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Invasive species may offer advanced phytoremediation of endocrine disrupting chemicals in aquatic ecosystems

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Abstract

One of the major areas of advancement in environmental science is bioremediation. Researchers have been using bacteria, fungi, algae and now macrophytes to remove pollutants from aquatic and terrestrial ecosystems. Here we share the results of a study on the macrophyte uptake of xenoestrogens from an urban river. We found that the invasive curly leaf pond weed (*Potamogeton illinoensis*) accumulated an average of 66% higher levels of estrogenic compounds and 94% more Bisphenol-A than the native Illinois pondweed (*Potamogeton crispus*) in an urban river, in the watershed for the greater Chicago, IL area. The invasive species accumulated 76% more estrone, 55% more 17 β -estradiol and 31% more 17 α -ethynylestradiol than the native species. The Non-native plants were also 72% larger than the native Illinois Pondweed. Managers may consider using invasive species to remove pollutants from ecosystems and restore ecosystem biogeochemistry.

Key words: Endocrine Disrupting Chemicals, Environmental Remediation, Invasive Species

Introduction

Examining ecosystem biogeochemistry and the alterations of biogeochemical cycles due to anthropogenic activities has become one of the most important fields within environmental science. The ever-increasing anthropogenic load on environments calls for innovative strategies to remediate and restore ecosystems. Polluted aquatic ecosystems present some of the largest challenges to managers because of the ability of nutrients, organisms and pollutants to vary in concentration at different depths and mediums. Urban aquatic ecosystems also have high rates of flux between the biosphere, hydrosphere and the atmosphere. Many pollutants flow into urban aquatic ecosystems from precipitation, run-off, and point sources. These pollutants may remain suspended in the water column, be taken up by aquatic organisms or accumulate in bottom sediments (i.e. Karickhoff et al., 1979).

Bottom sediments in aquatic ecosystems have

the ability to absorb, accumulate, and transform pollutants. However, many lakes and ponds undergo turnover events which may re-suspend pollutants in the water column (Karickhoff and Morris, 1985). It is desirable to remove pollutants from these aquatic ecosystems, since bottom sediments are not a sufficient sequestration pool. Phytoremediation is emerging as the most practical cleanup technology by using certain species of plants to sequester pollutants from sediments and water (Pilon-Smits, 2005; Suresh and Ravishankar, 2004). While much of the research has centered on specific species and their ability to remove particular pollutants from ecosystems (i.e. Kurilenko and Osmolovskaya, 2007), little attention has been paid to the role invasive species may play in regards to phytoremediation.

The utilization of natural biodegradation processes has gained traction in recent years. Their economic and environmental costs are much lower than the conventional incineration of large quantities of soil or the usage of chelating agents (Franks, 2006). Due to anthropogenic activities, pollution is a major driver of imbalance in native ecosystems. Under these adverse conditions, tolerance to these contaminants often leads to a change in the species that dominate ecosystems. Some ecosystems may then be vulnerable to invasion by non-native species. Non-native species

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may act as efficient phytoremediators; however, little research has been completed to assess this. Some studies have deployed non-native species to clean-up environmental pollutants, but this runs the risk of promoting further invasive entry of species (Li et al., 2009).

There is a growing awareness that organic contaminants such as endocrine disrupting chemicals (EDCs) are being found in wastewater, rivers and lakes are very difficult to remove (Farre, et al., 2007). Many of these active chemicals carry unknown risks, and may lead to interference with the endocrine systems of aquatic organisms (US EPA, 2010). EDCs are chemicals that mimic or upset natural hormones and include estrogen derivatives from anthropogenic sources such as treated and untreated sewage effluents, as well as xenoestrogens such as Bisphenol-A. These contaminants often lead to adverse health effects among species in aquatic and terrestrial ecosystems (Barceló and Petrovic, 2007). These effects include feminization, as well as other reproductive and developmental problems (Crain et al., 2007; Kang et al., 2006).

Three of the most common estrogen contaminants are Estrone, 17 β -estradiol and 17 α -ethynylestradiol. Having an environmental impact at concentrations in the nanograms per liter range, these compounds are hydrophobic, involatile, and practically insoluble in water. Furthermore, sewage treatment plants are unable to effectively reduce these estrogens, leading to adverse health effects amongst populations of aquatic and terrestrial species downstream. Estrone and 17 β -estradiol, naturally excreted sex hormones, have been shown to promote the production of vitellogenin (female egg proteins) among fish and brown frog populations (Crain et al., 2007; Rouhani et al., 2005). Furthermore, exposure to the potent synthetic birth control hormone 17 α -ethynylestradiol has been linked to decreased fertility of male rainbow trout and shown to alter vitellogenin production at sub-nanogram per liter concentrations (Schultz et al., 2003). Prolonged exposures to these EDCs have a significant effect on biodiversity and food webs (Franks, 2006).

Bisphenol A [BPA, 2, 2-bis (4-hydroxyphenyl) propane] has been found in many water-bodies across the world. The pervasiveness of BPA is due to the fact that it is involved in the production of numerous goods and materials such as epoxyphenolic resins, polycarbonates, polyacrylates and plastics. Many canned foods are also lined with plastics containing BPA (Kang et al., 2006). As the toxicity of BPA has reached the media and

the general public, individuals are now becoming aware of the potentially dangerous side effects of BPA and are therefore trying to remove it from human consumption (Takahashi et al., 2001). However, BPA is found in a wide variety of products from pesticides to flame retardants and rubber chemicals (Staples et al., 1998) and it therefore ends up in rivers, lakes and streams. BPA has become a target pollutant for removal due to its acute toxicity towards algae, invertebrates and fish (Alexander et al., 1988).

Several studies have targeted natural bioremediators for BPA. Ligninolytic fungi are known to effectively metabolize EDCs (Loffredo et al., 2012). Their ability to biodegrade lignin depends on extracellular enzymes having low substrate specificity. This property allows Ligninolytic fungi to utilize high concentrations of aromatic compounds, including EDCs, for this process (Loffredo et al., 2012; Tsutsumi et al., 2001). Li et al. (2009), found that the algae *Stephanodiscus hantzschii* could tolerate high concentrations of BPA and serve as a biodegradation agent. The recent work of many others has also determined that endocrine disrupting chemicals may be biologically transformed and/or sequestered by algae, fungi and many bacterial species (North and Halden, 2013). This ability of managers to utilize naturally occurring organisms to biodegrade EDCs from landfill leachates, wastewater, and natural bodies of water is encouraging. Although this research is still in the early stages, using macrophytes for biodegradation and bioremediation may prove to be an effective way to restore balance to polluted ecosystems.

This study examined the phytoremediation potential of two Pondweeds on estrogen derivatives and Bisphenol-A in the Des Plaines River near Chicago, IL. The Curly Leaf Pondweed, native to Eurasia, has been positively identified in North America. It is known to form dense mats displacing native aquatic plants, including the native Illinois Pondweed (Johnson, 2010). Samples of both the native Illinois Pondweed (*Potamogeton illinoensis*) and the invasive Curly Leaf Pondweed (*Potamogeton crispus*) were collected from six sites covering over 8 kilometers of river. Water samples were also collected to determine the current concentrations of estrone, 17 β -estradiol, 17 α -ethynylestradiol and BPA present in the river. Measurements of the accumulation of EDCs in the aquatic and plant samples, may point towards the role invasive species play in phytoremediation.

Materials and Methods

Study site

The Des Plaines River runs from north to south and bisects the Chicagoland area. This river receives large inputs from sewage treatment plants, road run-off and industrial complexes. The river is known to be highly polluted; however, little information has been published on the pollutants found therein. The Des Plaines River watershed covers the greater Chicago area, and about 854,669 acres of Cook, Dupage, Lake, and Will Counties. The majority of the land use in this area is urban (58.7%), followed by agricultural (33.2%), forest (5%), and the remainder being wetlands (Pescitelli and Rung, 2005). The Des Plaines River has at times received both untreated sewage inputs (Hawthorne, 2011) and regularly receives treated sewage inputs. As a river flowing through a major city, it bears a lot of environmental stress created by human activity, and in recent years, infiltration of this river by contaminants such as fire retardants, and oil sludge has been in the news (Dillon, 1999; The Huffington Post, 2009). The city of Chicago has an interest in maintaining forest preserves and wetlands; however, phytoremediation along the river has not yet been attempted. This study aims to test the viability of using phytoremediation on polluted urban river systems. These areas are of great importance because they provide habitat corridors that may allow flora and fauna to flourish in urbanized areas.

Water and plant samples were collected from the Des Plaines River just outside Chicago, IL on August 26-27, 2011. The samples were extracted from the upper Des Plaines River at six sites each approximately 1 km from the other. Sample locations were labeled for sites 1-6 with site 1 being the furthest upstream and approximately 9 km from a known sewage point source. Twenty liters of water samples were obtained from below the river surface about one meter from the shore and near the area where plants would be collected at each site. Water samples were placed into pre-cleaned amber glass bottles and were then stored at approximately 4°C in the absence of light. Six samples of both the native aquatic plant, Illinois pond weed (*Potamogeton illinoensis*), and the invasive, Curly Leaf Pondweed (*Potamogeton crispus*) were collected. Plants were dried in an oven at 80°C for 48 h until a constant dry weight was reached. The dried sample was cooled down to room temperature (ca. 20°C) in a desiccator before weighing each plant sample. Each plant was ground to a fine powder where a homogenized sub-sample of 1

gram of ground material was used for further extractions.

Estrogen and the Estrogen Derivative GC-MS Analyses

Estrogenic compounds and transformation products were identified using gas chromatography coupled with mass spectrometry (GC-MS). To prepare samples for GC-MS identification samples were dissolved in methanol and passed through an Oasis HLB 4 Plus solid phase extraction (SPE) pack (Waters Corp.), which had been preconditioned with 10mL of methanol and Milli-Q water. The SPE packs were eluted with 2 mL methanol over 5 min. The methanol was allowed to evaporate, and the residue was re-dissolved in 50 µL hexane followed by derivatization with 50 µL BSTFA (1% TMCS) at 60°C for 45 min.

GC-MS is a commonly used method for the detection of pharmaceuticals and hormones in water. This process involves the off-line purification of substances using solid phase extraction (SPE) and specific compounds for derivatization, prior to GC-MS analysis (i.e. Kelly, 2000; Kuch and Ballschmiter, 2000; Jeannot et al., 2002). To analyze plant and water samples for estrogenic compounds, off-line sample preparation was necessary. All stock solutions were prepared using HPLC-grade substances and followed the protocol designed by Quintana et al. (2004). Methanol and ethyl acetate were supplied by Sigma Aldrich (St. Louis, USA). Stock solutions of estrone, 17 beta-estradiol and 17 alpha-ethynylestradiol were also prepared using high quality products from Sigma-Aldrich (St. Louis, USA). MTBSTFA, BSTFA, BSTFA containing 1% of TMCS, MSTFA and TMSI, were obtained from Supelco (Bellefonte, PA, USA). We completed off-line preparation of samples using SPE cartridges containing 60 mg of Oasis HLB were obtained from Waters Corporation (Milford, MA, USA) and used as received.

GC-MS analyses were performed on a Varian 220-MS (Agilent, Santa Clara, California) linked to a Varian 431-GC (Agilent, Santa Clara, California) which was equipped with a 30 m x 0.25 mm x 0.25 µm Factor Four VF-5ms capillary column (Agilent, Santa Clara, California). The chromatographic conditions for estrogen and its derivatives are as follows: 5 µL split/splitless injection (injector temperature 250°C). The sample was held at 50°C for 1 min then the temperature was raised to 220°C at 20°C min⁻¹ and held for 27 minutes. The oven was then brought to 250°C at 20°C min⁻¹ and held for 20 minutes. The ultra-high purity helium carrier

gas was controlled at a velocity of 41.3 cm s^{-1} . The mass spectrometer was operated on auto electron impact ionization mode with an emission current of $10 \mu\text{A}$, transfer line temperature of 250°C , and an ion trap temperature of 200°C . The mass spectrometer was scanning from 40 to 650 amu with an inter-scan time of 1 s. Compound identification was based on the comparison of mass spectra with the NIST (NIST/EPA/NIH Mass Spectral Library NIST08). We measured standards and blanks periodically throughout the analyses to assure accuracy. Quantitative analyses of target compounds were achieved through the integration of selected gas chromatography chromatograms. Each sample was rerun three times and the detection limit was in the range of 1 ng/L.

BPA Analysis

BPA was extracted from dried plant material by mixing it with ethanol (10 ml for every 1.5 g plant dry weight) for 24 h prior to elution. Our Bisphenol-A standard (99%, solid crystal form) was purchased from Sigma-Aldrich (St. Louis, USA) and the stock solution was prepared in methanol at a concentration of 250 mg L^{-1} and stored in brown bottles at 20°C . Other reagents such as methanol, trichloroethene and carbon tetrachloride was purchased from Sigma-Aldrich (St. Louis, USA). All reagents were of analytical grade or above. Analyses proceeded following the method outlined in Fontana et al. (2011). GC-MS analyses were performed on a Varian 220-MS (Agilent, Santa Clara, California) linked to a Varian 431-GC (Agilent, Santa Clara, California) which was equipped with a $30\text{m} \times 0.25\text{mm} \times 0.25 \mu\text{m}$ Factor Four VF-5ms capillary column (Agilent, Santa Clara, California). The chromatographic conditions are as follows: 100°C ; ramping at $10^\circ\text{C min}^{-1}$ to 270°C ; ramping at $30^\circ\text{C min}^{-1}$ to a final temperature of 300°C ; and held for 2 min. Ultra high purity Helium was used as a carrier gas (purity = 99.999%) with a flow rate of 1.0 mL min^{-1} . The injector temperature was set at 280°C , and the injections were performed in the splitless mode. The mass spectrometer was operated in electron impact ionization mode at -70 eV . The temperatures of the trap, manifold, and transfer line were set at 220, 120, and 280°C , respectively. Compound identification was based on the comparison of mass spectra with the NIST (NIST/EPA/NIH Mass Spectral Library NIST08). Determination of constant error was established through the analysis of a blank sample. Quantitative analysis of target compounds was

achieved through the integration of selected gas chromatography chromatograms. Each sample was rerun three times and the detection limit was in the range of 1 ng/L.

Data analysis

Statistical analyses were performed with the software package SigmaPlot for Windows (SPSS Inc., Chicago, IL, USA). All data is expressed as mean \pm standard deviation. Prior to all statistical tests, data was examined for normality using a Shapiro-Wilk test and for homogeneity of variances using Levene's test. Depending upon the outcome of these tests, either parametric or non-parametric method was conducted to test for significant differences.

Results

Estrogenic compounds

The concentration of estrogenic compounds present in the water varied from 1.2-20.1 ng/L (Table 1). Estrone concentrations in river water varied from 14.2-20.1 ng/L, 17 β -estradiol concentrations varied from 10.2-15.1 ng/L and concentrations of 17 α -ethynylestradiol were within the range of 1.9-6.2 ng/L (Figure 1). The concentration of estrogenic compounds present in the non-native *P. crispus* and the native *P. illinoensis*, were very low compared to the concentration of estrogenic compounds present in the water (Figure 1). The non-native species, *P. crispus*, contained estrone in concentrations varying from 4.8-9.7 ng/L (Figure 1). The 17 β -estradiol concentrations varied from 3.3-4.9 ng/L, whereas the concentrations of 17 α -ethynylestradiol were very low, 0.39-1.1 ng/L (Figure 1). The native Illinois Pondweed (*P. illinoensis*) contained estrone in concentrations varying from 3.0 -5.9 ng/L (Figure 1). The 17 β -estradiol concentrations varied from 2.1-3.2 ng/L whereas the concentrations of 17 α -ethynylestradiol were very low, 0.3-0.6 ng/L (Figure 1).

Estrogen derivative concentrations were highest closest to the human effluent point source. Sample location 1 was $\sim 9\text{km}$ from the known sewage effluent point source and each successive sampling location was $\sim 1 \text{ km}$ from the previous site. As sample locations moved away from the point source the concentration of estrogenic compounds in water, *P. crispus* and *P. illinoensis* decreased (Table 1). The average concentration of estrogenic compounds in Des Plaines River water was $33.1 \pm 4.6 \text{ ng/L}$, while the range was between 26-41 ng/L.

Table 1. Estrogenic compounds found in water samples, the non-native species *P. crispus* and the native Pondweed, *P. illinoensis*. The most upstream sample was collected at location 1 while each sample location was ~1km further downstream. Sampling took place ~9km from sewage effluent point sources.

Sample Location	Estrogenic compounds		
	Water ng/L	<i>P. crispus</i> ng/L	<i>P. illinoensis</i> ng/L
1	41.4 ± 0.6	15.7 ± 0.9	9.7 ± 1.2
2	37.7 ± 0.8	14.7 ± 0.7	7.6 ± 0.6
3	34.0 ± 0.3	12.5 ± 0.4	7.1 ± 0.8
4	30.5 ± 1.1	11.2 ± 0.2	6.0 ± 0.5
5	28.5 ± 0.8	9.1 ± 0.3	6.7 ± 0.8
6	26.3 ± 0.5	8.7 ± 0.7	6.0 ± 0.9

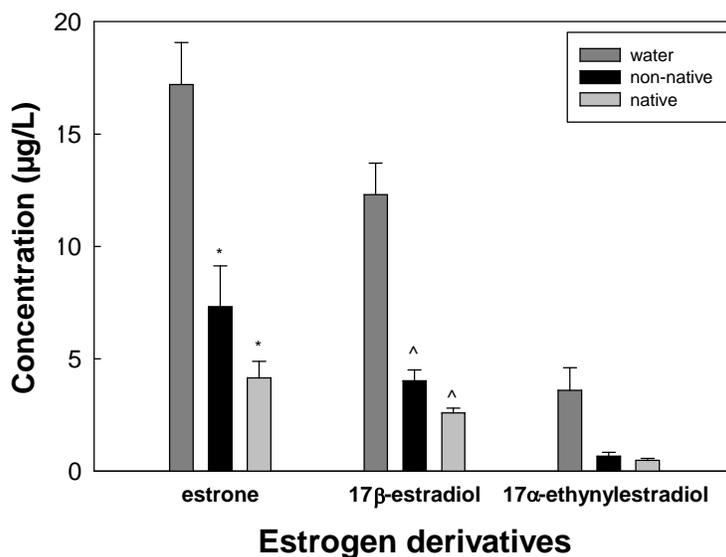


Figure 1. The non-native Pondweed sequesters higher amounts of estrogen derivatives in biomass. The non-native, *P. crispus*, contained significantly more estrone. * (P=0.009) and 17 β-estradiol[^] (P <0.001) than the native *P. illinoensis*.

Bisphenol-A

The concentration of Bisphenol A present in the Des Plaines River water varied from 2.6-3.8 µg/g. *P. crispus*, the invasive Pondweed, contained an average of 5.6 µg/g of BPA whereas the native Pondweed contained an average BPA concentration of 2.9 µg/g (Figure 2). At each sampling location 3 full plants were collected. The amount of BPA varied from 3.3-8.5 µg/g and 1.4-5.2 µg/g across sites for *P. crispus* and *P. illinoensis*, respectively (Table 2). We did observe higher levels of BPA in plants closer to the sewage input point source location, with decreasing concentrations down river (Table 2). However, the concentration of BPA in the river water was significantly less (P=0.02) than the concentration of BPA in the invasive Curly-leaf Pondweed (Table 2). The invasive pondweed also held significantly

more BPA per gram than the native Illinois Pondweed (P= 0.01; Figure 2). *P. crispus* plants were 72% larger overall (P=0.003) than the native Pondweed, *P. illinoensis* (Figure 3). At all locations, the invasive species were larger than the native species (Figure 3). Total plant biomass dry weight of *P. crispus* was 56%-100% larger than *P. illinoensis* (Figure 3). *P. crispus* possessed a root density ~two times greater than *P. illinoensis*.

As our sample locations moved further away from the point source of the estrogenic contaminations, the accumulation of BPA by these two pondweeds declined (Figure 3). This is likely caused by the decreasing concentrations of BPA in the river water. It is likely that some pulse events, such as the release of fire retardants (The Huffington Post, 2009), accounted for past spikes in BPA concentrations in this aquatic ecosystem.

Table 2. Bisphenol-A concentrations by sampling location in river water, the non-native species *P. crispus* and the native Pondweed, *P. illinoensis*. The most upstream sample was collected at location 1 while each sample location was ~1km further downstream. Sampling took place ~9km from sewage effluent point sources.

Sample Location	Bisphenol A		
	Water µg/g	<i>P. crispus</i> µg/g	<i>P. illinoensis</i> µg/g
1	3.8 ± 0.5	8.3 ± 0.2	2.9 ± 0.6
2	3.4 ± 0.8	8.1 ± 0.4	3.8 ± 0.9
3	3.2 ± 0.3	4.8 ± 0.5	3.2 ± 0.3
4	2.6 ± 0.3	4.6 ± 0.5	3.0 ± 0.4
5	3.3 ± 0.6	4.0 ± 0.2	2.4 ± 0.2
6	2.9 ± 0.5	3.7 ± 0.3	1.9 ± 0.3

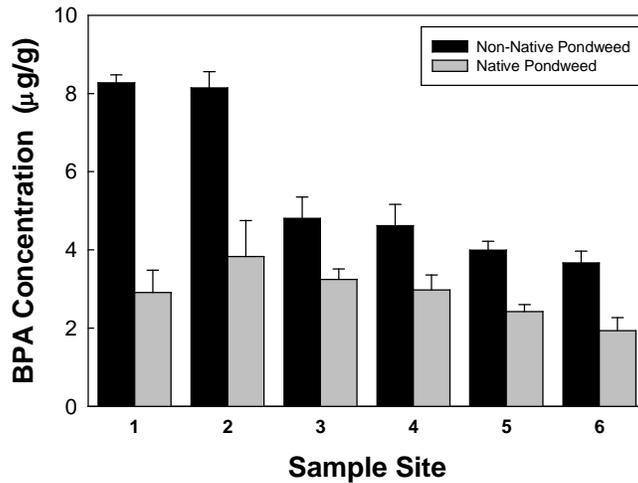


Figure 2. Uptake of Bisphenol-A was greater in the Non-native pondweed. The non-native, *P. crispus*, contained significantly more BPA ($P=0.01$) than the native *P. illinoensis*. The most upstream sample was collected at location 1 while each sample location was ~1km further downstream. Sampling took place ~9km from sewage effluent point sources.

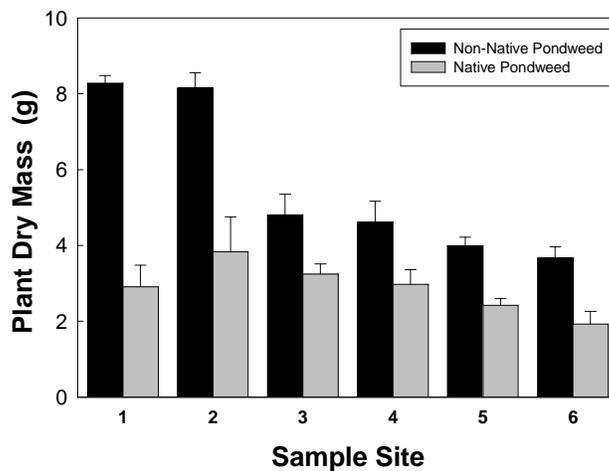


Figure 3. Dry mass of native and non-native Pondweed. The non-native, *P. crispus*, was significantly larger ($P=0.003$) than the native *P. illinoensis*. The most upstream sample was collected at location 1 while each sample location was ~1km further downstream. Sampling took place ~9km from sewage effluent point sources.

Discussion

Many environmental scientists are using the natural properties of plants to absorb and take up chemicals. Plants have very different strategies when it comes to uptake of nutrients, chemicals and metals (Tsao, 2003). Therefore, it is very important that manager's select the appropriate plants to remove specific pollutants. Certain plant species possess the ability to stabilize chemicals, whereas other plants may transform or concentrate the pollutants in specific plant tissues (Tsao, 2003). We found that the pondweed species did remove estrogen derivatives from the river water, but at very low concentrations. Studies have found that estrogen concentrations in surface waters can vary from 0-100 ng/L (reviewed in Song et al., 2009). The concentrations of estrogenic compounds in the Des Plaines River had an average total concentration of ~33ng/L. Both pondweed species had concentrations of estrogen lower than the river water, suggesting that they were not bioaccumulating any of the estrogen derivatives. The invasive *P. crispus* did sequester and average of 66% more estrogenic compounds than the native *P. illinoensis*. The *Potamogeton* species did take up small amounts of estrone 3-10 ng/L, 17 β -estradiol 2-5 ng/L and 17 α - ethynylestradiol 0.3-1 ng/L. However, our species showed relatively low uptake of estrogen derivatives when compared to other studies (i.e. Song et al., 2009) who have successfully removed 40-70% of estrogenic compounds from sewage effluent water using a native reed species. Therefore, we do not recommend the use of *Potamogeton* species for the removal of estrogen derivative compounds from aquatic ecosystems. In contrast, these plants appear to be successful at sequestering the xenoestrogen BPA.

In considering the characteristics necessary for phytoremediation, removal capability plays a significant role. In this study, pondweed was chosen as a model, comparing the ability of the non-native *P. crispus* and native *P. illinoensis* to accumulate EDCs. Comparing the native and non-native pondweed species, the non-native *P. crispus*, had up to three-fold greater accumulation of BPA. This sequestration of BPA is similar to the bioaccumulation potential of *Dracaena sanderiana* (Saiyood, 2010). The mass of these pondweeds and the associated BPA load suggests that these plants may accumulate BPA, a xenoestrogen. When assessing plants to determine their potential as phytoremediators, there are many factors to consider. Particular plant species are better at

removing and sequestering specific contaminants to certain tissues. For example, some fruit species have been shown to accumulate BPA and PCBs in their fruit (i.e. Kang et al., 2006; Zeeb et al., 2006). Studies have shown that examining the symbiotic associations of the plants with various bacteria and fungi may be important when determining the uptake capacity of phytoremediators as well.

The increased biomass of *P. crispus* and the larger roots on these plants may increase the surface area necessary for absorbing and assimilating contaminants. Moreover, the roots are sites of microbial attachment, where increased oxygen concentrations and excreted nutrients like carbohydrates symbiotically support microbial populations. In return, these microorganisms are capable of xenoestrogen degradation (Saiyood, 2010). The higher root density found in the *P. crispus* species allows a greater proportion of contact with microbial populations, which may facilitate greater microbial degradation and accumulation of estrogens (Bezbaruah and Zhang, 2004). This indicates that the larger mass of the non-native pondweed may carry an advantage in improved uptake of EDCs and improved recruitment of microbial populations responsible for EDC degradation. Considering its mass and accumulation of EDCs, the non-native pondweed is a significantly better phytoremediator and better adapted to the conditions presented in the Des Plaines River. Further research should be completed to determine how EDC sequestration may be enhanced by specific relationships between plants and their symbiotic bacterial and fungal colonies.

Successful phytoremediation is often the best way to remove nutrients and pollutants from aquatic ecosystems in a safe and cost effective manner. When using macrophytes such as the invasive curly-leaf pondweed or other alien species, it is essential that all of the macrophytes be successfully removed after remediation. Should the plants be left in place, the vast majority of the nutrients and pollutants that were sequestered in plant tissue will be returned to the water as the plants undergo decomposition. This study suggests that utilizing invasive species to remove pollutants may be a viable option, as long as the invasive species is confined to a target area and can be successfully removed after remediation.

Conclusion

Many ecologists recommend removal of exotic and/or invasive species from ecosystems. Our work shows that while non-native species may not be

desirable in a healthy ecosystem, they may serve a purpose in environmental remediation. Many urban aquatic ecosystems are plagued with endocrine disrupting chemicals and pharmaceutical and personal care products which may upset the natural balance of aquatic species. Further research into the effect of these anthropogenic chemicals on native and non-native macrophyte species should be completed to determine which species may help to mitigate and/or remediate anthropogenic pollutants.

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