions.

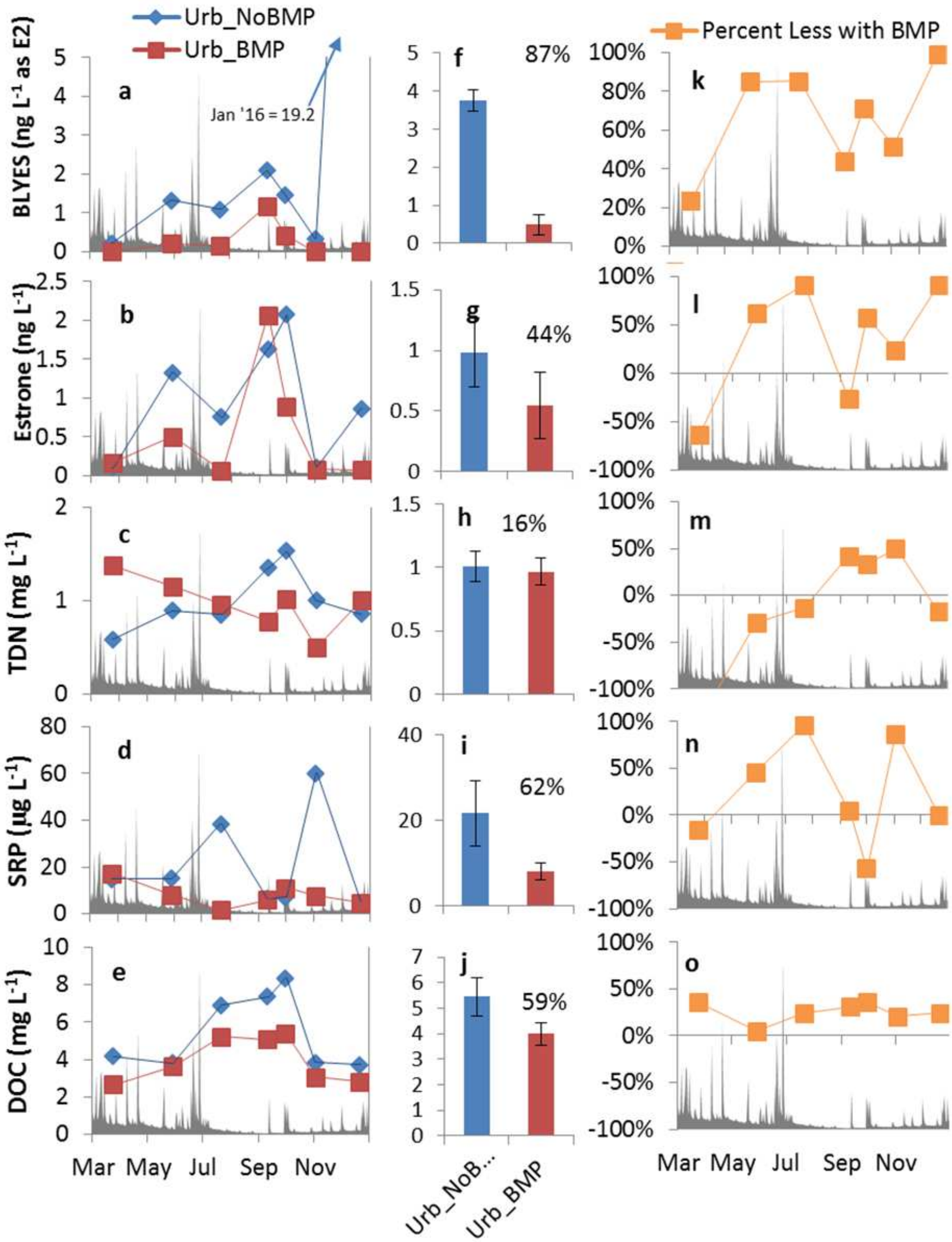


Figure 8. Concentrations and relative decreases of estrogenic activity, estrone, TDN, SRP, and DOC at urban sites (Urb) with and without BMPs. Potomac hydrograph in the background.

In general, our data showed that agricultural and urban best management practices (BMPs) can substantially enhance removal of estrogen, nutrients (mainly Phosphorous) and organic Carbon (Fig. 3 and 4). Successful cases of co-manageming nutrients and estrogens with agricultural BMPs was also reported by Shappell et al. (2010). In this study, BMPs at the agricultural site focused on restricting livestock access to the tributary, and consisted of new fencing and stream crossings and the development of springs to eliminate in stream cattle watering. It is reasonable that restricting livestock access to the tributary can reduce estrogen, N, P and organic C inputs from manure of the animals, thereby causing co-reductions of their concentrations with BMPs application. Conversely, the BMPs at the urban site were designed to minimize the impact of urban development and water runoff from a high level of impervious surfaces by construction of environmental overlay zone. Construction of environmental overlay zone could increase stormwater residence time and increase retention of estrogen, nitrogen, phosphorous and organic carbon at the same time.

4.1.2 Impacts of BMP implementation on estrogens, nutrients, and DOC – Point Sources

Figure 9 and Table 5 display the results of the grab sample analysis performed in bimonthly sampling throughout 2015, for EDCs (measured by BLYES and E1), as well as TDN, SRP, and DOC, and conductivity (as a conservative tracer) from the point sources studied. This included effluents from two wastewater treatment plants and one CSO, as described.

Trends observed in the wastewater treatment plants were interesting and in many ways counter to those observed from the non-point sources. For example, levels of EDCs (BLYES and E1) were consistently lower in the effluent of the Blue Plains AWTP than in background samples collected in the Potomac (both upstream and downstream), while levels of nutrients and DOC tended to be slightly higher in the effluent of each facility than in the background receiving streams. At the Blue Plains location, only conductivity regularly increased between the upriver and downriver sites, while longitudinal changes in EDCs, TDN, and SRP did not show this trends. In the summer under low flow conditions, DOC increased slightly in the downriver Potomac site below Blue Plains, correlating with elevated levels in the effluent, but this was not the case in other seasons with regular rains.

At WWTP2 (discharging into a much smaller tributary than the Potomac), the impact of the effluent could be observed more regularly, with effluent inputs of EDCs, nutrients, DOC, and conductivity contributing to elevated downstream levels of these parameters as compared to the upstream location. This trend was particularly apparent in the low-flow summer months, when the WWTP discharge could make up a significant portion of the stream flow.

The CSO provided an interesting case evaluation. Levels of all parameters were similar to the receiving stream under most operations, with the exception of the October sampling event. This sampling occurred by design, in the aftermath of a large storm event, and captured sewer overflow conditions. Resulting levels of all parameters (except conductivity) were one to two orders of magnitude greater than typical discharge levels. In addition, levels of EDCs observed during this discharge were two orders of magnitude greater than those observed in the treated WWTP2 effluent, indicating these CSOs could be a significant intermittent source of EDCs during high discharge events (when discharging diluted raw sewage with limited treatment.) While levels were extremely high at the point source during this sampling event, they did not impact levels in the receiving stream, likely due to large dilution effects from high background stream flow conditions.

To provide a longer-term, integrated evaluation of the inputs from the watersheds throughout 2015, the datasets were analyzed as a yearly composite, to evaluate the impacts of effluent on levels in the receiving waters. Table 5 displays as p-values for describe the likelihood of two means being statistically significantly different (up- and downstream, upstream and effluent, downstream and effluent), with $p<0.05$ indicating statistical significance that the null hypothesis (means being equal) is likely *not* true.

Table 5. Comparing statistical significance in differences of the means between effluent and background samples for Blue Plains AWTP and WWTP 2 for all parameters in Figure 5.

Blue Plains AWTP

a-1) BPAWTP EDCs		E1 (ng/L)		
		Up	Down	Effluent
BLYES (ng/L as E2)	Up		0.29 (no)	0.144 (no)
	Down	0.23 (no)		0.26 (no)
	Effluent	0.025 (yes)	0.14 (no)	

a-2) BPWWTP Nutrients		TDN (mg/L)		
		Up	Down	Effluent
SRP (µg/L)	Up		0.48 (no)	0.023 (yes)
	Down	0.46 (no)		0.016 (yes)
	Effluent	0.00014 (yes)	0.00009 (yes)	

a-3) BPWWTP water quality		DOC (mg/L)		
		Up	Down	Effluent
Specific Cond. (µS/cm)	Up		0.31 (no)	0.007 (yes)
	Down	0.201 (no)		0.023 (yes)
	Effluent	<0.00001 (yes)	0.00002 (yes)	

WWTP 2

b-1) WWTP 2 EDCs		E1 (ng/L)		
		Up	Down	Effluent
BLYES (ng/L as E2)	Up		0.48 (no)	0.49 (no)
	Down	0.36 (no)		0.47 (no)
	Effluent	0.47 (no)	0.04 (yes)	

b-2) WWTP 2 Nutrients		TDN (mg/L)		
		Up	Down	Effluent
SRP (µg/L)	Up		0.46 (no)	0.43 (no)
	Down	0.011 (yes)		0.47 (no)
	Effluent	0.0058 (yes)	0.071 (no)	

b-3) WWTP 2 water quality		DOC (mg/L)		
		Up	Down	Effluent
Specific Cond. (µS/cm)	Up		0.089 (no)	0.021 (yes)
	Down	0.061 (no)		0.20 (no)
	Effluent	0.00005 (yes)	0.012 (yes)	

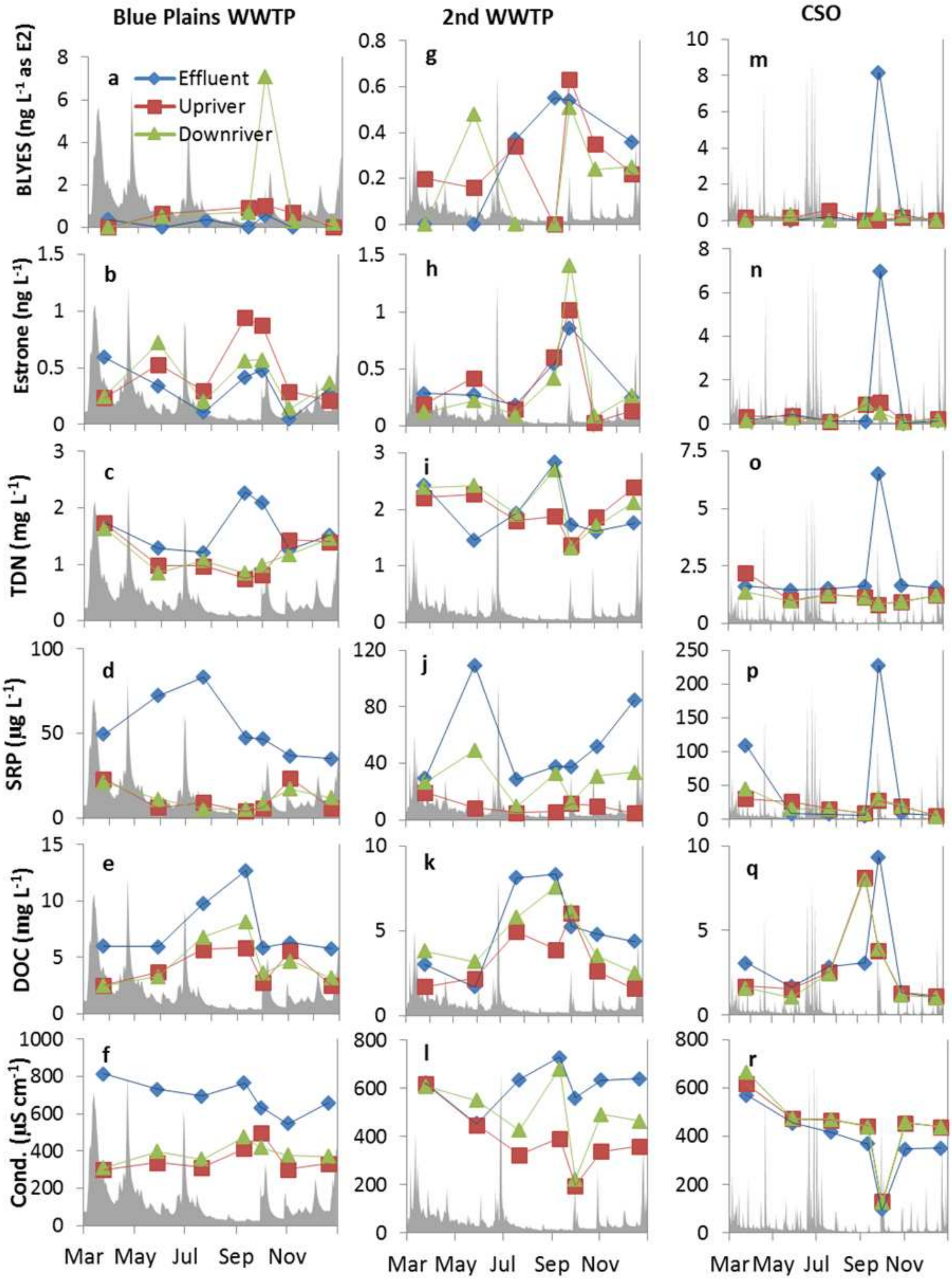


Figure 9: Concentrations of estrone, TDN, SRP and DOC of upriver, downriver, and in the WWTP or CSO effluent. Potomac hydrograph shown in the background.

Concentration of TDN, SRP, DOC and salinity (but not EDCs) in effluents from Blue Plains AWTP and WWTP2 were usually higher than background levels in their receiving streams. However, none of these effluent stream inputs resulted in a significant difference in the means between upstream and downstream levels at Blue Plains. For EDCs, levels in the Blue Plains effluent were observed to be less than those in the Potomac River, particularly for the bulk YES activity. A similar story can be told for WWTP2, where estrone levels were statistically significantly lower than those in the receiving stream. While levels discharged into the Potomac of TDN, SRP, DOC, and conductivity were statistically significantly higher than those of background, they did not result in a significant change in the background concentrations in the Potomac River between upstream and downstream locations. These results suggest that highly treated wastewater effluent may actually serve to dilute levels of EDCs in the receiving stream, when considered on a long-term basis.

4.1.3 Case Study: Blue Plains WWTP advanced nutrient control strategies for removal of EDCs

To investigate the impact of advanced nutrient control strategies implemented at the Blue Plains AWTP, a series of in-plant profile sampling events were performed. The results of the profiles are provided in Figure 10, and indicate that very large reductions in EDCs are achieved along with nitrogen reductions. On average, more than 99% of bulk estrogenic activity was removed between secondary effluent and post-advanced nitrogen removal at Blue Plains. While reduction was significantly higher in warmer months than cooler, this was simply a result of higher levels in the post-secondary effluent during these months. After nitrogen treatment, levels never exceeded 0.57 ng/L as E1, even with post-secondary levels exceeding 70 ng/L on several occasions. Even in colder weather, when nitrogen treatment can be impacted, observed levels of EDCs did not increase over background after the advanced treatment step. Similar reductions were observed for nitrogen removal, with less removal observed in organic carbon removal. Interesting, levels of SRP were observed to increase after advanced nitrogen removal treatment, but remained very low, never observed at greater than 100 μg/L (0.1 mg/L).

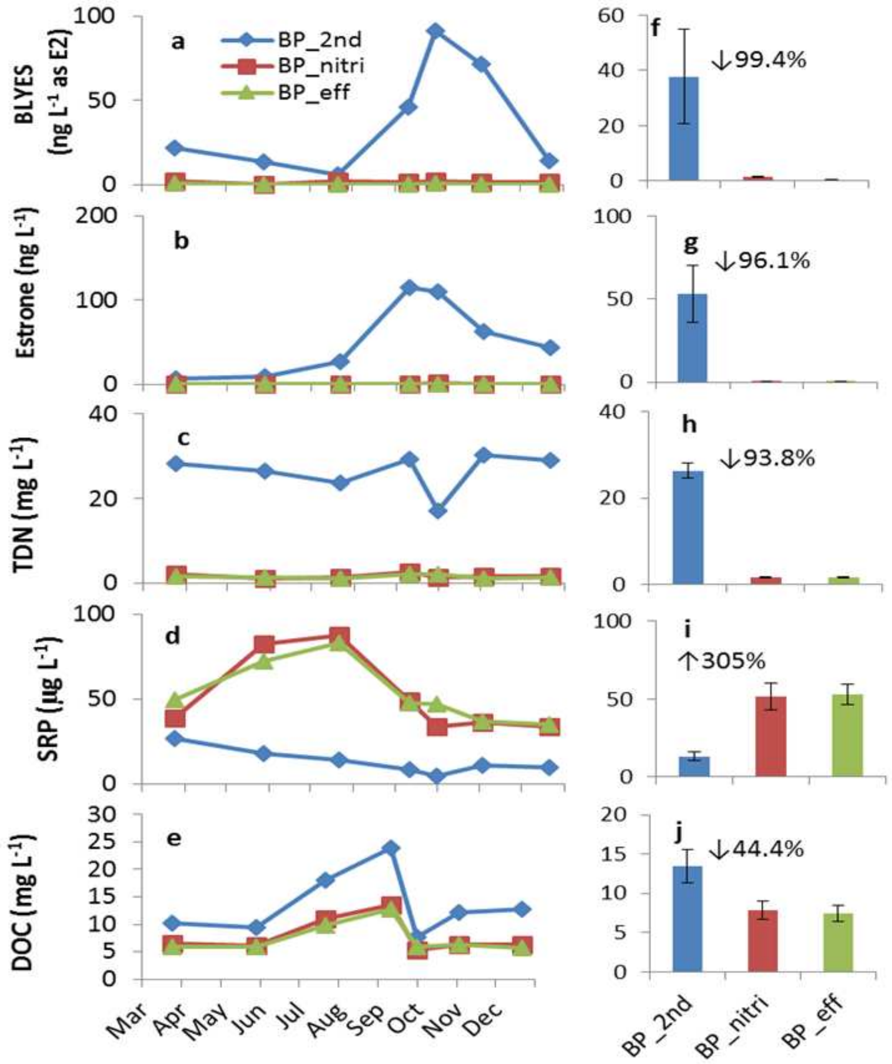


Figure 10: Seasonal and mean values of estrogenic activity, estrone, TDN, SRP and DOC, measured as a profile from Blue Plains (BP) post-second treatment (BP_2nd) to post-nitrification/denitrification (BP_nitr) to outfall (BP_eff).

WWTP2 also utilizes advanced nutrient control, although employing a different treatment strategy. Figure 11 compares directly only effluent EDC levels (total estrogenicity by BLYES and estrone concentration by LC-MS/MS). Both plants achieved non-detect levels (<0.16 ng/L as E1) at least once, with Blue Plains regularly discharging non-detect levels of EDC activity. Differences in effluent concentrations for either EDC activity (measured with BLYES) or estrone are not statistically significant. The chemical detection of low levels of E1 without a corresponding biological detection of total estrogenicity is not surprising because the BLYES assay reports estrogenic activity relative to estradiol (E2), which was not detected in the samples, and BLYES has a lower response to E1.

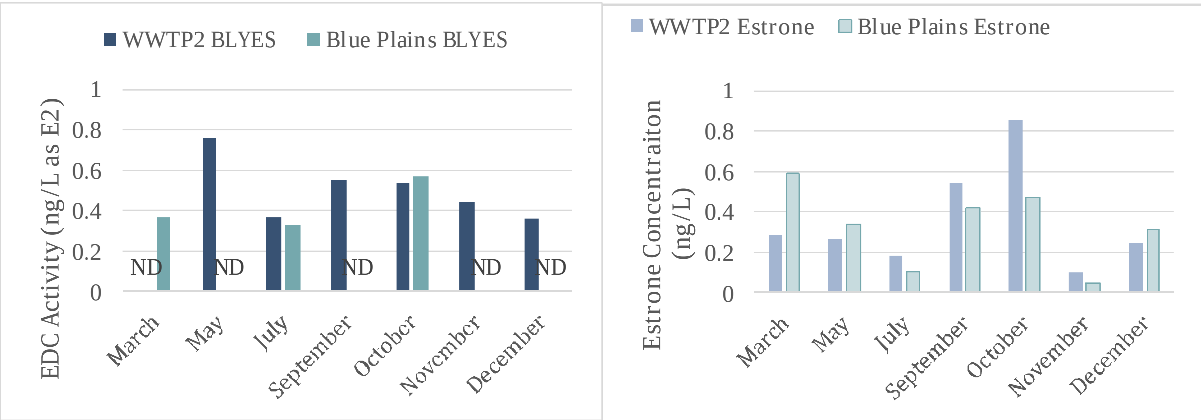


Figure 11: Effluent Concentrations of EDCs from Blue Plains and WWTP2. Both facilities employ advanced nutrient control technologies.

Finally, it must be realized that since both wastewater treatment plants are located in the sensitive Potomac River and Chesapeake Bay watersheds, they are subject to regulations forcing implementation of strict nutrient control technologies. This is not the case for all wastewater treatment plants in the United States. If the effluent of many wastewater treatment facilities not subject to nutrient control can be modeled as equivalent to post-secondary treatment at Blue Plains, with EDC levels expected to be much higher than those observed in the Blue Plains effluent. Table 6 can be used to compare means and standard deviations for EDCs (BLYES and E1), TN, SRP, and DOC. As described earlier, the mean levels observed in the effluent of Blue Plains and WWTP2 are not significantly different statistically for any of the parameters. However, both effluents are significantly lower than those of the Blue Plains secondary effluent. It can be surmised that advanced nutrient control on point sources successfully co-manages EDCs, reducing levels of potential discharge by more than 90%.

Table 6: Comparing concentrations of EDCs and nutrients in secondary treatment effluent, and effluent of facilities employing advanced nutrient control.

	Average Concentration +/- Standard Error			
	BLYES (ng/L as E2)	E1 (ng/L)	TN (mg/L)	SRP (μg/L)
Blue Plains Secondary	37.8 +/- 12.4	53.2 +/- 17.0	26.3 +/- 1.74	13.7 +/- 3.2
Blue Plains Effluent	0.6 +/- 0.2	0.32 +/- 0.07	1.62 +/- 0.16	56.7 +/- 9.9
WWTP 2 Effluent	0.5 +/- 0.06	0.36 +/- 0.10	2.11 +/- 0.17	48.9 +/- 12.5

This study clearly shows that approximately 99.4% of estrone, 93.8% of TDN, 44.4% of DOC were removed during the wastewater treatment, with most of the removal (99.3% of estrone, 93.6% of TDN and 41.6% of DOC) occurring during the nitrification/denitrification step. This suggests that it is possible to co-manage Nitrogen, organic Carbon, and estrone during wastewater treatment, and this co-management occurs during nitrification/denitrification step. It is known that nitrification is oxidizing the nitrogen from ammonia to nitrate via biological process using aerobic microbes. Denitrification converts nitrate to nitrogen gas, achieved in anoxic conditions with methanol added as the carbon source. Possibly, organic C including estrogens that is generally associated with organic carbon (Gong et al. 2012) was assimilated and thus removed simultaneously with Nitrogen during this step. Other studies (e.g., Wang et al., 2010; Qiang et al., 2013) have also shown that estrogens could be effectively removed by the biological treatment processes, while physical treatment processes had no or little effect on removal of EDCs. So, WWTP upgrades to enhance biological treatment processes could potentially improve efficiencies of WWTP and thus reduce point source inputs of estrogen and Nitrogen to the Potomac River. Other studies also reported that additional treatment, e.g., activated sludge treatment (Johnson et al., 2001), granular activated carbon upgrade (Grover and Sumpter, 2011) and ferrate (VI) treatment of secondary wastewater effluents (Yang et al. 2012) provide alternatives for estrogen removal in WWTPs.

However, it seems phosphorous is not similarly reduced because SRP concentrations increased by 300% (occurred mainly during nitrification/denitrification; Fig. 5c and 5g). The reason for SRP release during nitrification/denitrification step is not clear but seems to be attributed to anoxic condition occurring during denitrification (https://www.dcwater.com/news/publications/Blue_Plains_Plant_brochure.pdf). It is known that particulate P can be mobilized as SRP under anoxic condition when Fe-oxides, where P absorbed onto, are reduced to soluble Fe^{2+} form (House and Denison, 2002). However, the effect of WWTP upgrades on phosphorous discharge from WWTPs should be further evaluated because we did not examine the changes in total phosphorous during wastewater treatments.

4.2 Objective 2: Assess the relative contribution of EDCs from WWTPs performing biological nutrient removal

To address the question "What is the contribution of EEDCs of point vs. non-point sources to receiving waters?", four approaches to data analysis were utilized to compare the relative contributions from Point and Nonpoint sources. Quantitative measures, along with a qualitative "fingerprinting" technique, were employed to characterize the relative source contributions to the Potomac system, as follows:

- Comparing point-source discharge of EDCs with non-point and background Potomac levels
- Passive, 30-day, sampling (POCIS) of EDCs in urban Washington DC, to evaluate impact of Blue Plains effluent in the Potomac
- Estimation and comparison of EDC "loads" from point- and non-point sources into the Potomac.
- Nutrient Isotope and DOC "fingerprinting" to correlate EDCs with WWTP impacts.

4.2.1 Comparing EDC discharges from Point and Non-point sources

Tables 7 and 8 have been developed to provide a summary of EDC and nutrient data, for point and non-point sources throughout the Potomac Watershed. Table 7 presents mean values and standard errors (SE) for estrone (E1), total estrogenicity equivalent to E2 (BLYES), TDN, SRP, and DOC, while Table 8 provides a summary of the statistical significance of differences in the mean values for EDC discharges from the various point and non-point sources, presented both for estrone and total estrogenic activity.

Table 7: Arithmetic mean concentrations of estrone, estrogenic activity, TDN, SRP, and DOC in the Potomac River (above Blue Plains WWTP) and selected nonpoint and point sources.

	Source Type	Estrone (ng L^{-1})		Total Estrogenic Activity (ng L^{-1} as E2)		TDN (mg L^{-1})		SRP (µg L^{-1})		DOC (mg L^{-1})	
		Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Potomac	Background	0.48	0.12	0.59	0.15	1.15	0.14	11.2	3.2	4.10	0.59
Agr. Runoff (No BMPs)	Non-point	0.88	0.31	0.88	0.23	5.01	0.60	76.3	24.7	5.50	2.54
Agr. Runoff (BMPs)	Non-point	0.28	0.08	0.23	0.02	4.22	0.32	31.9	3.71	2.28	0.36
Urb. Runoff (No BMPs)	Non-point	0.98	0.28	3.76	2.69	1.01	0.12	21.6	7.6	5.45	0.76
Urb. Runoff (BMPs)	Non-point	0.55	0.28	0.49	0.23	0.97	0.10	8.61	2.13	3.98	0.46
CSO	Point	1.14	0.97	2.88	2.62	2.28	0.71	53.5	32.3	3.19	1.07
2nd WWTP	Point	0.40	0.10	0.50	0.06	2.11	0.17	54.0	11.7	5.54	0.75
BP WWTP	Point	0.33	0.07	0.42	0.07	1.62	0.16	53.1	6.8	7.48	1.02

Table 8: Statistical analysis comparing significance of differences in mean E1 (above the match line) and estrogenic activity (below the match line) values. Cells highlighted in green indicate $p<0.05$ for E1 and $p<0.1$ for estrogenic activity data. Cells highlighted in yellow indicate $p<0.1$ for E1 and $p<0.15$ for estrogenic activity

		E1 (ng/L)							
		Background Potomac	Blue Plains	WWTP 2	CSO	Agr. (No BMPs)	Agr. (BMPs)	Urb. (No BMPs)	Urb. (BMPs)
Estrogenic Activity (ng/L as E2)	Background Potomac		0.304	0.387	0.033	0.004	0.177	0.0005	0.181
	Blue Plains	0.246		0.411	0.211	0.055	0.314	0.022	0.231
	WWTP 2	0.430	0.024		0.219	0.067	0.264	0.029	0.266
	CSO	0.121	0.187	0.241		0.401	0.197	0.438	0.219
	Agr. (No BMPs)	0.494	0.087	0.348	0.267		0.042	0.408	0.219
	Agr. (BMPs)	0.191	0.158	0.001	0.169	0.038		0.017	0.183
	Urb. (No BMPs)	0.004	0.107	0.121	0.207	0.128	0.102		0.147
	Urb. (BMPs)	0.495	0.309	0.205	0.208	0.193	0.144	0.112	

Table 7 and Table 8 provide information on the relative levels of EDCs with land use, as compared to background Potomac levels. The first set of observations from these tables are that urban discharge (no BMPs) and CSO discharge of EDCs are significantly greater than background levels found in the Potomac with a high degree of confidence for both estrone and YES activity. Similarly, for the agriculture discharge (no BMPs), estrone is significantly higher than background Potomac levels, although the same is not seen for the bulk estrogenic activity measurement. Levels of EDCs, measured either as estrone or total estrogenic activity, from the other potential sources (both WWTPs, agriculture and urban discharges with BMPs) were not significantly different from levels in the background Potomac. This indicates that continued upward pressure on EDC levels in the Potomac at this time are likely occurring from CSOs, agriculture, and urban sources that are not implementing BMPs. Interestingly, treatment plants with advanced nutrient control, appear to not be contributing levels different than those observed in the background Potomac.

Statistical differences in EDCs levels between the sources can also be elucidated from the data analysis as well. Blue Plains AWTP and WWTP2 both discharged levels of estrone significantly lower than either the agriculture or urban discharges (without BMPs), but were not significantly different than CSOs, or agriculture or urban discharges implementing BMPs. Additionally, although there was no statistically difference in the estrone discharged by the two wastewater treatment plants, the bulk estrogenic activity was significantly lower in Blue Plains effluent than in the effluent of WWTP2, although this difference could not be confirmed with statistical significance for estrone. Both wastewater treatment plants were discharging significantly less estrone *and* estrogenic activity than agricultural or urban sources without BMP implementation, and statistically significant differences were observed with implementation of BMPs for the agriculture sources (although this trend was not as apparent with the urban sources).

Results on estrogens concentrations of point and nonpoint sources at selected sites of the Potomac River are comparable to prior studies at this and other watersheds. For example, mean estrone levels of the Potomac River (0.48 ng/L) and watershed (0.28 – 0.98 ng/L) were close to the estrone concentrations (0.1-1.6 ng/L) found in peri-urban creeks and rivers in Melbourne, Australia (Chinathamby et al., 2013), the lower Mississippi River (< 5 ng/L; Boyd et al. 2004) and rivers in Pearl River delta (<LOQ to 1.58 ng/L; Yang et al. 2014). However, the values were lower than that of the heavily contaminated Pearl River of China (<1.5- 14 ng/L; Gong et al., 2009, 2012; Xu et al. 2014) and other rivers (5-12 ng/L; Laganà et al., 2002). Meanwhile, estrone levels of the two WWTP effluents (0.33 and 0.38 ng/L) were also lower than those reported in other WWTPs of central Italy and south China (5-30 ng/L, Laganà et al., 2002; 8.1-35.6 ng L^{-1}, Wang et al., 2010; 26 ng/L, Xu et al., 2014). This difference might be related to difference in technologies used and efficiencies of estrone removal of these WWTPs. It was not surprising that the highest estrone level was observed in the CSO (1.14 ng/L), considering the extremely high estrone concentrations occurring during the storm event (Fig. 6k). Previous studies (Xu et al. 2014) reported that high concentrations of estrogens were detected in rivers receiving untreated sewage discharge.

4.2.2 Passive sampling (POCIS) of EDCs in urban Washington DC, to evaluate impact of Blue Plains effluent in the Potomac

To further evaluate the potential contributions of EDCs form the Blue Plains effluent, passive sampling devices (POCIS) were deployed at several locations in the Potomac, centered around Blue Plains. The passive samplers were deployed for 30-day increments in the fall and spring, during periods of base flow, as highlighted in Figure 12. As described in the methods section, relatively hydrophobic compounds (including estrogens and estrogen mimicking compounds) passively adsorb to the POCIS adsorbent material. The technique was utilized to attempt to better assess the very low levels of EDCs measured in the monthly grab samples, by providing essentially 30 days-worth of material for EDC analysis.

Results summarizing the sum of estrogens (including conjugates), estrone, along with the BLYES response representing total estrogenic activity in each POCIS sample deployed are provided below. While generally higher levels of measured estrogens and YES response were observed in the spring 2016 deployment, levels were generally within 50%, indicating that at base flow similar inputs of EDCs are expected in the Potomac. One striking observation is the apparent reductions in measured estrogens and estrogenic activity between Hains Point and National Harbor. The Blue Plains AWTP discharges into the Potomac between these two locations, contributing to this reduction, suggesting that highly treated wastewater flow can actually reduce levels of estrogens and/or estrogenic activity into the system over extended periods.

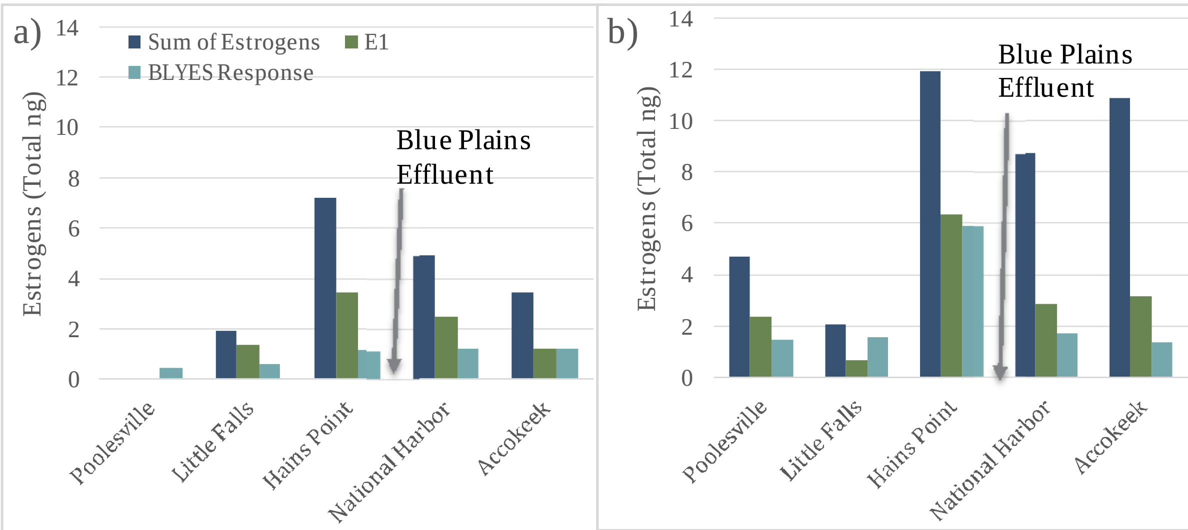


Figure 12: Concentrations of E1, Sum of Estrogens, and BLYES for 30-day Passive Sampling (POCIS) deployments in October/November 2015 (a) and March/April 2016 (b).

4.2.3 EDC and Nutrient Loading in the Potomac

To estimate annual contributions from the various sources of contaminants into the Potomac watershed, a load analysis was performed. By multiplying flow-average concentration with mean annual flow, we roughly estimated annual loads of EDCs (BLYES activity and estrone), TDN, SRP and DOC of the Potomac River and contribution from listed point versus non-point sources. Annual flow of the Potomac River at Little Falls USGS sites was from https://en.wikipedia.org/wiki/PotomacRiver, and the flow for the whole watershed was obtained by scaling the values according the drainage area. Water flows from agricultural and urban land use was estimated from areas of these land use (http://www.washingtonpost.com /wp-srv/metro/daily/111307/fullreport.pdf), assuming surface runoff was evenly distributed across land use. We also arbitrarily assumed 30% of agricultural and 50% of urban land was restored with BMPs. Blue Plains WWTP effluent flow was estimated from https://www.dcwater.com/news/publications/ Blue_Plains_Plant_brochure.pdf, while the volume of WWTP effluent of the whole watershed was estimated from http://www.washingtonpost.com/wp-srv/metro/daily/111307/fullreport.pdf. We assumed that concentrations of estrone, nutrients and DOC of the 2nd WWTP effluents represented the effluents of the rest WWTPs. The volume of CSO only included those in Washington DC (https://www.dcwater.com/workzones/projects/pdfs/ltcp/Control_Plan_Highlights.pdf) and Alexandria VA (http://greatergreaterwashington.org/tag/Combined+Sewer+Overflow/).

According to our estimates, the Potomac River annually exported 6.06 kg estrone, 16,741 × 10^3 ton TDN, 0.185 × 10^3 ton SRP and 45,608× 10^3 ton DOC. Of total EDC load, nonpoint sources of agricultural and urban runoff accounted for 60% and 20%, respectively, while 3%, 1% and 2% of the load can be ascribed to the inputs from Blue Plains WWTP, other WWTPs and CSOs. The relative unimportance of point source inputs from WWTP are also supported by the lower estrone concentrations in the WWTP effluents than in agricultural runoff, urban runoff and CSOs effluents. Similarly, TDN and SRP concentrations in agricultural runoff was highest among the other inputs, and their inputs dominated over other sources, consistent with previous studies on N and P sources of the Potomac River (Ator et al. 2011). The higher SRP inputs from agricultural runoff, as compared to the total flux of the Potomac can be attributed to either P transformation to particulate form or unrepresentative of the selective streams.

Table 9 Estimated annual fluxes of EDCs (BLYES and estrone), TDN, SRP and DOC of the Potomac River and annual nonpoint and point inputs to the river

		Annual Average Concentrations					Annual Load				
	flow	BLYES	Estrone	TDN	SRP	DOC	BLYES	Estrone	TDN	SRP	DOC
	$10^6 m^3 y^{-1}$	ng L^{-1} as E2	ng L^{-1}	mg L^{-1}	µg L^{-1}	mg L^{-1}	g as E2	g	ton	ton	ton
Potomac River	13182 [1]		0.46	1.27	14	3.46		6064	16741	185	45608
Agricultural runoff no BMP	2584 [2]	0.88	1.2	3.82	108	5.2	2273	3100	9869	279	13435
Agricultural runoff BMP	1107 [2]	0.23	0.48	3.55	37.7	2.87	254	531	3931	42	3178
Agricultural subtotal							2527	3632	13800	321	16612
Urban runoff no BMP	639 [2]	3.76	1.31	1.14	14.6	6.28	2403	837	729	9	4015
Urban runoff BMP	639 [2]	0.49	0.56	1.06	10.3	4.33	313	358	678	7	2768
Urban subtotal							2716	1195	1406	16	6783
Nonpoint Source subtotal							5243	4827	15206	337	23395
Other WWTP effluents	144 [4]	0.50	0.38	1.97	54.0	5.09	72	55	283	8	732
Blue Plains WWTP effluents	531 [3]	0.42	0.33	1.62	53.1	7.47	223	175	860	28	3966
Combined sewer overflow	22 [5]	2.88	4.87	4.95	164	7.05	64	107	109	4	155
Point sources subtotal							359	337	1252	40	4853

1. Potomac River annual flow was adapted from https://en.wikipedia.org/wiki/PotomacRiver, with flow at Little Falls USGS sites expanding to the whole watershed basing on area ratio.
2. Estimated from land use percent of the Potomac watershed (http://www.washingtonpost.com /wp-srv/metro/daily/ 111307/fullreport.pdf), and assumed 1) surface runoff was evenly distributed across land use and 2) 30% of agricultural and 50% of urban land were restored with BMPs.
3. Blue Plains WWTP effluent flow was estimated from https://www.dcwater.com/news/publications/Blue_Plains_Plant_brochure.pdf,
4. Total WWTP flow of the watershed was estimated from http://www.washingtonpost.com/wp-srv/metro/daily/111307/fullreport.pdf, and assumed that concentrations of EDCs, nutrients and DOC of the 2nd WWTP effluents represented the effluents of the rest WWTPs.
5. Combined sewer overflow only included those in Washington DC (https://www.dcwater.com/workzones/projects/pdfs/ltcp/Control_Plan_Highlights.pdf) and Alexandria VA (http://greatergreaterwashington.org/tag/Combined+Sewer+Overflow/)

4.2.4 Nutrient Isotope and DOC "fingerprinting" to correlate EDCs with WWTP impacts

Techniques exist for parsing out likely sources of nutrient and organic carbon inputs using advanced analytical techniques to characterize nutrients (particularly nitrate) and organic material. By using stable isotope analysis (δ^{15}N-NO3 and δ^{18}O-NO3) and fluorescence excitation emission matrices (EEMs) techniques, potential sources and character of these two parameters were suggested. Brief descriptions of these two techniques are provided below, followed by analysis performed for this study using the techniques.

Nutrient Isotopes: Nitrates originating from different pollution sources generally have a distinctive nitrogen (N) or oxygen (O) isotopic signature (ratio of the heavy to light stable isotope), as described in Figure 13. Therefore, stable isotope analysis of nitrate in water can be used to identify the nitrate sources and to estimate their contribution to nitrate pollution. In addition, biological cycling of N often affects the isotopic composition in a predictable pattern, which can be used to detect and quantify natural nitrate removal processes (denitrification) (Kendall, 1998).

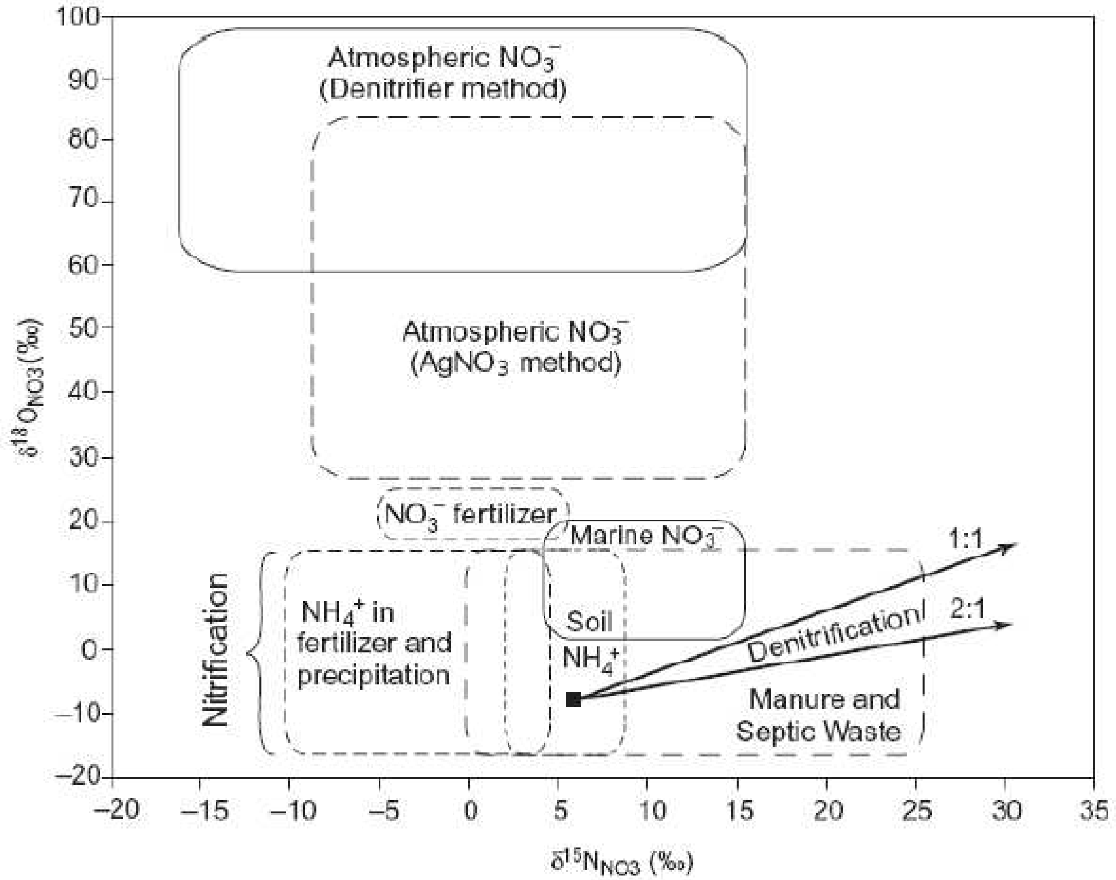


Figure 13. Typical values of N and O isotope ratios (expressed in δ^{15}N-NO_3 and δ^{18}O-NO_3 values) of nitrate derived from various N sources. Taken from Kendall et al, 2007.

These techniques are used when multiple potential nitrate sources exist, to identify the main sources and estimate contributions to implement effective, source-oriented remediation measures. This information cannot be assessed from current water quality monitoring data alone (concentration measurements). In addition, interpretation of the relation between nitrate concentrations and nitrate input from the sources is complicated by time delaying mechanisms (i.e., percolation through soil, groundwater flow) and biogeochemical processes altering the concentration during nitrate transport. Isotope data is used as a tool, complementary to existing monitoring data, since they enable to identify the nitrate sources, assess relative contributions to nitrate pollution, and quantify nitrate transport and removal processes.

Fluorescence Excitation-Emission Matrices (EEMs): Fluorescence techniques (i.e. excitation-emission matrix spectroscopy) have been widely used to investigate the sources and optical properties of dissolved organic material (DOM) or humic substances in aquatic environment. For example, the use of EEMs permits discrimination of DOM sources based on the relative abundances of different fluoromophores, including humic-like, protein-like, and pigment-like fluorescences (Coble, 2007; Stope et al, 2014). Typical EEM spectra are shown in Figure 14, highlighting areas of fluorescence known to correlate with humic and protein-like fluorescence of DOM.

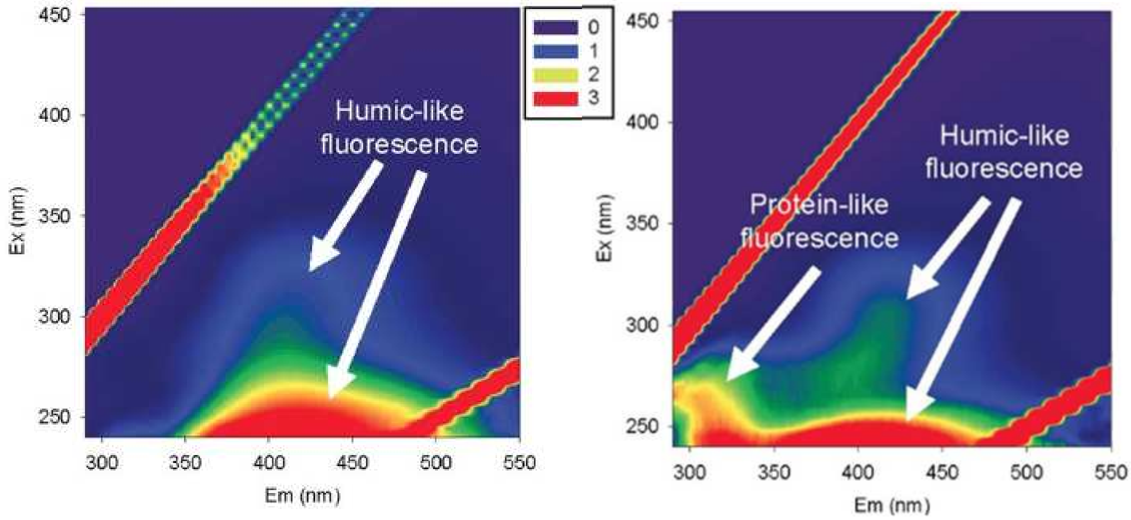


Figure 14: Example EEMs. Taken from Fu et al (2015)

Similar EEMs, collected from each sample location, were analyzed in a similar manner to determine Fluorescence Index (FI), humification index (HIX), and biological index (BIX). Descriptions of the analysis used to determine these indexes are provided in the methods section. McKnight et al (2001) proposed FI as a proxy for the relative amount of DOM derived from terrestrial and microbial/algal sources, determining that FI values of 1.4 or less correspond to terrestrially derived organics and higher aromaticity, while values of 1.9 or higher correspond to microbial sources and lower aromatic carbon content. BIX, also allows an estimation of the contribution of autochthonous biological activity. An increase in BIX is related to an increase in the contribution of microbial derived organics. For example, high values (>1) have been shown to correspond to a predominately biological or microbial origin of DOM and to the presence of OM freshly released into the water, whereas values of <0.6) contain little biological material. HIX was introduced by Zsolnay et al (1999), to estimate the degree of maturation of DOM. During the humification process, the aromaticity of organic matter increases and its microbial availability decreases. Thus high HIX values (>10) correspond to strongly humified or aromatic organics, principally of terrestrial origin, while low values (<4) are indicative of autochthonous or microbial origin organics (Birdwell and Engel 2010, Tedetti et al 2011, Zsolnay et al. 1999).

These techniques were utilized for analysis of the collected data, and are presented in Figure Z, which presents nitrate isotopes and fluorescence analysis for A) Blue Plains AWTP effluent (along with Potomac River above and below the discharge), B) WWTP2 effluent (along with receiving stream above and below the discharge), and C) non-point sources (with and without BMPs). Annotations describing the degree of enrichment as well as the qualitative descriptors of FI and HIX are provided in the figures. Observations from Figure 15 are as follows.

- Impact of Blue Plains Effluent on the Potomac. Figure 15, A-1 and A-2 show significant differences between the nature of the nitrate and DOM discharged from Blue Plains as compared to the Potomac receiving waters. While the Potomac above the outfall is terrestrial derived, with a mixture of autochthonous and aromatic carbon, describing a mixture of DOM “freshness”, and the nitrate shows very little enrichment, input of the highly enriched nitrate and highly biological, highly autochthonous DOM from Blue Plains visibly changes the nitrate and fluorescent “fingerprint” of the Potomac River below the outfall, indicating a significant impact of the effluent on the nature of the River below the outfall. For comparison, the nature of Blue Plains secondary treated water is also shown, indicating a more similar nutrient profile to the Potomac, but an even more significantly “different” nature of the organics.
- Impact of WWTP2 Effluent on its receiving stream. Figure 15, B-1 and B-2 show less significant differences between the nature of the nutrients discharged, but still significant differences between the nature of the DOM discharged from WWTP2 as compared to the receiving waters. While the receiving stream above the outfall is obviously terrestrial derived and aromatic, the WWTP2 effluent is much more highly microbial derived and autochthonous. Organics present below the outfall resemble the outfall, displaying an obvious impact in the nature of the organics by the WWTP2 effluent. However, with respect to the nature of the nitrate, the impact is much more subtle. The nitrate in the effluent is much less enriched as compared to Blue Plains effluent, speaking to differences in the removal process for the advanced nutrient control implemented by each facility. As a result, the impact on nitrate character in the receiving stream below the outfall is not significantly changed by the input from WWTP2.
- Nature of nitrate and organics from nonpoint sources. Figure 15, C-1 and C-2 describe the nature of nitrate and organics inputs from nonpoint sources, both agricultural and urban runoff. Non-point sources provide a less enriched nitrate fingerprint, with little difference between the sources, and a terrestrial derived, aromatic skewing organic profile. The implementation of BMPs showed little change in the nutrient fingerprint, and a slight skewing toward more aromatic carbon. Interestingly, the Potomac above Blue Plains displays nutrient and organic matter profiles similar to urban (without BMPs), while the background fingerprinting of the WWTP2 receiving stream display a potential impact of agricultural impacts.

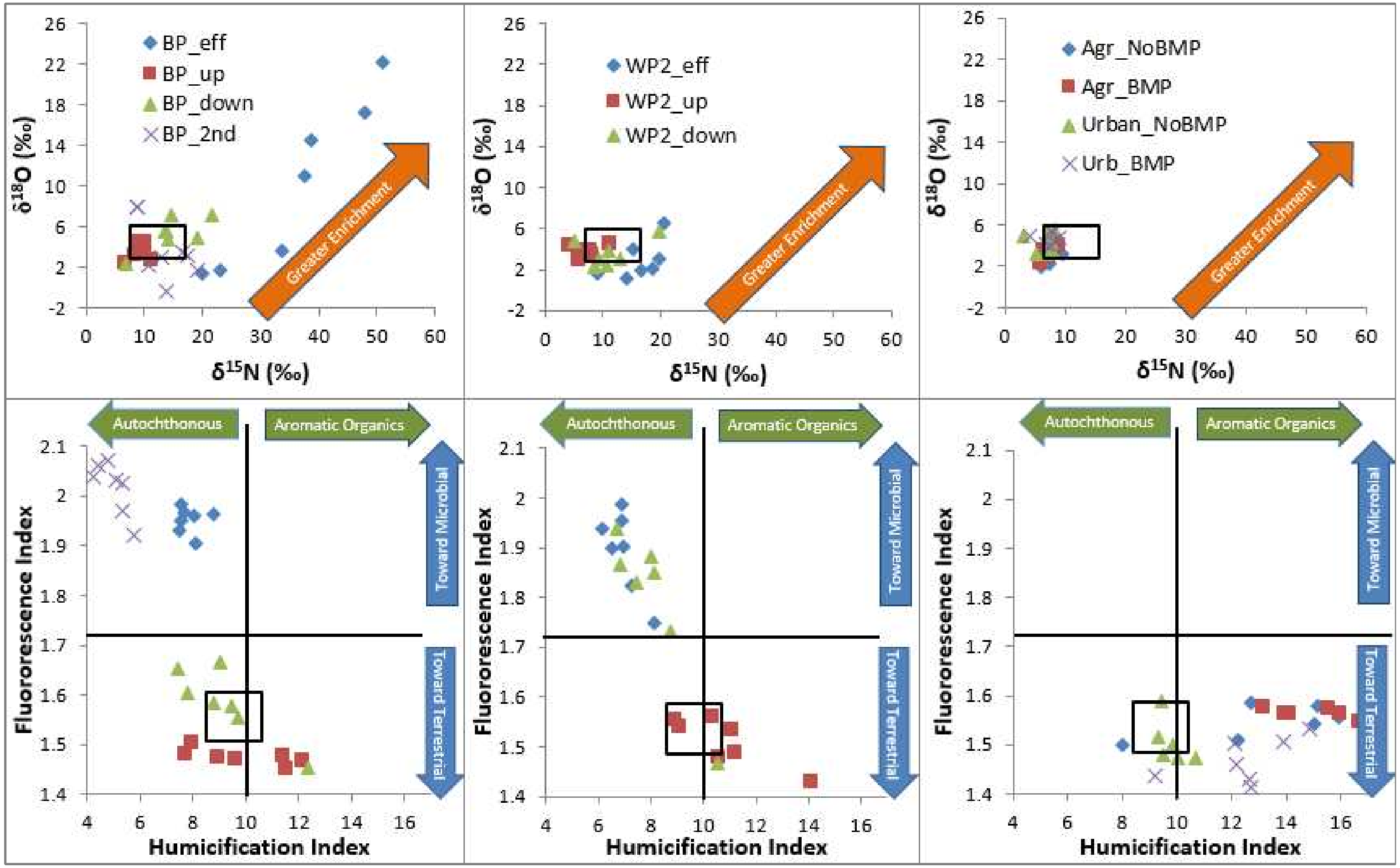


Figure 15: Nutrient and DOM fingerprinting techniques for Blue Plains (BP), WWTP2 (WP2), and Agriculture (Agr) and Urban (Urb) non-point sources to the Potomac.

Mean isotopic values of nitrate and spectral ratios of DOC were shown in Figure 16 Potomac River nitrate and possible sources can be separated by their isotopic composition (Figure 16-a). In general, nitrate from agricultural runoff, urban runoff and CSOs was relatively depleted in both ^{15}N and ^{18}O, while nitrate from Blue Plains AWTP was enriched in both in ^{15}N and ^{18}O. Conversely, nitrate from the Seneca WWTP was only slightly enriched in ^{15}N relative to that in Blue Plains AWTP. Nitrate isotopic compositions of the Potomac River above the Blue Plains AWTP were close more to nitrate from agricultural runoff, urban runoff or CSOs. The $\delta^{15}N$ and $\delta^{18}O$ values of the Potomac River below Blue Plains AWTP increased toward to nitrate isotopic values of the Blue Plains AWTP effluent.

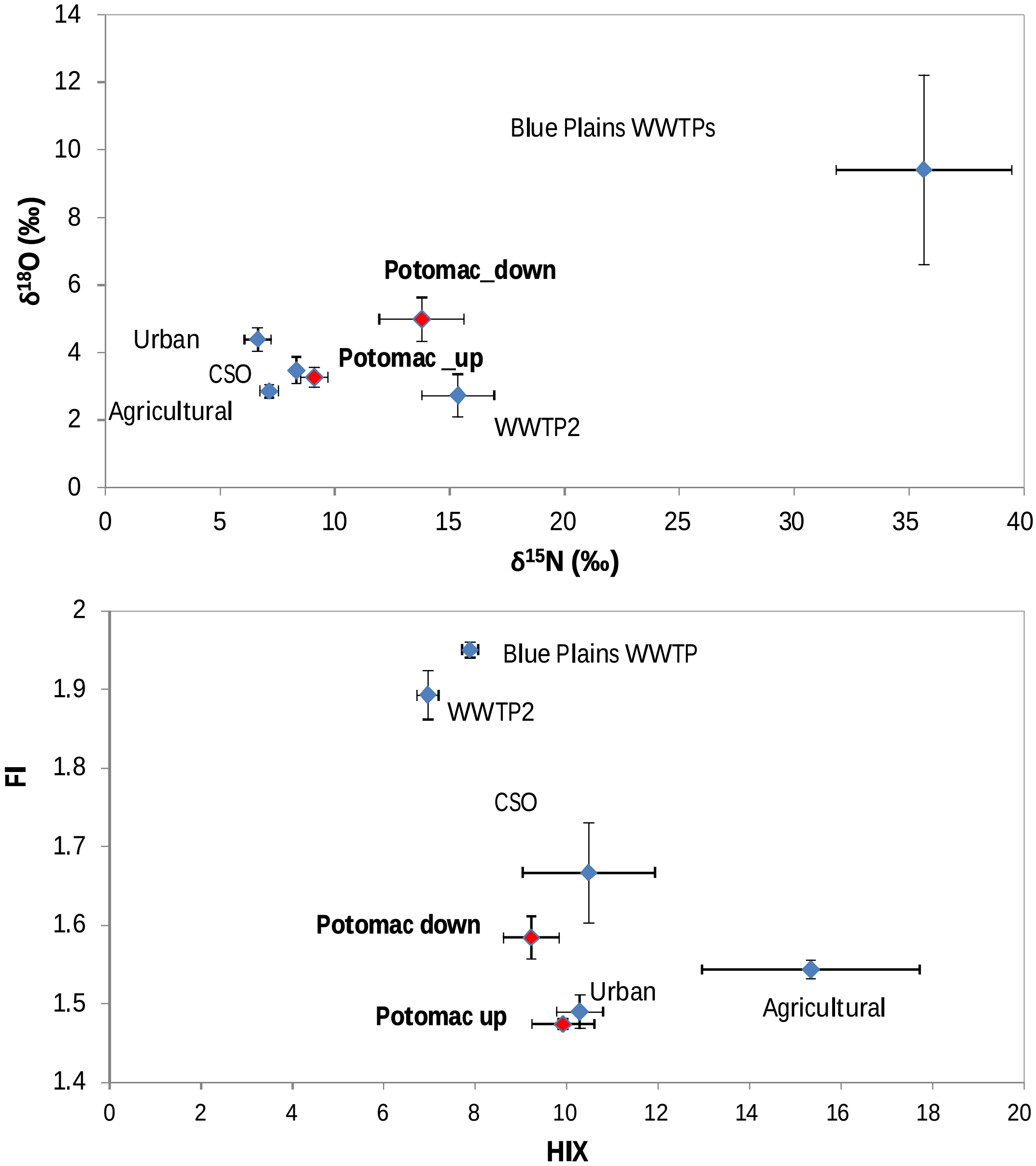
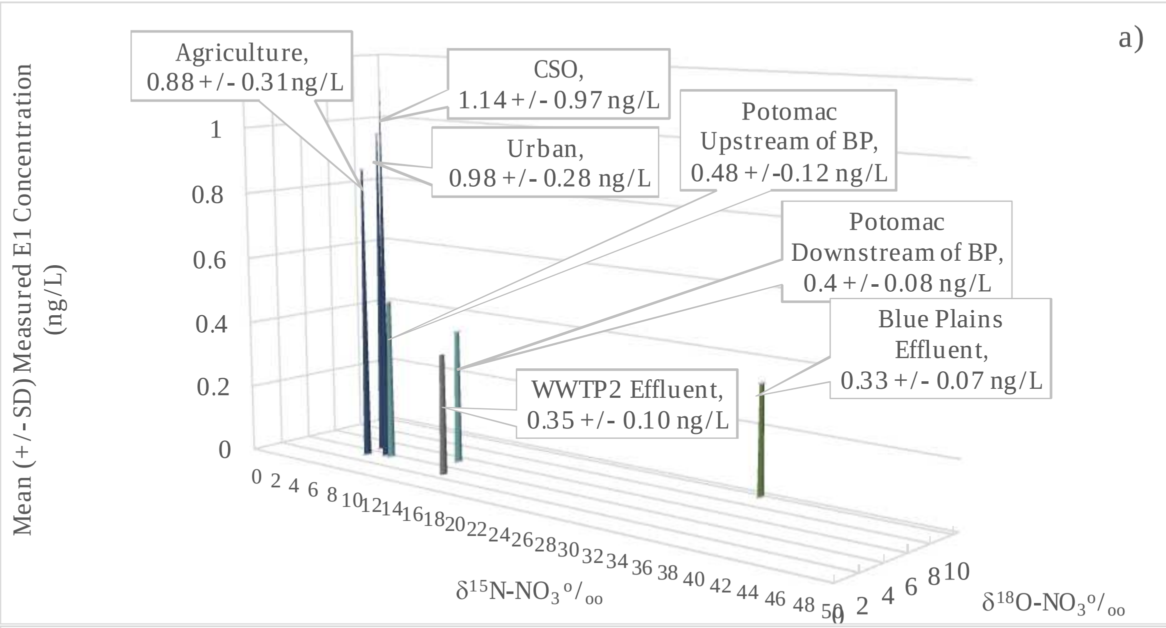


Figure 16: Average nitrite and organic matter "fingerprints" for sources in the Potomac River.

Finally, levels of EDCs were superimposed on the nutrient "fingerprints" in Figure 17, for estrone (17-a) and YES estrogenic activity (17-b). These figures display changes in nutrient composition along with impacts on EDC concentrations with various inputs. Interestingly, the data suggest that the input of Blue Plains changes the nutrient fingerprint of the Potomac River from one influenced heavily by urban and agriculture to that of the Blue Plains effluent, and also slightly reduces EDC concentrations in the river.



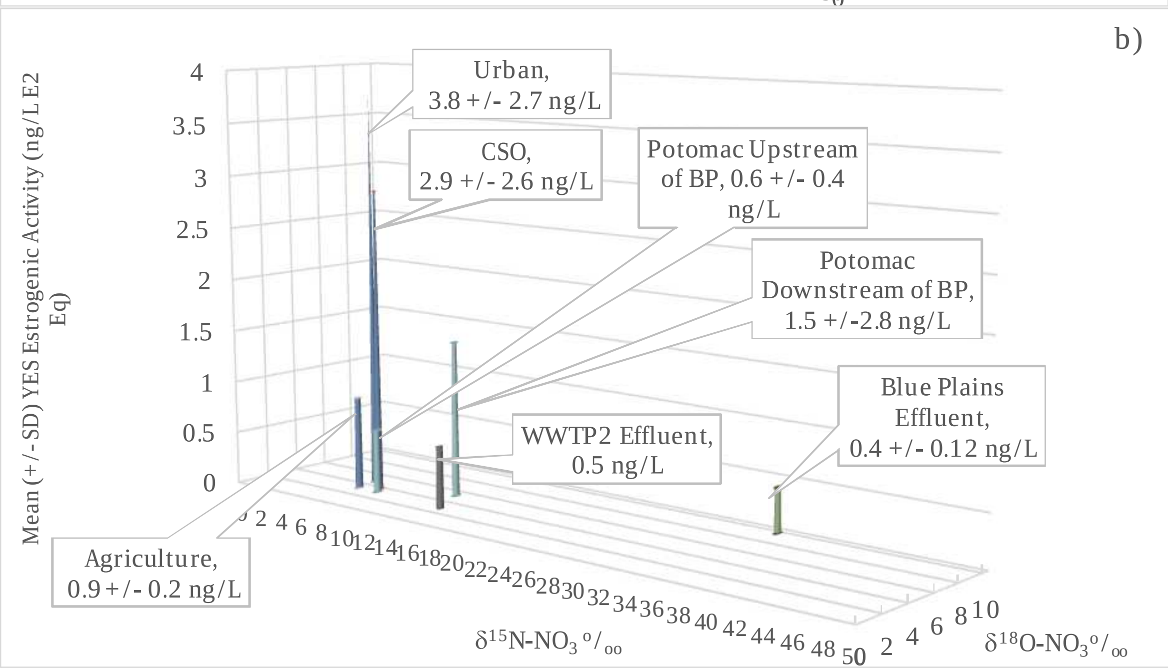


Figure 17: Comparing Nutrient Fingerprints with EDCs (a) estrone, and b) BLYES activity)

5. Conclusions

The following conclusions can be drawn from this research.

Objective 1: Upstream and Downstream Impacts on EDCs from "best-in-class" nutrient control, agriculture management, stormwater management, and wastewater treatment strategies.

- In general, implementation of BMPs showed significant reductions in EDC inputs to the Potomac Aquifer from agriculture and urban runoff, with statistically significantly lower EDC levels input into the Potomac from both Agriculture and Urban runoff with BMPs implemented. BMPs studied included:
    - Agriculture: restricting livestock access to streams, planting grasses for stream shading and improving streambank stability.
    - Urban: maintaining shaded habitat, reducing impervious area, restoring stream habitat and riparian, and creating wetlands.
- Reductions in EDCs with implementation of BMPs correlated well with reductions in soluble reactive phosphate, less-so with organic carbon, and not with nutrient control, suggesting co-management of EDCs with phosphorous control methods may be more effective for agriculture and urban inputs.
- Advanced nutrient control for two wastewater treatment plants in the Potomac Watershed (Blue Plains and WWTP2) resulted in lower EDCs discharged in the effluent from these two facilities than were present in the background receiving streams, suggesting effective co-management of nutrients and EDCs in wastewater treatment.
- Blue Plains profile sampling revealed large reductions in EDCs with advanced nutrient control. Levels of EDCs (measured as bulk estrogenic activity and estrone), were both reduced by greater than 99% on average with advanced nutrient control. This suggests that point-sources of EDCs from wastewater treatment facilities is likely low in the Potomac watershed, where nutrient control is highly prioritized, but may not be the case in watersheds with inputs from wastewater plants only performing secondary treatment.

Objective 2: Assess the relative contribution of EDCs from WWTPS performing biological nutrient removal to the Potomac.

- A statistical comparison of the mean concentrations measured during the discrete grab sampling events indicated only CSOs, agriculture inputs (without BMPs) and urban inputs (without BMPs) displayed statistically significantly greater levels of estrone (E1) than the background Potomac. The same analysis for bulk EDC activity indicated urban inputs (without BMPs) were statistically significantly greater than background Potomac levels, and CSOs were borderline. Wastewater treatment plant inputs (Blue Plains and WWTP2) were not statistically significantly different than background Potomac EDC levels.
- Results from two, 30-day, passive sampling campaigns indicated:
    - Higher levels of EDCs were observed at 5 locations in the Potomac during the spring of 2016 deployment when compared with the fall of 2015.
    - The input of Blue Plains Effluent correlated with reductions in observed EDC mass between Hains Point and National Harbor in both fall 2015 and spring 2016 deployments.

- An annual load analysis indicated non-point sources accounted for over 80% of EDC load to the Potomac, with Blue Plains contributing only 3% of the EDC load to the Potomac.
- The relative minor contribution of EDCs and nutrients from WWTPs were also supported by the downstream changes in concentrations, and suggested co-management of EDCs in Wastewater Treatment may be matched to nitrogen control, rather than SRP as was the case for agriculture and urban BMPs.
    - EDCs and TDN concentrations in both WWTP effluents were lower than their receiving water, and no downstream increases in estrone and TDN concentrations were observed when the receiving stream/river passed these WWTPs.
    - Conversely, SRP and DOC concentrations in Blue Plains and WWTP2 effluents were higher than their receiving water, with apparent downstream increases in SRP and DOC concentrations were observed in the WWTP2 receiving stream.
- Nutrient and DOM fingerprinting analysis suggested the WWTP effluents both had significant impact on the nature of DOM on their receiving waters, while only Blue Plains impacted the receiving water's nutrient fingerprint.
- Changes in nutrient fingerprint associated with Blue Plains Effluent correlated with a reduction in EDC concentration in the Potomac River below the outfall, suggesting the high level of nutrient management employed at Blue Plains is effectively co-managing EDC inputs to the system.

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