SUSCEPTIBILITY OF RIPARIAN WETLAND PLANTS TO PERFLUOROOCTANOIC ACID (PFOA) ACCUMULATION

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Abstract

As plants have been shown to accumulate organic compounds from contaminated sediments, there is a potential for long-lasting ecological impact as a result of contaminant accumulation in riparian areas of wetlands, particularly the accumulation of non-biodegradable contaminants such as perfluorooctanoic acid (PFOA). In this study, commonly found riparian wetland plants including reeds, i.e., *Xanthium strumarium, Phragmites australis, Schoenoplectus corymbosus, Ruppia maritime; Populus canescens, Polygonum salicifolium, Cyperus congestus; Persicaria amphibian, Ficus carica, Artemisia schmidtiana, Eichhornia crassipes*, were studied to determine their susceptibility to PFOA accumulation from PFOA contaminated riparian sediment

with a known PFOA concentration, using liquid chromatography/tandem mass spectrometry (LC/MS/MS). The bioconcentration factor (BCF) indicated that the plants affinity to PFOA accumulation was; *E. crassipes, > P. salicifolium, > C. congestus, > P. x canescens, > P. amphibian, > F. carica, > A. schmidtiana, > X. strumarium, > P. australis, > R. maritime, > S. corymbosus.* The concentration of PFOA in the plants and/or reeds was in the range 11.7 to 38 ng/g, with a BCF range of 0.05 to 0.37. The highest BCF was observed in sediment for which its core water had a high salinity, total organic carbon and a pH which was near neutral. As the studied plants had a higher affinity for PFOA, the resultant effect is that riparian plants such as *E. crassipes, X. strumarium,* and *P. salicifolium*, typified by a fibrous rooting system, which grow closer to the water edge, exacerbate the accumulation of PFOA in riparian wetlands.

KEY WORDS: Perfluorooctanoic acid (PFOA); Perfluorinated compounds; wetland plants; reed grass; Bioconcentration factor (BCF).

INTRODUCTION

Perfluorinated compounds (PFCs) are one of several persistent organic pollutants (POPs) found in the environment as a result of pollution from anthropogenic activities (Quante et al. 2011; Yang et al. 2011). PFCs are used in aqueous fire fighting foams, while their lipid-and-water-repellent properties serve as stain repellents on carpets, textiles, leather, home furnishing, paper products, non-stick cookware and cleaning products (Prevedouros et al. 2006; Ahrens et al. 2009). For example, PFOA is used as an emulsifier and a surfactant in mining and oil well drilling (EFSA, 2008). Consequently, numerous studies have reported PFCs in the natural

environment, and like their predecessors, PFCs bioaccumulate in the environment and cause ecological degradation due to their adverse effects on biota (Chen et al. 2012; Calafat et al. 2006; Karrman et al. 2006; Kelly et al. 2009; Liu et al. 2009; Quinete et al. 2009; Rumsby et al. 2009; Suja et al. 2009; Naile et al. 2010; Pan et al. 2010; Shi et al. 2010). Perfluorooctanoic acid (PFOA) is one of the most prevalent and studied PFCs and as a result, have been detected in aquatic matrices such as river sediment (Pico et al. 2012), marine animals (Quinete et al. 2009) and plants (Stahl et al. 2009; Lechner and Knapp 2011), with PFOA being the predominant bioaccumulative organic chemical in plants (Lechner and Knapp 2011). Although, several treatment strategies have been implemented to bio-remediate contaminated matrices, a new remediation approach involves the use of artificial wetlands in which aquatic and/or riparian wetland plants are used for the removal of PFCs from either contaminated water or sediment (Chen et al. 2012), suggesting that some of the common wetland plants have an affinity to accumulate PFCs including PFOA, thus susceptible to bioaccumulate PFOA in the natural environment. Several studies have reported that wetland plants accumulate organic pollutants such as atrazine, chlortoluron, chloroacetanilide, nitro-glycerine (Cherian and Oliveira 2005; Pilon-Smits and Freeman 2006; Reinhold et al. 2010). Marchand et al. (2010) concluded that the accumulation of pollutants depends on the type of wetland, riparian area, sediment core water characteristics and the rooting system of individual plants. Furthermore, the carryover of PFOA from contaminated soil to plants varies, depending on the concentration of the contaminant in the sediment (Stahl et al. 2009); including the total area of transfer as a function of the rooting system of each plant (Schachtschneider et al. 2010).

Therefore, the aim of this study was to determine the susceptibility of commonly found riparian wetland plants and reeds exposed to natural environmental effects to PFOA accumulation from known PFOA concentration in riparian wetland sediment. Thus far, most studies have utilised controlled laboratory studies, which in effect do not represent natural environmental conditions, particularly for studies which focus on the bioaccumulation of PFOA.

MATERIALS AND METHODS

Chemical reagents

All reagents used in this study were of analytical grade standard. Methanol and analytical standards of PFOA were purchased from Sigma-Aldrich (St. Louis, MO, USA) and stored at -20°C with appropriate dilutions being used to prepare required concentrations when required. Sodium Hydroxide was purchased from Saarchem (Wadeville, South Africa).

Sample collection/sites and plants/reeds grass

Plant samples used in this study were randomly collected from three Western Cape rivers, namely the Diep, Eerste and Salt Rivers (Fig. 1). The three rivers were selected as they are, 1) part of the three biggest catchments areas in the Western Cape with a large surface water network (Ogutu 2007); 2) have a similar mean annual runoff range of 1305 to 2085e10^6 m^3 (Midgley et al. 1990) and several tributaries; and 3) have similar soil/sediment characteristics, i.e., structured with a sandy soil which facilitates subsurface accumulation of organic matter, and thus contaminant accumulation. For consistency, the location co-ordinates of each sampling site were recorded using a Global Positioning System (GPS) and were, for Eerste River: Lat: S34°01' (49.9 to 51.1"), Long: E18°44' (51.8 to 52.1); for Diep river: Lat: S33°49' (47.4" to 53.3''),

Long: E18°31' (13.2" to 14.9"); and for Salt River (Lat: S33°56' (04.6" to 59.9"), Long: E18°28' (48.3" to 89.2"). The plant species randomly selected were: for Diep River: *X. strumarium*, *P. australis*, *S. corymbosus*, and *R. maritime;* for Eerste river: *P. x canescens, P.salicifolium,* and *C. congestus;* and for Salt river: *P. amphibian, F. carica, A. schmidtiana* and *E. Crassipes*. Thus, using a stainless scissors, vegetative compartments (leaves) of each of the plants were harvested and transferred into methanol pre-rinsed polypropylene (PP) bottles.

Although some of the species sampled are identified as alien vegetation species in South Africa, their prevalence remains high in the area under investigation and they are commonly found in riparian wetland areas of major national and international rivers. All the sediment samples were collected using methanol rinsed 2L PP bottles, and a stainless AMS Multi Stage Sediment Sampler (AMS Samplers, USA), up to a depth of 40 cm below the sediment surface, 80 cm from the shoreline. The riparian water level from where the sediment samples were collected was at 30 cm depth. The core water characteristics were determined using an YSI multi-function probe (YSI, USA) to quantify salinity and pH, while the sediment characterisation, i.e., granulometry/classification, was performed using the American Society for Testing and Materials method (ASTM) coupled with the Unified Soil Classification System (USCS) chart. The Loss-On-Ignition (LOI) method, to quantify the percentage total organic carbon (%TOC) involved the thermal destruction of organic matter in the sediment at approximately 950°C without the addition of accelerants (Heiri et al. 2001).

Solid Phase Extraction and Sample cleanup for PFOA quantification

Prior to analysis, plant sample pre-treatment was performed using the method described by Quinete et al. (2009) and adapted by Stahl et al. (2011) with minor changes. Prior to processing, both the plant and sediment samples were oven dried for 24h, at 60°C. Thereafter, plant samples were milled into a powder form while sediment samples were used without screening. Subsequently, 2 g from each of the dry samples, plants and sediment, were transferred to a clean 15 mL PP centrifuge tube to which 5 ml Milli-Q water was added for plant samples while for sediment samples, 10 ml of a 1% (v/v) acetic acid solution was used, homogenizing all samples using a high powered piston probe sonicator (Sonics, vibra-cell sonicator, 20 KHz ± 50 Hz) for 1 min, at ambient temperature. The probe was rinsed with analytical grade methanol between sample treatments to reduce cross-contamination. Subsequent to sonication, 1 ml of sonicated plant extracts were transferred into a second clean 15 ml PP centrifuge tube to which an additional 11 ml of 0.01N NaOH/MeOH solution was added. The mixture of the plant extracts and 0.01N NaOH/MeOH solution was then sonicated for 1 min, after which all samples for both plants and sediment extracts were centrifuged at 3,000 rpm, for 4 min. The recovered acetic acid-based sediment extracts/supernatant was decanted into an empty 50 ml PP tube. An aliquant of 3 ml of a 90:10 (v/v) methanol and 1% (v/v) acetic acid mixture was then added to the original tube to resuspend the sediments and the contents were again sonicated for 1 min, before being centrifuged and decanted into the second PP tube with the extracts from the first centrifugation cycle. This process was repeated for sediment samples using a 10 ml acetic acid (1% v/v acetic acid) solution. All the recovered supernatants from both plant and sediment extracts without debris were filtered using PP 0.22 µm, Cameo syringe filters (Sigma Aldrich). A volume of 10

ml (plant extracts) and 25 ml (sediment extracts) was used for the Solid Phase Extraction (SPE) process.

The SPE process was performed using a method similar to the one proposed by So et al. (2004, 2006a) for which a Supelco-Select HLB SPE cartridges (500 mg solid phase, 12 ml tubes) were used. The process was used taking in to consideration recommendations from the ISO 25101 (2009) and OSPAR (2010), whereby the cartridges were preconditioned by eluting 5 ml of methanol and then 5 ml of Milli-Q water at a flow rate of 1-2 drops per second. The solid phase was then kept moist in order to obtain optimum extraction. Thereafter, the plant and sediment extracts were eluted at a flow rate of 1-2 drops a second. All cartridges used were then washed with 5 ml of a 40% (v/v) solution of methanol in Milli-Q water, as reported by Naile et al. (2010), to ensure that contaminants were washed-off from the solid phase. Subsequently, the cartridges were allowed to run dry while being kept cool. The recovered filtrates were then discarded, before the use of analytical grade methanol for the recovery of PFOA from the SPE cartridges. The cartridges were eluted with 10 ml methanol, after which nitrogen gas was used to further reduce the volume of methanol/PFOA eluents collected to 1 ml for LC/MS/MS analysis.

Analytical instrument parameters for PFOA quantification

A liquid chromatography (LC) system (Nexera UHPLC, LC/MS-8030, Shimadzu, Japan) coupled with a tandem mass spectrometer (MS) to determine PFOA concentration in each of the samples was used. A volume of 10 µl from the concentrated eluents was injected into the instrument for a total run time of 6.5 min. The mobile phase constituents were 100% Acetonitrile (ACN) and 2mM Ammonium acetate (NH_4OAc) at a flow rate of 0.3 mL/min. The gradient used

was; t = 0.01 min, 2% ACN; t = 4 min, 98% ACN; t = 6 min, 98%; which was reduced to 2% ACN thereafter. The separation column (Shimpack FC-ODS, 150 x 2 mm, 3.0 µm; Shimadzu, Japan) was maintained at 40°C. Quantitative analysis was performed in the multiple reaction monitoring mode and the collusion gas was used at an energy of 10v (PFOA).

For the mass spectrometer (MS) the transition mode (MRM settings) was 413.10 > 368.90 m/z for PFOA. The MS was operated employing electrospray ionisation (ESI) in a negative mode. The ionization source-specific parameters were; nebulizer gas at a flow rate of 3 L/min and dry gas at a flow rate 15 L/min; temperature of the heat block was 400°C, while the DL temperature was 250°C. After the processing of each sample, a methanol rinse was performed to limit cross contamination after each injection. For calibration standards, the concentration of 0; 0.5; 1; 5; 10 and 50 ng/L for PFOA was used, achieving a correlation co-efficient (R^2) of 0.99 for each run. The limit of detection (LOD) was 0.5 ng/g for PFOA for extracts used in the analysis, at an S/N ratio ≥ 3, while the recovery of PFOA was > 70% using multiple injections. The mean of two injected samples was used for every sample processed. From each of the sampling sites, PFOA concentration was quantified in the sediment and therefore known, with an average of six sediment samples used for each of the sampling sites, with duplicate samples for each of the plants and reeds used in the analysis. A similar PFOA concentration was observed in the sediment and core water samples.

Results and Discussion

PFOA concentration in different riparian wetland plant/reed species

It has been previously indicated that the uptake of pollutants from soil to plants dependents on the type of plants used in the sorption of organic pollutants (Marchand et al. 2010). Furthermore, the sorption of a particular contaminant is highly dependent on its chemical structure and concentration (EPA 1990). Some plant species showed better pollutant accumulation potential than others (Schachtschneider et al. 2010). However, according to the reviewed literature, studies on PFCs uptake by plants remain meagre (Yoo et al. 2011).

In this study, the susceptibility of eleven commonly found riparian wetland plants and reeds to PFOA accumulation was investigated. PFOA was observed in all the species studied, from all the three rivers under observation. Overall, plant accumulation of PFOA was higher in *E. crassipes* (38.3 ± 1.2 ng/g) species from the Salt River, followed by *X. strumarium* species (20.0 ± 0.17 ng/g), from the Diep River, and then *P. salicifolium* species from the Eerste River (17.5 ± 4.56 ng/g). The current study was conducted on plants taken directly from a natural and riparian environment. The results showed higher uptake of PFOA compared to the results reported by other researchers who studied PFOA uptake by plants obtained from constructed wetlands (e.g. Stahl et al. 2009; Lechner and Knapp 2011; Chen et al. 2012; Yoo et al. 2011). In the present study, the observed high uptake of PFOA by the plants was attributed to the high levels of PFOA in the sediment. The average (n = 6) PFOA concentration found in the sediment in the present study were 104.6 ± 1.98 ng/g, 297.5 ± 7.15 ng/g; and 95.0 ± 3.71 ng/g for Salt, Diep and Eerste_Rivers respectively. This trend was also observed by Stahl et al. (2009) whose study indicated that the higher the concentration of PFOA in the sediment, the higher the pollutant accumulation in the plants, particularly the vegetative parts of the plants. Concentration uptake of PFOA (8.9 ± 0.6 and 9.8 ± 2.1 ng/g) by Kentucky bluegrass (C9) and Bermuda grass

(C10), respectively (Yoo et al. 2011) have also been reported. The lowest concentration of PFOA was found in the following plant species; *S. corymbosus* (13.8 ± 0.6 ng/g), *C. congestus* (13.7 ± 0.52), *P. amphibian (*13.4 ± 0.22), *P. x canescens* (13.3 ± 1.44 ng/g), *F. carica* (12.5 ± 0.19) and *A. schmidtiana* (11.7 ± 0.6 ng/g). This represented a significantly higher PFOA concentration in the sediment in comparison to that found in the plants.

Sorption capacity as a function of bioconcentration factor (BCF), root system and sediment characteristics

The bioconcentration factor (BCF), which is a ratio between the concentrations of the contaminant in the plant in comparison to that in the sediment, can be represented as either a fraction or a percentage, to quantify the bioaccumulation of the contaminant in the plants. Furthermore, as the rooting system can have an influence on the uptake rate and persistence of a contaminant in the vegetative parts of the plants under evaluation, it was imperative to also have analysed the rooting system for each of the plants. Thus, the root system characterization was based on observation. For the studied riparian plants, the %BCF, PFOA concentration including the rooting systems for each of the plants, are summarized in Table 1. The %BCF for the different plant species was; 6.7% (*X. strumarium*), 5.2% (*P. australis*), 4.8% (*R. maritime*) and 4.6% (*S. corymbosus*) for the Diep River. For the Eerste River, the percentages were 18.4% (*P. salicifolium*), 14.4% (*C. congestus*) and 14.0% (*P. x canescens*); while for Salt River it was 36.6% (*E. crassipes*), 12.8% (*P. amphibian*), 11.9% (*F. carica*) and 11.1% (*A. schmidtiana*). The results obtained in this study concur with results previously obtained by Yoo et al. (2011), Marchand et al. (2010), and Stahl et al. (2009) which indicated that, PFOA was readily absorbed

by various plants. Since plant roots are an important part for the direct uptake of organic compounds from different sediments with varying pollutant concentrations in the riparian areas (Bell 1992; E.A., 2006), plant species with fibrous roots seemed likely to have a higher, but inconsistent, %BCF for PFOA in comparison to those with a tap root system.

The sediment from all the three rivers was classified as poorly graded and/or gravelly with most of the sediment being retained by sieve no's 10 to 400 (2000 to 106 µm grain size). Therefore, the retainment of PFOA would have been minimal. In this study, core water quality parameters such as pH, salinity (mg/L), and %TOC were also analysed. Some of the Salt River's plants, particularly *E. crassipes,* were observed to have the highest %BCF, indicating that this plant had the highest accumulated PFOA concentration of 38.3 ± 1.2 ng/g, in comparison to all the other species evaluated. The top soil in the sediment of the Salt river had the highest %TOC associated with plant decay, observed in-situ, which can have a bio-augmentation effect in the sediment, which can facilitate the uptake rate of organic contaminants into the plants thus their accumulation(Theobald et al. 2012) . From the results, it was also clear that the frequent submersion of some of the plants may result in higher sorption capacity of PFOA. Furthermore, although the Diep River had a higher PFOA in the sediment, it was likely that the salinity and a higher pH resulted in reduced uptake of PFOA into the plants. Generally, there is limited knowledge about the effect of soil ecological parameters such as bio-augmentation, including eutrophication, on organic chemical sorption and accumulation in riparian wetland plants. Since it was expected that a high %BCF was to be observed in species obtained from the Diep River, which had the highest average PFOA concentration in the sediment (297.5 ± 7.15 ng/g) the observed results suggested that other parameters might have had a significant role, even for *S.*

corymbosus species with a fibrous rooting system in comparison to that of *E. crassipes*, which had the highest PFOA accumulation, a phenomenon attributed to the plant's growth site preference of individual plants. In this case, plants such as *E. Crassipes* which prefer to grow in constantly submerged areas might have had a high PFOA accumulation, which suggests that PFOA contaminated water is more likely to cause increased the uptake of PFOA, than polluted sediment.

Highly saline environments can decrease the absorption of long-chain organic compounds with a complex structure, as plants' rooting system absorbs as solutes via osmosis, thus, having a saline environment would have resulted in a negative osmosis potential (Dogar et al. 2012), with plant species having a higher affinity to other solutes with a simpler chemical structure than PFOA. Other species considered to be alien species in the South African environment, e.g., *P. salicifolium*, tended to have had a higher affinity for PFOA, however, their presence in the environment should be carefully monitored as the rooting system of these species is regarded as water-hungry, which can further increase the sorption of organic pollutants from the sediment core water. Additionally, the limited sorption of PFOA by plants with a tap root system such as *P. Australis* and *P. x canescens* indicated their limited biosorption capacity for PFOA. These plants generally grow further away from the water edge, with less submersion frequency.

Although, wetland riparian plants can be used as biological remediation agents for chemically contaminated sediments in constructed wetlands (Hellström 2004), the contamination of water sources and riparian wetlands in an environment in which these plants are prevalent, would suggest that an escalation of the ecological impact of the contaminants would have been

magnified thus further endangering wetland eco-systems and the food chain associated with the contaminated wetlands. From an environmental perspective, the results observed in this study indicated that the accumulation of PFOA by the wetland plants under observation was largely higher than any other wetland plants from previous studies (Yoo et al. 2011; Stahl et al. 2009). Since other studies use laboratory grown plants, which negate environmental factors including seasonal variations (Mudumbi et al. 2012), this study presents an assessment of PFOA accumulation in riparian wetlands, which in-turn will contribute to the effective management of wetlands in which PFOA is a major pollutant.

Conclusion

There is limited information about riparian wetland plants including reeds susceptibility to PFOA accumulation. In this study, PFOA was observed in all the eleven plant species randomly collected from riparian areas with *E. crassipes* having the highest bioconcentration factor in comparison to others. Although the study was conducted in riparian areas with a high PFOA concentration, the resultant higher sediment PFOA contamination was not directly proportional to a high plant PFOA absorption and/or accumulation. Wetlands are the main sources of water and they support ecological systems in riparian areas, thus the susceptibility of some of the wetland plants to pollutant accumulation will have a negative effect in the food chain of the riparian areas. Additionally, for riparian areas with sediment core water with less saline properties, plants and reeds with a fibrous rooting system had a high susceptibility to PFOA accumulation in comparison to those growing in saline areas. These results can, therefore,

contribute to the establishment of a database for monitoring the accumulation of PFCs, in particular PFOA, for riparian vegetation.