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Urban Insecticides in Surface Water: Runoff Source Identification and Large-Scale Constructed Wetland Treatment

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Urban Insecticides in Surface Water: Runoff Source Identification and Large-Scale Constructed Wetland Treatment

A Dissertation submitted in partial satisfaction
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in

Environmental Toxicology

by

Zachary Michael Cryder

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ABSTRACT OF THE DISSERTATION

Urban Insecticides in Surface Water: Runoff Source Identification and Large-Scale Constructed Wetland Treatment

by

Zachary Michael Cryder

Doctor of Philosophy, Graduate Program in Environmental Toxicology
University of California, Riverside, June 2020
Dr. Jay Gan, Chairperson

Fipronil and pyrethroids are insecticides applied at high rates in urban environments for indoor and outdoor elimination of pests such as ants, termites, roaches, and fleas. Applied residues of fipronil and its degradation products (hereafter: fiproles) as well as pyrethroids are easily transported offsite in surface runoff and wastewater. In surface water, fiproles and pyrethroids elicit toxicity in non-target aquatic and benthic organisms. Failing to alleviate this pollution will result in ecological impacts and as-yet-unknown human health impacts following reuse of scarce water resources. This dissertation addresses the need to reduce urban insecticide contamination of surface water by investigating urban runoff sources of fiproles and examining the comparative efficacy of a full-scale vegetated constructed wetland and a unit-process open water constructed wetland in treating surface water contaminated with fiproles and pyrethroids. If the sources of fiproles in urban surface runoff are identified, it will be possible to modify pesticide application practices to mitigate surface water contamination by these pollutants. Therefore, runoff, soil, urban dust, and concrete wipe samples were collected from fipronil-treated homes to identify the contributions of these compartments to urban runoff of fiproles. Concrete surfaces and urban dust were shown to be the dominant contributors of fiproles to surface runoff, indicating that limiting application on these matrices would substantially reduce contamination. In addition to mitigating urban insecticide pollution, it is also necessary to uncover strategies to treat contaminated water bodies. To this end, water, sediment, and plant samples from a vegetated constructed wetland were monitored for fiproles and pyrethroids. All contaminants were effectively treated by the wetland via partitioning into sediment and subsequent degradation as well as uptake by or adsorption to aquatic macrophytes. To compare the effect of constructed wetland design on removal of fiproles and pyrethroids, water and sediment samples were also collected from a unit process open water constructed wetland. Despite the absence of macrophytes, this system was still effective for contaminant removal. Overall, this research highlights the feasibility of combining preventative mitigation strategies with more resource-intensive treatment strategies to attain the goal of reducing urban insecticide contamination in surface water.

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Chapter 1 Introduction

1.1 Background

Modern pesticides are utilized for the management of a vast array of pest species spanning multiple taxonomic groups—including insects, weeds, fungi, and rodents—in agricultural and urban environments. Of particular interest to this research are urban-use insecticides, which are applied in indoor and outdoor settings to eliminate a variety of nuisance insects such as ants, termites, roaches, and spiders. Such organisms, if not managed, have the potential to compromise the integrity of urban structures, damage landscape features, and present risks to human health. High rates of urban insecticide use occur in densely populated regions like Southern California with stable climates that allow pest populations to thrive for the majority of the year. Indoor applications include the use of consumer insecticide sprays for direct pest elimination, bait stations, and veterinary medications for domestic animal parasites such as fleas. In addition, urban insecticides are commonly applied outdoors for structural pest control and landscape protection.

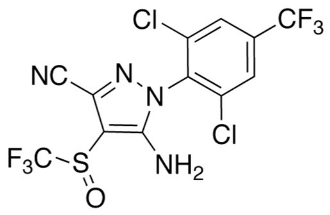
Urban insecticides are an environmental concern due to their offsite transport following outdoor and indoor use. Rainfall and irrigation mobilize applied insecticide residues in outdoor settings, allowing for runoff transport to surface water where these contaminants can exert adverse effects on sensitive aquatic organisms (Gan et al., 2012; Weston et al., 2013; Weston and Lydy, 2012, 2014). In addition, insecticides may enter wastewater as a result of indoor use and subsequent washing down the drain, or as a result of high-volume stormwater runoff flowing into sewers, and eventually reach surface waterways via deposition of treated wastewater effluent (Sadaria et al., 2017; Weston et al., 2013). The high incidence of impervious surfaces in urban environments facilitates the runoff transport of insecticides applied outdoors: approximately 50% and 90% of residential and commercial surface areas, respectively, are comprised of impervious surfaces (Arnold and Gibbons, 1996). Predevelopment conditions allow for infiltration of water into soil, but the implementation of impervious surfaces in urban environments has inadvertently compromised water quality by largely preventing runoff infiltration and, consequently, contaminant retention. Runoff transport of urban insecticides via impervious surfaces will only increase with the anticipated tripling of the worldwide urban land area from the early 2000s to the year 2030 (Seto et al., 2012). Furthermore, the human population is expected to experience rapid growth particularly in urban areas, a trend which will likely lead to a concomitant increase in the indoor use of insecticides (McDonald et al., 2011). Therefore, insecticide washoff into sewage systems following higher rates of indoor use will increase the loading of these compounds into wastewater, while a greater prevalence of impervious surfaces will enhance the transport of insecticides in runoff as a result of outdoor use. Greater insecticide contamination of surface water via these transport pathways will increase the risks presented to sensitive aquatic organisms and exacerbate the scarcity of high-quality freshwater resources that is expected to intensify due to the effects of global climate change (Kummu et al., 2010; McDonald et al., 2011).

Fipronil and the synthetic pyrethroids are among the most commonly utilized urban insecticides on the market. These compounds presented a solution to the persistence of organochlorine insecticides as well as the toxicity posed by organophosphate and carbamate insecticides to aquatic life and humans alike (Gan et al., 2012; Weston et al., 2005, 2009). Fipronil and the synthetic pyrethroids are frequently more effective than the compound classes that they replaced, degrade more readily than organochlorine insecticides, and are less acutely toxic to mammals than their organophosphate counterparts (Brander et al., 2016; Elliot et al., 1978; van den Berg et al., 2012). These properties ensured that fipronil and the synthetic pyrethroids were widely adopted in urban environments. The high use rates of these insecticides in urban environments make them ideal model compounds for investigation of the pollution mitigation strategies and water treatment initiatives that are necessary to reduce the risks urban insecticides present to aquatic organisms and to secure quality freshwater supplies.

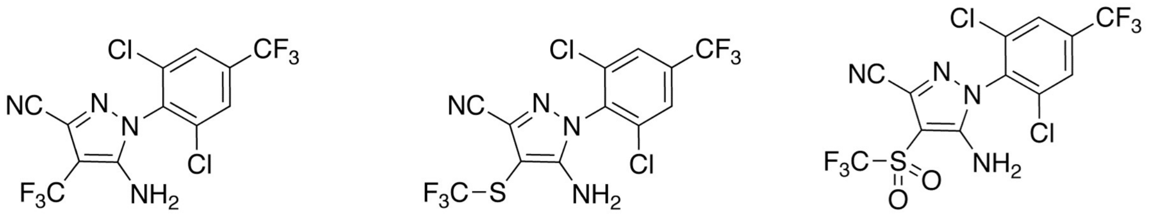
1.2 Fiproles

1.2.1 Properties and Use

Fipronil is a phenylpyrazole insecticide whose outdoor use is restricted to licensed pest management professionals (Figure 1.1). In California, application of fipronil formulations is limited to urban environments where population density is at its maximum, particularly in the southern portion of the state. Despite the lack of agricultural fipronil applications in the state of California, this compound is applied for myriad urban pest control scenarios, including perimeter treatments to manage structural pests such as ants and termites, underground foundation injections, topical flea and tick treatments for pets, impervious surface crack and crevice gel treatments, insect control



Fipronil



Fipronil Desulfinyl Fipronil Sulfide Fipronil Sulfone

Figure 1.1: Structures of fipronil and its primary degradation products.

baits, and landscape maintenance (Ensminger, 2014; Sadaria et al., 2017; Teerlink et al., 2017; United States Environmental Protection Agency, 2011). According to the California Department of Pesticide Regulation's Pesticide Use Reporting database, over 15,000 kg of fipronil active ingredient (a.i.) were applied in the state of California in 2017 (https://calpip.cdpr.ca.gov/main.cfm). Fipronil's insecticidal activity is a result of its interaction with γ-aminobutyric acid (GABA)-gated chloride channels in neuron cell membranes, interrupting nervous system signal transmission and causing neural hyperexcitation, paralysis, and death (Aajoud et al., 2003; Bobe et al., 1998; Cole et al., 1993; Gant et al., 1998). The efficacy of fipronil as an insecticide coupled with its high rate of application is what makes it a substantial threat to non-target aquatic organisms following offsite transport. Fipronil degrades in the environment to primarily form fipronil desulfinyl, fipronil sulfide, and fipronil sulfone (hereafter collectively referred to as fiproles) via photolysis, reduction, and oxidation, respectively (Figure 1.1; Gunasekara and Truong, 2007). These degradation products have been shown to be as toxic or more toxic than the parent compound to various aquatic organisms, so it is important to consider their fate and transport in conjunction with fipronil (Chandler et al., 2004; Maul et al., 2008; Schlenk et al., 2001; Weston and Lydy, 2014). Fiproles are moderately hydrophobic, with log K_{OW} values ranging from 3.5-4.8 (Table 1.1; Gunasekara et al., 2007). In addition, fiproles are moderately soluble in water—particularly the parent compound—and possess low vapor pressures, indicating that adsorption to solids likely plays an important role in their aqueous transport and volatilization plays a negligible role in their fate (Table 1.1).

Table 1.1: Properties of fipronil and its primary degradation products.

Compound	Log K_{OW}	Solubility (mg L^{-1})	Vapor Pressure (mm Hg)
Fipronil[a]	3.5	1.9	1.13×10^{-9}
Fipronil Desulfinyl[b]	4.2	0.49	1.77×10^{-7}
Fipronil Sulfide[b]	4.8	0.09	1.79×10^{-8}
Fipronil Sulfone[b]	4.4	0.13	5.8×10^{-10}

[a] Values for fipronil were drawn from Gunasekara et al. (2007)
[b] Values for fipronil degradation products were estimated using United States Environmental Protection Agency's EPI Suite

1.2.2 Occurrence and Transport

The moderate hydrophobicity and water solubility of fiproles allows for their adsorption to urban matrices such as concrete, urban dust, and soil as well as their subsequent transport during runoff events. Previous research has observed that 81.1-96.7% of fipronil mass applied to concrete surfaces was present in the aqueous phase of runoff samples (Jiang et al., 2014). There is also evidence that fipronil's degradation products are primarily freely dissolved in runoff water following washoff from concrete surfaces (Demcheck and Skrobialowski, 2003; Jiang and Gan, 2016). In addition, literature data suggest that fipronil residues remain on concrete long after application, indicating that fiproles are capable of continually transferring to runoff and contaminating surface water (Jiang et al., 2010). Urban dust, which refers to the collection of fine particles residing on surfaces in outdoor urban environments, has also been implicated as an important source of fiproles in runoff. One study of insecticide occurrence in urban dust collected from residential areas observed fiproles in up to 75.5% of samples (Richards et al., 2016). Furthermore, fiproles can become enriched in loose urban dust particles that are easily transported offsite in runoff (Jiang et al., 2016). These findings collectively reveal that runoff transport of fiproles likely results from a combination of contaminated solid mobilization and direct release of residues from solid matrices into the aqueous phase.

Fiproles are often detected in surface water present in urban environments following runoff transport of residues applied outdoors. In a study of runoff collected from storm drain outfalls serving residential areas, median total fiprole concentrations of 204-441 ng L^{-1} and 13.8-20.4 ng L^{-1} were measured for Southern and Northern California sites, respectively (Gan et al., 2012). The same study reported 90th percentile total fiprole concentrations of 338-1169 ng L^{-1} and 62.6-65.3 ng L^{-1} in Southern and Northern California, respectively (Gan et al., 2012). A more recent study, which monitored urban creeks, urban rivers, and storm drain outfalls, detected fipronil sulfide, fipronil sulfone, fipronil desulfinyl, and fipronil in 8%, 63%, 65%, and 75% of samples, respectively (California Department of Pesticide Regulation, 2016). It is apparent that fiproles are transported to urban surface water via runoff with ease, especially in more populated regions like Southern California.

Indoor use of pest control products containing fipronil has resulted in detection of fiproles in wastewater. In a recent study of a municipal wastewater treatment plant (WWTP), fipronil was detected at an average daily concentration of 17-31 ng L^{-1} and 13-21 ng L^{-1} in raw sewage and treated effluent, respectively (Supowit et al., 2016). A regional study of WWTPs in northern California revealed 100% detection frequency of fipronil, fipronil sulfone, and fipronil sulfide in influent and effluent samples (Sadaria et al., 2017). In the same study, fipronil and fipronil sulfone influent concentrations were 8.6-74.9 ng L^{-1} and 1.1-11.9 ng L^{-1}, respectively, while effluent concentrations were 14.3-48.6 ng L^{-1} and 1.1-16.3 ng L^{-1}, respectively (Sadaria et al., 2017). Clearly, conventional WWTPs are ineffective in removing fiproles emanating from indoor pest control product applications.

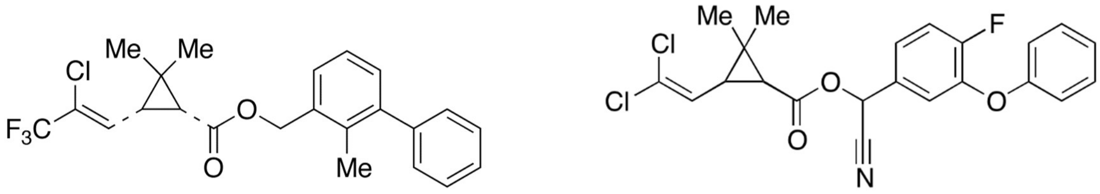
1.2.3 Toxicity

There is a great deal of evidence regarding the toxicity of fiproles to a variety of non-target aquatic organisms. Several studies have documented LC_{50} acute toxicity values for fipronil, with representative values including 320 ng L^{-1} for grass shrimp (*Palaemonetes pugio*), 140 ng L^{-1} for mysid shrimp (*Neomysis americana*), and 180-310 ng L^{-1} for the black fly (*Simulium vittutum*; Key et al., 2003; Overmyer et al., 2005, 2007; United States Environmental Protection Agency, 1996). A particularly sensitive species, *Chironomus dilutus*, exhibited a mean 96 h EC_{50} value (sublethal endpoint: ability to thrash when prodded) of 32.5 ng L^{-1} for fipronil and 7-10 ng L^{-1} for its degradation products (Weston and Lydy, 2014). Since fipronil's degradation products can elicit toxicity that is equal to or greater than the parent compound, it is necessary to include them in this research (Chandler et al., 2004; Maul et al., 2008; Schlenk et al., 2001; Weston and Lydy, 2014). Evidently, sensitive aquatic organisms are at risk of experiencing adverse effects as a result of exposure to fiproles at environmentally relevant concentrations, which may elicit lethality or effects that otherwise compromise their survivability.

1.3 Pyrethroids

1.3.1 Properties and Use

Synthetic pyrethroid insecticides are widely used, by professionals and consumers alike, to combat urban and agricultural pests. Insecticides that belong to the pyrethroid class are utilized for structural pest control and serve as the active ingredients in many consumer pesticide products (Weston et al., 2009). A survey of California home improvement stores conducted in 2010 found that 46% of insecticide products contained at least one pyrethroid (Osienski et al., 2010). In addition, it has been estimated that pyrethroids constitute up to 74% of pesticide use in urban environments (Budd, 2010). Common pyrethroid active ingredients include bifenthrin, cyfluthrin, deltamethrin, esfenvalerate, lambda-cyhalothrin, permethrin, fenpropathrin, and cypermethrin; bifenthrin and cyfluthrin were selected for consideration in this dissertation (Figure 1.2). The Pesticide Use Reporting database states that 150,299 kg of bifenthrin and cyfluthrin a.i. were applied in California for all professional uses in the year 2017 (https://calpip.cdpr.ca.gov/main.cfm). It is important to note that this number does not include consumer applications of bifenthrin and cyfluthrin, despite the fact that pyrethroids are present in many of the insecticide products available for purchase (Weston et al., 2005, 2009). Since pyrethroids are the dominant active ingredients in retail pest control products, the actual urban use of these insecticides is likely much greater (Weston et al., 2013). Pyrethroids exert their toxic effects via interaction with voltage-gated sodium channels in the nervous systems of sensitive organisms, which induces neural hyperexcitation and death (Breckenridge et al., 2009). Pyrethroid insecticides are highly hydrophobic, with log K_{OW} values ranging from 5.7-7.6 (Table 1.2; Laskowski, 2002). In addition, these compounds exhibit low water solubility and relatively low vapor pressure values (Table 1.2). It is therefore likely that the fate of pyrethroids in urban settings is dictated by their hydrophobicity, which ensures that the majority of pyrethroid residues in the environment are found adsorbed to sediments or other solid phases. However, levels of these compounds sufficient to elicit acute toxicity



Bifenthrin Cyfluthrin

Figure 1.2: Structures of select pyrethroids.

in aquatic organisms have been reported in water columns of urban streams and in wastewater effluent.

Table 1.2: Properties of select pyrethroids.

Compound	Log K_{OW}[a]	Solubility (mg L^{-1})[a]	Vapor Pressure (mm Hg)[b]
Bifenthrin	6.4	1.4×10^{-5}	1.8×10^{-4}
Cyfluthrin	5.97	2.3×10^{-3}	2.03×10^{-9}

[a] Log K_{OW} and solubility values were taken from Laskowski (2002)
[b] Vapor pressure values were sourced from the United States Department of Health and Human Services (2003)

1.3.2 Occurrence and transport

The high hydrophobicity of pyrethroid insecticides indicates that they undergo sorption to various solids in urban settings following application, which has a profound effect on their subsequent offsite transport. Concrete surfaces and urban dust particles are of particular importance to the fate of pyrethroids according to previous research. Concrete has been shown to act as a reservoir of applied pesticide residues such as pyrethroids, allowing for gradual desorption during runoff events (Jiang et al., 2012). Another study of insecticides on concrete surfaces found that the washable fraction of pyrethroids applied as liquid formulations rapidly decreased within the first 7 d of the experiment, suggesting irreversible sorption of pyrethroid residues to the porous concrete interior (Jiang et al., 2010). Urban dust has also been reported to play an important role in the urban fate of pyrethroids. A study of residential dust samples detected pyrethroids at higher frequencies than other monitored compounds, with 88% of dust samples containing pyrethroids at concentrations ranging from 5-500 ng g^{-1} (Jiang et al., 2016).

Another study found that more than 80% of pyrethroids present in runoff from concrete surfaces were bound to particles larger than 0.7 µm, and the investigators concluded that pesticide-contaminated particles likely originated from urban dust that was present on concrete surfaces before insecticide treatment (Jiang and Gan, 2012). Overall, these data highlight the importance of sorption to solid surfaces in the offsite transport of pyrethroid insecticides.

Detections of pyrethroids in runoff and surface water from urban environments are quite frequent as a consequence of outdoor applications of these compounds. One study detected pyrethroids in 100% of samples collected from storm drain outfalls (Weston et al., 2009). The same study detected bifenthrin in 96% of samples, with median and maximum concentrations of 5-17 ng L^{-1} and 73 ng L^{-1}, respectively (Weston et al., 2009). The investigators also measured cyfluthrin, permethrin, and cypermethrin at maximum concentrations of 23 ng L^{-1}, 125 ng L^{-1}, and 26 ng L^{-1}, respectively (Weston et al., 2009). Another investigation, which focused on creeks of the American River in northern California, observed maximum concentrations of 106.4 ng L^{-1}, 20.5 ng L^{-1}, 9.4 ng L^{-1}, and 21.1 ng L^{-1} for bifenthrin, cyfluthrin, cypermethrin, and permethrin, respectively (Weston and Lydy, 2012). A recent monitoring study detected bifenthrin, cyfluthrin, and permethrin in 80%, 40%, and 43%, respectively, of samples collected from urban creeks, urban rivers, and storm drain outfalls in Southern California (California Department of Pesticide Regulation, 2016). These observations indicate that outdoor application of pyrethroid formulations results in offsite transport of insecticide residues to surface water.

Pyrethroids have been detected in wastewater following down-the-drain washoff of residues applied indoors or infiltration of contaminated surface runoff into wastewater systems (Weston et al., 2013). A European study of wastewater treatment systems detected permethrin in secondary treated effluent at a concentration of 20 ± 7 ng L^{-1} (Kupper et al., 2006). Another study conducted in Europe measured influent and effluent concentrations of permethrin to be 331 ng L^{-1} and 16 ng L^{-1}, respectively (Turner et al., 2011). A Northern California investigation detected bifenthrin, cyfluthrin, cypermethrin, and permethrin in 39%, 6%, 6%, and 33% of wastewater samples, respectively, with maximum observed concentrations of 6.3 ng L^{-1}, 1.7 ng L^{-1}, 17.0 ng L^{-1}, and 17.2 ng L^{-1}, respectively (Weston and Lydy, 2010). More recently, influent from a municipal wastewater treatment plant in San Francisco, California contained bifenthrin, cypermethrin, and cyhalothrin at concentrations of 10-30 ng L^{-1}, 21-44 ng L^{-1}, and 9-31 ng L^{-1}, respectively (Weston et al., 2013). The corresponding effluent concentrations were 1-5 ng L^{-1}, 12-45 ng L^{-1}, and 1-5 ng L^{-1} for bifenthrin, cypermethrin, and cyhalothrin, respectively (Weston et al., 2013). Although comparison of the aforementioned influent and effluent measurements indicates that municipal WWTPs are effective in reducing pyrethroid concentrations in wastewater, the reductions are insufficient to eliminate toxic effects in sensitive organisms (Weston et al., 2013).

1.3.3 Toxicity

Several studies have demonstrated that pyrethroids are very toxic to sensitive aquatic organisms, especially the amphipod *Hyalella azteca*. Toxicity values for this organism include an EC_{50} (sublethal end point: paralysis) of 3.3 ng L^{-1} for bifenthrin, a 96 h LC_{50} of 21.1 ng L^{-1} for permethrin, and an EC_{50} of 2.3 ng L^{-1} for lambda-cyhalothrin (Anderson et al., 2006; Maund et al., 1998; Weston and Jackson, 2009).These potent toxicity values are an even greater cause for concern in the presence of pyrethroid mixtures, since there is evidence of additive toxicity (Trimble et al., 2009). The occurrence data presented above confirm that multiple pyrethroids are often present in urban surface water, which confirms that mixture toxicity will be vital when considering the risk of pyrethroids. The available evidence indicates that sensitive aquatic organisms are at risk of experiencing adverse effects when exposed to pyrethroid insecticides present in urban waters following transport from their outdoor and indoor sites of application.

1.4 Water Quality Improvement Strategies

Contamination of surface water by urban insecticides such as fiproles and pyrethroids must be addressed to prevent the ecotoxicological impacts that have been documented to occur following their offsite transport as well as potential human health effects caused by reuse of polluted water resources. Mitigation strategies seek to reduce initial contamination of surface water by identifying major causes of pollution and adjusting application methods or disposal practices to minimize offsite transport. In addition, various treatment approaches have been evaluated to improve the quality of stormwater and wastewater before they enter surface water systems. Most types of stormwater treatment have been developed more recently, while wastewater treatment is a well-established process in developed countries as well as in many developing nations.

1.4.1 Stormwater Treatment

Urban stormwater runoff is typically collected in catchment systems and deposited directly into surface water without treatment (McIntyre et al., 2014). However, structures designed to mimic the hydrologic and filtration capacity of predevelopment conditions by slowing and filtering stormwater runoff are known as green stormwater infrastructure (GSI) or low impact development (LID) systems and are increasing in popularity and prevalence (Anderson et al., 2016; DeBusk and Wynn, 2011; McIntyre et al., 2014, 2015). A key metric for assessing the efficacy of these systems is a reduction in the stormwater concentrations of toxic chemicals (McIntyre et al., 2015). Some regions have begun to require the implementation of GSI/LID practices in all new developments while others have instituted financial incentives to encourage developers to install such systems (DeBusk and Wynn, 2011).

Types of GSI/LID stormwater treatment systems include sedimentation basins, retention ponds, bioretention systems, infiltration basins, media filtration systems, bioswales, and constructed wetlands (CWs; Anderson et al., 2016; Haile et al., 2016). Bioswales have been shown to reduce concentrations of several pollutants: a recent study observed reductions of 81%, 81%, 82%, and 74% for suspended solids, metals, hydrocarbons, and pyrethroid insecticides, respectively (Anderson et al., 2016). The same study found that bioswales reduced runoff toxicity to *Hyalella azteca* and *Chironomus dilutus*, the organisms most sensitive to pyrethroids and fiproles, respectively (Anderson et al., 2016). Another study that evaluated a retrofit bioretention cell for the treatment of parking lot runoff observed mass reductions of sediment, total nitrogen, and total phosphorous >99% each (DeBusk and Wynn, 2011). An investigation of experimental soil bioretention columns containing sand, compost, shredded bark, and drinking water treatment residuals found that the columns reduced the concentrations of Zn, Cu, Ni, Pb, Cd, and total polycyclic aromatic hydrocarbons (PAHs) by 99%, 72%, 31%, 91%, >95%, and 95%, respectively (McIntyre et al., 2014). Furthermore, these columns significantly reduced the observed toxicity in zebrafish following runoff water filtration (McIntyre et al., 2014). A subsequent study observed that identical soil bioretention columns prevented all runoff-induced mortality and sublethal toxicity in coho salmon and reduced metals, PAHs, and organic matter by 30-99%, >92%, and >40% respectively (McIntyre et al., 2015). Overall, the data presented regarding treatment of stormwater reveals that such systems are effective in reducing concentrations of many classes of contemporary contaminants. It is thus probable that there exists a treatment technique that is effective in reducing concentrations of fiproles and pyrethroids in stormwater as well as their associated toxicities, namely, CWs.

1.4.2 Wastewater Treatment

Municipal wastewater treatment was developed to remove pollutants such as suspended solids, nutrients, biochemical oxygen demand (BOD), and fecal coliforms (Weston et al., 2013). However, modern wastewater contains several classes of pollutants that conventional WWTPs were not designed to treat, including pharmaceuticals, personal care products, metals, and pesticides (Weston et al., 2013). Recent studies have nonetheless examined the efficacy of WWTPs in removing some of these compounds. A treatment plant in Sacramento, California removed approximately 90% of pyrethroids from wastewater, but effluent concentrations were still sufficient to elicit toxicity in sensitive aquatic invertebrates (Weston et al., 2013). A conventional WWTP provided no significant removal of total fiprole compounds despite partially removing 25% of fipronil present in influent; parent compound removal was offset by transformation to other toxic fiproles (Supowit et al., 2016). The existing data emphasize the fact that traditional WWTPs are inadequate in the treatment of organic micropollutants such as fiproles and pyrethroids. It is therefore clear that additional treatment technologies, like CWs, must be implemented to complement WWTP processes.

1.4.3 Constructed Wetlands

CWs are utilized to improve water quality via treatment of surface water, stormwater, or wastewater. Regarding wastewater treatment, CWs may be implemented as a polishing process for highly treated wastewater effluent or as a sole treatment strategy in areas lacking centralized WWTPs. Studies of stormwater treatment using CWs have discovered that concentrations of metals and nutrients are significantly reduced by the process (Adhikari et al., 2011; Walker and Hurl, 2002). CWs are also gaining popularity as components of wastewater treatment systems since they can effectively treat the targets of conventional wastewater treatment as well as modern pollutants. A review of CW use for wastewater treatment states that removal efficiencies of 60.7%, 80.7%, 63.2%, 68.1%, 39.4%, 21.1%, and 40.9% were observed for BOD < 40 mg L^{-1}, BOD > 40 mg L^{-1}, chemical oxygen demand, total suspended solids, total nitrogen, ammonia nitrogen, and total phosphorous, respectively (Vymazal, 2009). Additional investigations have revealed that CW treatment reduced Zn concentrations by 72% and

97.3%, depending on the influent concentration (Song et al., 2001; Yang et al., 2006). Another review reported highly variable removal of As, with a range of 33-99% (Kadlec and Wallace, 2008). The data presented clearly demonstrate the efficacy of CWs for treatment of water containing current pollutants that either resist treatment in WWTPs or are directly discharged into surface water via runoff.

There are several types and variations of CWs that have been utilized to facilitate treatment of a wide array of contaminants in an abundance of applications. The two major types of treatment wetlands are surface flow CWs (also known as free water surface CWs) and subsurface flow CWs (Kadlec and Wallace, 2008). An example of a surface flow CW is shown in Figure 1.3. Surface flow CWs may contain floating plants, submerged plants, emergent plants, or some combination of the three, and possess areas of open water (Kadlec and Wallace, 2008). Subsurface flow CWs may employ horizontal flow or vertical flow; the former usually consists of a vegetated gravel bed with water flowing horizontally below the bed surface from inlet to outlet while the latter involves water treatment as it percolates through the plant root zone of a vegetated sand or gravel bed (Kadlec and Wallace, 2008). Hybrid CWs are typically composed of a surface flow CW and subsurface flow CW operated in series. Recently, unit process CWs have been implemented in an effort to isolate the ideal conditions necessary to treat particular pollutants in independent ponds, with the goal being to operate distinct ponds in series to treat multiple contaminant classes within the same treatment chain (Jasper et al., 2013). Examples of unit process CWs are open water cells, macrophyte-dominated wetland cells, and bivalve filtration wetland cells (Jasper et al., 2013). An example of a unit process open water CW is depicted in Figure 1.4. CW treatment is usually the result of a combination of abiotic degradation, microbial degradation, sorption, and phytoremediation, depending on the specific contaminant and the design of the CW cell (Kadlec and Wallace, 2008).

Figure 1.3: A vegetated surface flow constructed wetland at the Prado Wetlands in Corona, California.

There has been some research focused on the treatment of fiproles and pyrethroids by CWs in urban and agricultural settings. A recent study of a treatment wetland receiving treated effluent from a WWTP found that the CW removed 44% of fipronil and 47% of total fiproles (Supowit et al., 2016). Another study of an agricultural drainage wetland observed removal rates of 52-94% for pyrethroids (Budd et al., 2009). It is clear that CWs have the potential to remove urban insecticides such as fiproles and pyrethroids from surface water, but the precise mechanisms and ideal conditions for their treatment remain unclear. Wetlands are among the most biologically productive ecosystems on Earth, allowing them to transform many ubiquitous pollutants at relatively low cost (Kadlec and Wallace, 2008). For these reasons, CWs present an ideal option for the treatment of fiproles and pyrethroids in surface water.

Figure 1.4: A unit process open water constructed wetland at the Prado Wetlands in Corona, California.

1.5 Problem Statement

Stormwater and wastewater effluent are contaminating urban streams with toxicologically relevant concentrations of urban-use insecticides. It is essential to study novel water treatment strategies to improve the quality of surface water that has been polluted by stormwater and wastewater discharge. Alternatively, identifying sources of urban insecticides and modifying their application practices may serve to reduce the mass loadings of these compounds in surface runoff. If water quality is not improved, sensitive aquatic organisms will continue to encounter adverse effects, potentially causing detrimental effects on ecosystem services and local food webs. Furthermore, water reuse initiatives may uncover as-yet-unknown human health consequences presented by use of recycled water containing insecticides. More information is needed to develop strategies to reduce insecticide concentrations being deposited into surface water via stormwater and wastewater treatment. It is imperative to understand the urban sources of insecticide contamination to reduce runoff transport of these contaminants by altering application practices. Furthermore, CWs hold a great deal of promise for reducing urban insecticide concentrations in stormwater, wastewater, and surface water. It is vital to assess the removal efficacy of fiproles and pyrethroids passing through CW treatment systems. Pursuing these goals will assist in the development of improved mitigation and treatment practices for water contaminated with urban pollutants.

1.6 Research Objectives

The ultimate goal of the research described in this dissertation was to reduce input of fiproles to surface water by identifying the urban media contributing to their presence in runoff and to determine the efficacy of CWs in removing fiproles and pyrethroids from surface water in an urban watershed. The specific objectives are outlined below:

Objective 1: Identify the contribution of concrete surfaces, soil, and urban dust to surface runoff of fiproles in residential environments following treatment with a professional fipronil formulation.

Objective 2: Evaluate the effect of a large-scale vegetated surface flow constructed wetland on concentrations and mass loadings of fiproles and pyrethroids. Toxic unit values were also calculated to determine the change in adverse effects presented to sensitive aquatic invertebrates.

Objective 3: Assess the fiprole and pyrethroid treatment efficacy of a unit process open water constructed wetland and compare results to those generated in the study of the vegetated surface flow constructed wetland. Toxic unit values were calculated to demonstrate the effect of insecticide treatment on potential ecotoxicity.

1.7 Dissertation Organization

This dissertation is compiled into five chapters. Chapter 1 is a review of the current literature that is relevant to the aforementioned research objectives. Chapters 2, 3, and 4 are each dedicated to one of the research objectives. The first manuscript (Chapter 2) was published in *Environmental Pollution* (Cryder et al., 2019), the second manuscript (Chapter 3) is in review in *Environmental Pollution*, and the third manuscript (Chapter 4) is being prepared for submission. Chapter 5 provides conclusions and recommendations for future research.

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Chapter 2 Fiproles in Urban Surface Runoff: Understanding Sources and Causes of Contamination

2.1 Introduction

Urban-use insecticides are primarily applied to eliminate structural pests such as ants, termites, roaches, and spiders. Extensive outdoor urban pesticide use is a cause for concern since surface runoff of these biologically active compounds into urban waterways following rainfall or irrigation has the potential to exert adverse effects in non-target aquatic organisms (Gan et al., 2012; Weston et al., 2013; Weston and Lydy, 2012, 2014). The high incidence of impervious surfaces (i.e., concrete pavement, asphalt roads, roofs) in urban environments, which may account for 50-90% of residential and commercial surface areas, prevents infiltration of water into soil and facilitates runoff and offsite transport of pollutants such as pesticide residues to urban streams (Arnold and Gibbons, 1996). Perpetual urban expansion is projected to triple the global urban land area between the early 2000s and 2030 (Seto et al., 2012), exacerbating the issue of urban pesticide runoff and contamination to surface water.

Fipronil is a moderately hydrophobic (log Kow = 3.9-4.1; Demcheck and Skrobialowski, 2003) phenylpyrazole insecticide applied for a multitude of urban pest control purposes by licensed applicators. Applications include perimeter and underground injection treatments to manage ants and termites, veterinary flea and tick treatments, insect control baits, and landscape maintenance (Ensminger, 2014; Sadaria et al., 2017; Teerlink et al., 2017; United States Environmental Protection Agency, 2011). After application, fipronil degrades primarily into fipronil desulfinyl, fipronil sulfide, and fipronil sulfone following photolysis, reduction, and oxidation, respectively (Gunasekara and Truong, 2007). According to the Pesticide Use Reporting database, over 24,000 kg a.i. of fipronil were applied in 2016 in California, where use is confined to urban areas (http://cdpr.ca.gov/docs/pur/purmain.htm). Consequently, fipronil and its degradation products (collectively termed fiproles hereafter) are frequently detected in surface water, such as in regions like California.

In a study of runoff discharge collected from residential storm drain outfalls in Southern and Northern California, median total fiprole concentrations were found to be 204-441 ng L^{-1} and 13.8-20.4 ng L^{-1}, respectively, and 90th percentile total fiprole concentrations were 338-1169 ng L^{-1} and 62.6-65.3 ng L^{-1}, respectively (Gan et al., 2012). In a recent study surveying urban creeks, rivers, and storm drain outfalls, fipronil sulfide, fipronil sulfone, fipronil desulfinyl, and fipronil were detected in 8%, 63%, 65%, and 75% of samples, respectively (California Department of Pesticide Regulation, 2016). The presence of fiproles in surface water is significant, since these compounds have been shown to exert toxic effects in a variety of non-target aquatic organisms with an LC_{50} (fipronil) of 140-320 ng L^{-1} for *Palaemonetes pugio*, *Neomysis americana*, and *Simulium vittutum*, and an EC_{50} of 32.5 ng L^{-1} (fipronil) and 7-10 ng L^{-1} (degradation products) for *Chironomus dilutus* (Key et al., 2003; Overmyer et al., 2005, 2007; United States Environmental Protection Agency, 1996; Weston and Lydy, 2014). Therefore, in regions such as California, fiproles are ubiquitously present at toxicologically relevant levels in urban surface water ecosystems. However, little is presently known about the principal sources of fiproles in urban surface runoff, which hampers watershed-scale risk assessment as well as the development of effective strategies for mitigation. In the context of this study, runoff sources are defined as environmental matrices that contribute fiprole residues to surface runoff following known pesticide application.

Primary objectives of this study were to characterize the affinity of fiproles for common urban matrices, to investigate persistence of fiproles in urban compartments, and to identify potential sources of fiproles in urban runoff. Bench sorption experiments were conducted for fiproles in urban dust, soil, and concrete. In addition, runoff water, urban dust, soil, and concrete wipe samples were collected from multiple fipronil-treated homes in Southern California from July-December 2016. This study represents the first systematic investigation of potential runoff sources of fiproles in urban residential environments. Results may be used to direct mitigation efforts of these compounds and to guide future pollution prevention initiatives for similar contaminants in urban watersheds.

2.2 Materials and Methods

2.2.1 Chemicals and Materials

Fipronil (98.9%), fipronil desulfinyl (97.8%), fipronil sulfide (98.8%), and fipronil sulfone (99.7%) were obtained from the United States Environmental Protection Agency's National Pesticide Standard Repository (Fort Meade, MD). Ethiprole (97.4%) was obtained from the Shanghai Pesticide Research Institute (Shanghai, China). Isotopically labeled fipronil ($^{13}C_4$-$^{15}N_2$-fipronil, 99.1%) was purchased from Cambridge Isotope Laboratories (Andover, MA). Solvents and other chemicals were of pesticide or GC-MS grade. Small concrete cubes used in the sorption isotherm experiment were made in the laboratory via a process described elsewhere (Jiang et al., 2011). The following stock solutions were prepared and stored in a freezer before use: 100 mg L^{-1} fiproles in acetone, 1 mg L^{-1} $^{13}C_4$-$^{15}N_2$-fipronil in hexane, and 5 mg L^{-1} ethiprole in acetone.

A sandy loam soil was collected for use in the sorption isotherm experiment from the 0-30 cm depth at the South Coast Research and Extension Center in Irvine, California. Soil was air dried and sieved through a 2 mm mesh prior to use. Soil pH was 7.7, measured using a 1:2 (v/v) soil slurry (Donohue, 1992). Soil particle size composition was 66.0% sand, 16.7% silt, and 17.3% clay, as determined using the 12-h hydrometer method (Klute, 1986). Soil organic matter content was 1.1% (w/w), measured via loss-on-ignition by heating 5 g of soil at 375 °C in a muffle furnace for 24 h (Gavlak et al., 2003). Total organic carbon content (0.66%) was derived using high temperature combustion on an Elementar Vario MAX C/N Analyzer (Elementar Americas, Mt. Laurel, NJ) following the addition of HCl for carbonate removal (Schumacher, 2002).

Samples of urban dust for the sorption isotherm experiment were collected from three homes in Orange County, CA, pooled before use, and fractionated as described elsewhere (Richards et al., 2016). Dust was comprised of the following size fractions: 2 mm-425 μm (15.9% w/w), 425-250 μm (12.4%, w/w), 250-149 μm (4.22%, w/w), and 149-45 μm (67.4%, w/w). Whole dust samples were stored at 4 °C prior to use.

2.2.2 Sorption Isotherm Construction

Sorption isotherms were constructed over a period of five days by mixing concrete cube, urban dust, or the sandy loam soil (collectively: urban solids) samples in aqueous solutions simultaneously spiked with all four fiproles at 5, 20, 50, 100, 200, or

500 µg L^{-1}. NaN_3 was amended at 200 mg L^{-1} in the solution to suppress microbial activity and $CaCl_2$ was added at 100 mg L^{-1} to adjust the solution's ionic strength. Samples were prepared by adding 10 mL of the solution to a 40 mL amber glass vial containing one concrete cube, 2 g of dust (dry weight), or 2 g of soil (dry weight) and mixing on a horizontal shaker at 120 rpm for 5 d (steady state determined through preliminary experiments). No statistically significant fiprole losses occurred over the course of the incubation. Sample vials were centrifuged at 1500 rpm for 30 min to separate the aqueous and solid phases. Aqueous phases were collected and extracted with 10 mL hexane by mixing on a horizontal shaker at 200 rpm for 30 min a total of two times. Solid phase samples were mixed with 1 g NaCl and 4 g anhydrous Na_2SO_4 and then extracted with 10 mL of 8:2 acetone:hexane (v/v) by mixing at 200 rpm for 30 min a total of two times. The solvent extract of the aqueous or solid phases was evaporated to dryness under a gentle stream of nitrogen at 40 °C, and the condensed extracts from solid phase samples were subject to clean up using Florisil cartridges according to the procedure listed in the supporting information (SI). Cleaned extracts were evaporated to dryness at 40 °C under a stream of nitrogen, and all extracts were reconstituted in 1.0 mL hexane before analysis.

2.2.3 Collection and Analysis of Runoff and Solid Samples from Residential Homes

Five homes in Riverside, CA, received standard perimeter spray treatments of a professional fipronil formulation (0.06% w/v) diluted from a suspension concentrate per the label instructions in July 2016, and the treatment was similar to the conventional treatment described in Greenberg et al. (2010). Pre-treatment runoff concentrations of fiproles revealed low-level background contamination of all four compounds (24-134 ng L^{-1}). However, these concentrations were two to three orders of magnitude lower than 1 d runoff concentrations and were similar to 153 d runoff concentrations. Therefore, this background contamination likely did not impact the conclusions presented in this study. Fiproles in runoff, soil, urban dust, and concrete were monitored at five time points during July-December 2016. Weather during this period was very warm and dry until light rainfall occurred in December prior to the collection of the final set of samples. The results reported herein were likely not impacted by this rainfall since the final samples still contained fiproles at concentrations similar to those measured at the previous time point. Runoff samples, one from each home at each time point (n=25), were collected by building a temporary water berm approximately 6 m away from the home's garage door. Berm dimensions and composition were described in detail in Greenberg et al. (2017). Each driveway was rinsed with a hose to generate a volume of runoff sufficient for the collection of a 1 L water sample in an amber glass bottle. Sample bottles were transported to the laboratory on ice within 3 h and stored at 4 °C until extraction.

Extraction of runoff water samples was adapted from the methods in Gan et al. (2012). Briefly, water samples were combined with 30 mL NaCl and liquid-liquid extraction was performed with 60 mL dichloromethane a total of three times. Extracts were then evaporated using a Büchi RE121 Rotovapor (Flawil, Switzerland), solvent exchanged into 9:1 hexane:acetone (v/v), and cleaned up by loading into a Florisil cartridge preconditioned with hexane and eluting with 9:1 hexane:acetone (v/v). Cleaned extracts were evaporated to dryness under a gentle stream of nitrogen at 40 °C and reconstituted in 1.0 mL hexane.

At each house and sampling time point, the following urban solid samples were simultaneously collected: one soil sample from the home perimeter, two dust samples from paved surfaces, and two concrete wipe samples from concrete walkways near the driveway. Soil samples (n=25) from the 0-3 cm depth were collected into 40 mL amber glass vials. Total organic carbon content for the home soil samples was determined to be 3.80% using the aforementioned method (Schumacher, 2002). Urban dust samples (n=50) were collected using a method similar to Richards et al. (2016). Briefly, dust was sampled using a handheld vacuum fitted with a metal housing and mesh containing a Whatman GF/A glass fiber filter paper (1.6 µm pores; Maidstone, U.K.). The area to be vacuumed was marked off using a 0.5 m^2 frame. If additional dust was needed to obtain a sufficiently large sample, the frame was moved to an adjacent region of the concrete surface for vacuuming. Filter papers were subsequently removed from the vacuum and stored in 40 mL amber glass vials. The total organic carbon content of the urban dust samples was measured to be 6.54% utilizing the previously described method (Schumacher, 2002). Concrete wipe samples (n=50) were collected from the vacuumed areas using cotton wipes soaked in 70% (v/v) isopropanol (Jiang and Gan, 2012). A 0.04 m^2 frame was used to mark off the area to be wiped, and samples were then placed into 40 mL amber glass vials. All samples were transported on ice to the laboratory within 3 h and stored at 4 °C until extraction.

Soil, urban dust, and concrete wipe samples were extracted via sonication in a Fisher Scientific FS110H sonication water bath (Waltham, MA), using 20 mL aliquots of 1:1 dichloromethane:acetone (v/v) (Richards et al., 2016). Samples were placed in the sonication water bath for 15 min and centrifuged at 1500 rpm for 30 min. This extraction process was repeated a total of four times. Extracts were evaporated using a Büchi RE121 Rotovapor (Flawil, Switzerland), solvent exchanged into 9:1 hexane:acetone (v/v), and then cleaned up by loading onto a Florisil cartridge preconditioned with hexane and eluting with 9:1 hexane:acetone (v/v). Cleaned extracts were evaporated to dryness under a gentle stream of nitrogen at 40 °C and reconstituted in 1.0 mL hexane.

2.2.4 Chemical Analysis

All samples were analyzed on an Agilent 6890N/5973N GC/MSD equipped with a 30 m x 0.25 mm x 0.25 µm DB-5MS column. Helium was used as the carrier gas at 1.2 mL min^{-1} and sample aliquots (2 µL) were injected at 200 °C. The GC oven temperature program is described in the SI. Quantification was performed using an internal standard-normalized 8-point calibration curve, with concentrations ranging from 1 to 500 µg L^{-1}. Coefficients of determination for all calibration curves fulfilled the requirement of $R^2 \geq 0.99$.

2.2.5 Quality Control and Data Analysis

Several steps were taken to ensure the quality of results generated in this study. Instrumental controls involved running a calibration standard every 10 samples during GC/MSD analysis, adding $^{13}C_4$-$^{15}N_2$-fipronil as an internal standard to each extract immediately before GC/MSD analysis, and determining method detection limits (MDLs) using EPA Method 40 CFR Part 136, Appendix B. The calculated MDLs for fipronil desulfinyl, fipronil sulfide, fipronil, and fipronil sulfone were 1, 2, 2, and 2 μg L^{-1}, respectively. In addition, several procedural controls were utilized. Blank samples were analyzed with every set of three (sorption isotherm experiment), seven (residential runoff), and five samples (residential urban solids). In the case of the sorption isotherm samples, which utilized field samples to determine fiprole sorption and thus were potentially contaminated with fiproles prior to spiking, low level matrix blank detections were used to correct sample concentrations as needed. Reagent blanks for the other sample types revealed no fiprole detections. Matrix spike samples were analyzed to determine extraction efficiencies, which are listed in the SI. In addition, ethiprole was added to all samples prior to extraction to assess surrogate recoveries, which were 101 ± 15% for aqueous samples from the sorption experiment, 101 ± 22% for solid phase samples from the sorption experiment, 96 ± 17% for the residential runoff water samples, and 88 ± 27% for the residential urban solid samples. Additional procedural controls are described in the SI. Statistical analyses were performed using SAS® 9.4 (SAS Institute, Cary, NC). Linear regression was performed to calculate Freundlich sorption coefficients and to assess potential correlation between pesticide concentrations in runoff water and those in various urban solids.

2.3 Results and Discussion

2.3.1 Sorption of Fiproles in Different Urban Matrices

Chemical concentrations in the aqueous phase and on the solids at equilibrium were fit to the Freundlich equation:

$$\log C_s = \log K_f + n \log C_w \qquad (1)$$

where C_S is the chemical concentration on the solids, in µg m^{-2} for concrete or ng g^{-1} for dust and soil, C_w is the aqueous concentration in µg L^{-1} for concrete or ng mL^{-1} for soil and dust samples, n is the non-linear factor representing the slope of the logarithmic regression line, and K_f is the Freundlich sorption coefficient.

Sorption isotherm data for concrete, dust, and soil are summarized in Table 2.1. It is apparent that the sorption capacities of the four fipronil compounds differed substantially within each matrix. Fiproles with larger K_f values exhibit a greater affinity for that matrix and are thus less likely to desorb than compounds with smaller K_f values. Likewise, a larger K_f value would indicate that a compound is relatively more likely to be bound to the specific solid phase as opposed to being dissolved in the aqueous phase when exposed to runoff water.

Table 2.1: Freundlich sorption coefficients of fipronil and its degradation products in different matrices from residential homes.

Matrix	Compound	K_f	Units of K_f	R^2
Concrete	Desulfinyl	9.64	L m^{-2}	0.90
	Sulfide	0.908		0.70
	Fipronil	91.2		0.91
	Sulfone	2.22		0.82
Dust	Desulfinyl	43.7	L kg^{-1}	0.82
	Sulfide	182		0.70
	Fipronil	21.9		0.71
	Sulfone	93.3		0.74
Soil	Desulfinyl	12.9	L kg^{-1}	0.88
	Sulfide	21.4		0.90
	Fipronil	4.75		0.84
	Sulfone	16.6		0.90

According to the K_f values generated in this study, fipronil sulfide has the greatest sorption capacity for soil (21.4 L kg^{-1}), followed by fipronil sulfone (16.6 L kg^{-1}), fipronil desulfinyl (12.9 L kg^{-1}), and the parent compound fipronil (4.75 L kg^{-1}). Freundlich sorption coefficients for the Irvine sandy loam soil used in this study were very similar to values generated in soils from Australia (Ying and Kookana, 2001). These results indicated that fipronil sulfide adsorbs to soil particles more strongly than the other fiprole compounds under the same conditions, with the parent fipronil exhibiting the weakest sorption. This implies that soil may serve as an important runoff source of fipronil compounds if they are able to desorb from soil particles or if the particles themselves are physically washed away during large volume runoff events; for the parent compound, transport in the dissolved form may be important, while for metabolites such as fipronil sulfide with higher K_f values, movement as soil particles may be more significant. It is also possible that the strong sorption of fiproles onto soil particles may preclude their offsite transport if runoff volumes are insufficient to mobilize contaminated soil particles, especially with soils containing high total organic carbon content like the residential soil collected in this study (3.80%). This is important because degradation products of fipronil are known to elicit toxicity equal to or greater than that of fipronil itself (Chandler et al., 2004; Maul et al., 2008; Schlenk et al., 2001; Weston and Lydy, 2014). In addition, surface soil may be susceptible to wind or traffic-induced erosion, and loose soil particles may be transported and deposited onto impervious surfaces to become available for offsite movement by runoff water. Alternatively, when a runoff event produces a sufficient volume of water, surface soil may be inundated and mobilized from its origin in residential environments, resulting in potential transport of fipronil compounds either in the dissolved form or attached to soil particles.

Sorption coefficients for fiproles in dust followed a trend similar to that in soil. Fipronil sulfide displayed the largest K_f value (182 L kg^{-1}), followed by fipronil sulfone (93.3 L kg^{-1}), fipronil desulfinyl (43.7 L kg^{-1}), and then fipronil (21.9 L kg^{-1}). As with soil, fipronil sulfide adsorbed to dust particles with greater affinity than the other compounds, and fipronil showed the lowest relative affinity. Since the units of the dust and soil K_f values were the same, they may be directly compared. Freundlich sorption coefficients for the dust samples were greater than the soil for the same compounds. Therefore, fiproles were sorbed to the dust particles more strongly than the soil solid phase. A recent study showed that fiprole concentrations in dust had the tendency to increase with decreasing particle size (Richards et al., 2016). Since the dust utilized for the sorption isotherm construction in this study was composed predominantly of fine particles, it may be reasonable to assume that increased sorption to dust samples was due to larger specific surface area of dust particles. In addition, the urban dust collected in this study had a total organic carbon content of 6.54% and a previous study showed that the mean organic carbon content of dust collected in southern California was 8.3%, much higher than the organic matter content of most soils in the region (Richards et al., 2016). Since organic matter content is typically the main driver of pesticide sorption, this finding may help explain the higher sorption of fiproles in dust compared with soil. The relatively strong sorption of fiproles to dust suggested that this environmental compartment may serve as an important source for the offsite transport of these compounds following application, since dust particles on impervious surfaces are easily translocated during a runoff event. Past studies suggested that dust particles on impervious surfaces served as a critical source for runoff loads of pesticides and polycyclic aromatic hydrocarbons (Murakami et al., 2004; Richards et al., 2016).

Sorption coefficients for concrete surfaces cannot be directly compared to those of soil and dust due to differences in concentration units (mass/surface area for concrete and mass/mass for soil or dust particles). In contrast to the trends observed with soil and dust samples, fiprole degradation products displayed smaller sorption coefficients than the parent compound on the concrete. It is likely that the sorption trend for concrete diverges from that of soil and dust due to its alkaline nature and complex interior pore system introduced by the curing and hydration processes utilized in its formation (Jiang and Gan, 2016). Concrete's alkaline pH likely affects the ionization states of fiproles, thus altering their relative sorption affinities. Furthermore, it is possible that fiprole residues became irreversibly sorbed to the inner porous network of the concrete cubes, further differentiating sorption to this matrix from that of soil and dust. For fipronil, the K_f value was 91.2 L m^{-2}, which was considerably greater than that for fipronil desulfinyl (9.64 L m^{-2}), fipronil sulfone (2.22 L m^{-2}), or fipronil sulfide (0.908 L m^{-2}). This finding suggested that fipronil, relative to its degradates, may be sorbed strongly to concrete matrices once it is applied and will be more likely to persist within such matrices. Thus, a continuous source of fipronil and its degradation products following the transformation of fipronil on and within the concrete may emanate from concrete surfaces over extended periods of time. Concrete surfaces, in the form of abraded particles, were also suggested as a source of polycyclic aromatic hydrocarbons in urban runoff, suggesting that surfaces such as concrete may serve as a reservoir of multiple classes of contaminants in urban environments (Hoffman et al., 1984).

2.3.2 Distribution of Fiprole Residues in Residential Compartments

Levels and descriptive statistics of fiproles in runoff water, urban dust, soil, and concrete wipe samples collected from actual residential homes are summarized in Table 2.2. The variation of concentrations within each environmental compartment was high, likely due to uncontrolled differences in pesticide use history, land cover, and landscape characteristics (Gan et al., 2012; Jiang et al., 2016a). All four fiprole compounds were detected in 100% of runoff water samples over the five month study interval. This finding was in agreement with a previous study that showed detection of fipronil in runoff water seven months after treatment (Jiang et al., 2014). Similarly, a study surveying residential stormwater outfalls in California showed occurrence of fipronil and its metabolites year-round, with detection frequencies of ≥ 81% in Northern California and ≥ 98% in Southern California (Gan et al., 2012). An examination of relative concentrations of fiproles in the runoff water in this study further confirmed the persistence of these compounds in residential environments (Figure 2.1). Mean fiprole concentrations in runoff at 30 d after application showed an initial decrease to 10-30% of those observed after 1 d. After 30 d, mean fiprole concentrations remained at 5-60% of the 1 d values, with some fluctuations. There was an apparent increase in fiprole mean runoff concentrations from the 30 d to 79 d sampling points, which may be an artifact of the high overall variability. It is also likely that soil or dust particles containing adsorbed

Table 2.2: Descriptive statistics for urban solid and urban runoff samples.

Matrix	Units	Compound	DF%	Median	75th Percentile	90th Percentile	Min	Max
Water[a]	ng L^{-1}	Desulfinyl	100	82.1	293	4850	15.2	380000
		Sulfide	100	27.6	216	3450	5.78	163000
		Fipronil	100	161	517	2420	11.5	166000
		Sulfone	100	104	247	939	10.5	161000
Dust[b]	ng g^{-1}	Desulfinyl	22	<DL[e]	<DL	45.4	<DL	6960
		Sulfide	52	1.39	6.57	20.4	<DL	140
		Fipronil	82	32.8	88.5	734	<DL	4750
		Sulfone	40	<DL	7.76	50.5	<DL	311
Soil[c]	ng g^{-1}	Desulfinyl	32	<DL	9.63	25.1	<DL	86.7
		Sulfide	56	1.97	12.6	39.3	<DL	42.2
		Fipronil	72	11.0	53.2	128	<DL	215
		Sulfone	64	5.23	73.4	157	<DL	562
Concrete[d]	ng m^{-2}	Desulfinyl	27	<DL	395	2490	<DL	9640
		Sulfide	58	59.6	80.0	781	<DL	3190
		Fipronil	92	320	504	4070	<DL	25400
		Sulfone	65	40.5	444	2150	<DL	4960

[a] n=25, [b] n=50, [c] n=25, [d] n=50, [e] Below detection limit

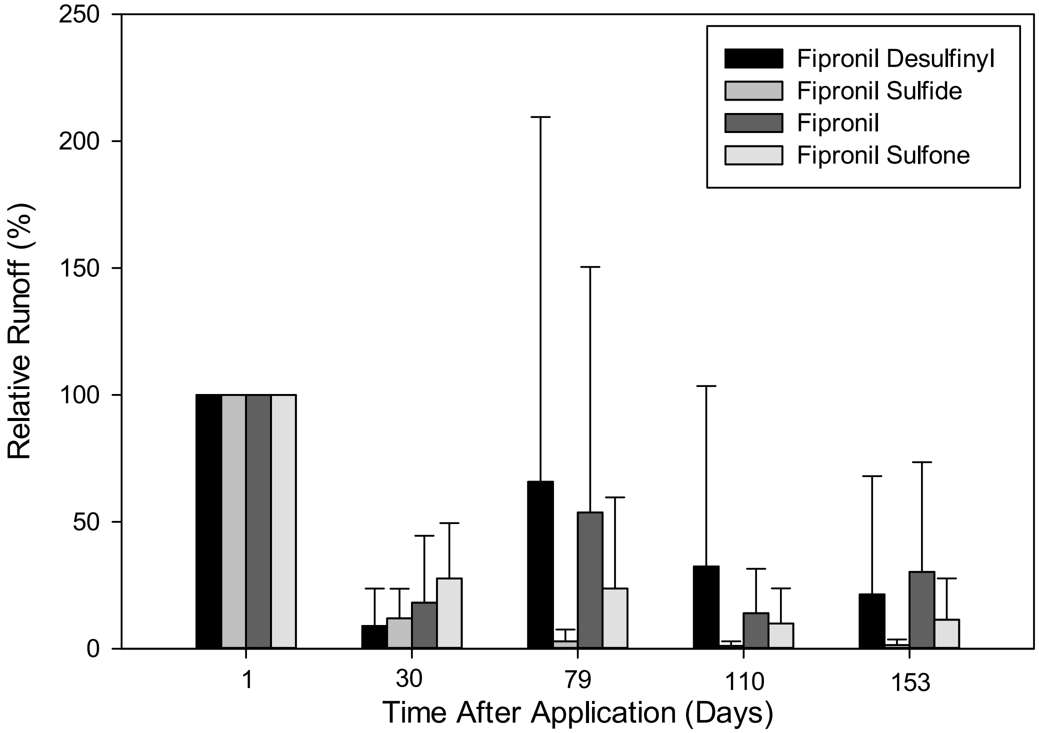

Figure 2.1: Relative runoff concentrations (expressed as percent of 1 d values) of fiproles in residential runoff following fipronil application. Error bars are mean ± 1 SD.

fiprole residues were transported from nearby treated homes onto the pavement that was sampled for runoff water during this sampling interval. This assumption was consistent with the finding that pesticide-laden fine urban dust particles were readily redistributed in residential areas, becoming uniformly present on various impervious surfaces over time (Richards et al., 2016). Together, these results indicated that mean runoff concentrations of fiproles decreased rapidly initially, but low-level emissions may continue for many months. Similar to fipronil, detectable levels of pyrethroids were observed in runoff water from concrete after repeated simulated rainfall events, suggesting that concrete surfaces may serve as a sustained reservoir of hydrophobic pesticides (Jiang et al., 2012).

The persistence of fiproles in runoff water following a single structural pesticide application highlights the importance for mitigation at the source (Table 2.2). Fiprole runoff loads in real-world scenarios could be substantially higher, since multiple pesticide applications may be performed to maintain pest control efficacy (Greenberg et al., 2014). The sustained presence of fiproles within dust, soil, and concrete necessitated an understanding of the relative contributions of these matrices to fiprole loads in runoff, so that the primary origin of contamination may be known and targeted in mitigation practices.

Mean concentrations of fipronil and fipronil desulfinyl in the dust showed an initial peak at 1 d after application, which was followed by a sharp decrease at 30 d and relatively low but detectable levels thereafter (Figure 2.2). This suggested that rapid initial degradation of fipronil took place after application, likely due to photolysis to

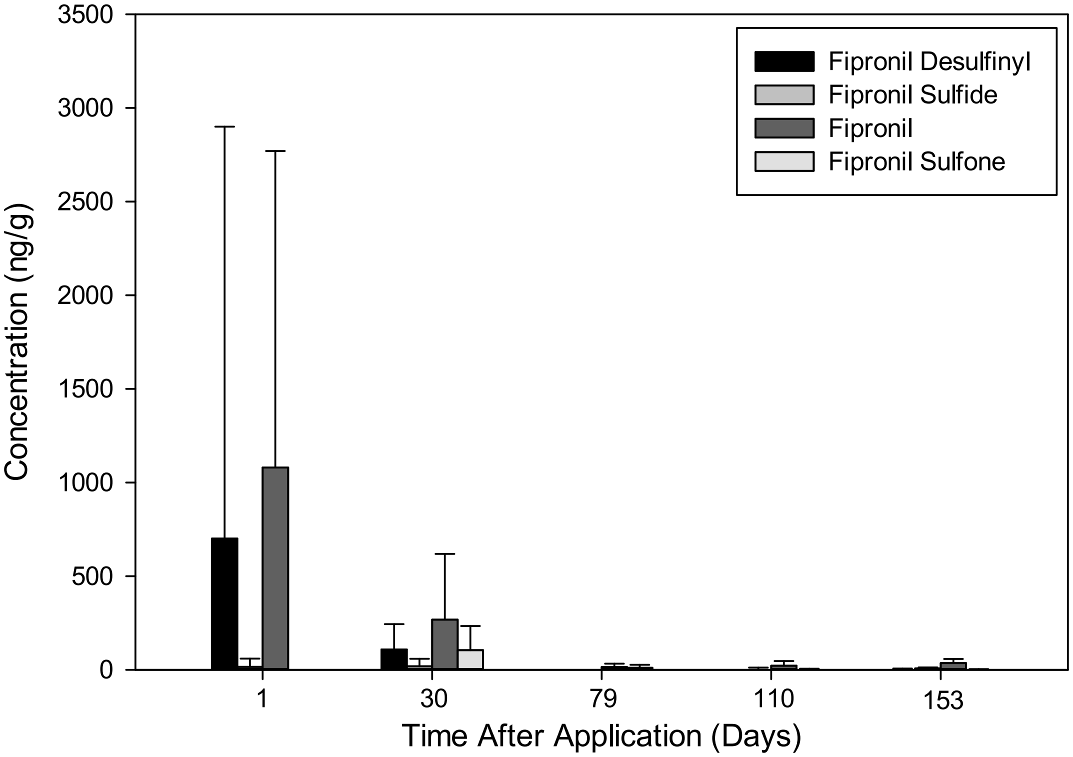

Figure 2.2: Concentrations of fiproles in urban dust collected from residential areas following fipronil application. Error bars are mean ± 1 SD.

fipronil desulfinyl and other abiotic transformations during the intense summer of southern California where temperatures often exceed 38 ºC (Gunasekara and Truong, 2007). Gradual formation of fipronil sulfone was apparent beginning at the 30 d sampling point. The mean concentration of fipronil sulfide remained relatively low and constant throughout the study period. Total fiprole concentrations remained approximately the same at 79 d, 110 d, and 153 d. Fipronil sulfide was the most frequently detected degradation product in the dust (52%), but its maximum concentration (140 ng g^{-1}) was smaller than that of fipronil desulfinyl (6960 ng g^{-1}) or fipronil sulfone (311 ng g^{-1})

(Table 2.2). The parent compound fipronil was the most frequently detected (82%) compound and was present at mean concentrations greater than those of its degradation products throughout the entire sampling duration (Figure 2.2). Fipronil desulfinyl and fipronil were observed to have the highest maximum concentrations of 6960 and 4750 ng g^{-1}, respectively (Table 2.2). A study of the occurrence of pyrethroids and fiproles in urban dust samples collected from the driveways, gutters, and streets of residential areas showed median fiprole concentrations of 1-2 ng g^{-1} and maximum concentrations of 1069-6188 ng g^{-1}, with detection frequencies of 50.6-75.5% (Richards et al., 2016). These detection frequencies and maximum concentrations were similar to those observed in this study (22-82%; 140-6960 ng g^{-1}). However, degradation product concentrations and detection frequencies were lower in the present study, likely attributable to the fact that samples were collected from homes treated with a single application of fipronil. Together, results from this and other studies indicated that dust particles exposed to fipronil may retain fipronil and its degradation products for many months after application. This suggests that urban dust may serve as a source of fiproles, especially fipronil and fipronil desulfinyl, in urban runoff long after the conclusion of pest treatment activity, barring removal or offsite transport of the dust prior to the occurrence of a runoff event.

Fipronil was present in soil at similar mean concentrations throughout the sampling period (Figure 2.3). Fipronil sulfone levels gradually increased from 1 d to 153 d, with mean concentrations ranging from 1.43-209 ng g^{-1}. Fipronil desulfinyl and fipronil sulfide mean concentrations also generally increased over time, ranging from

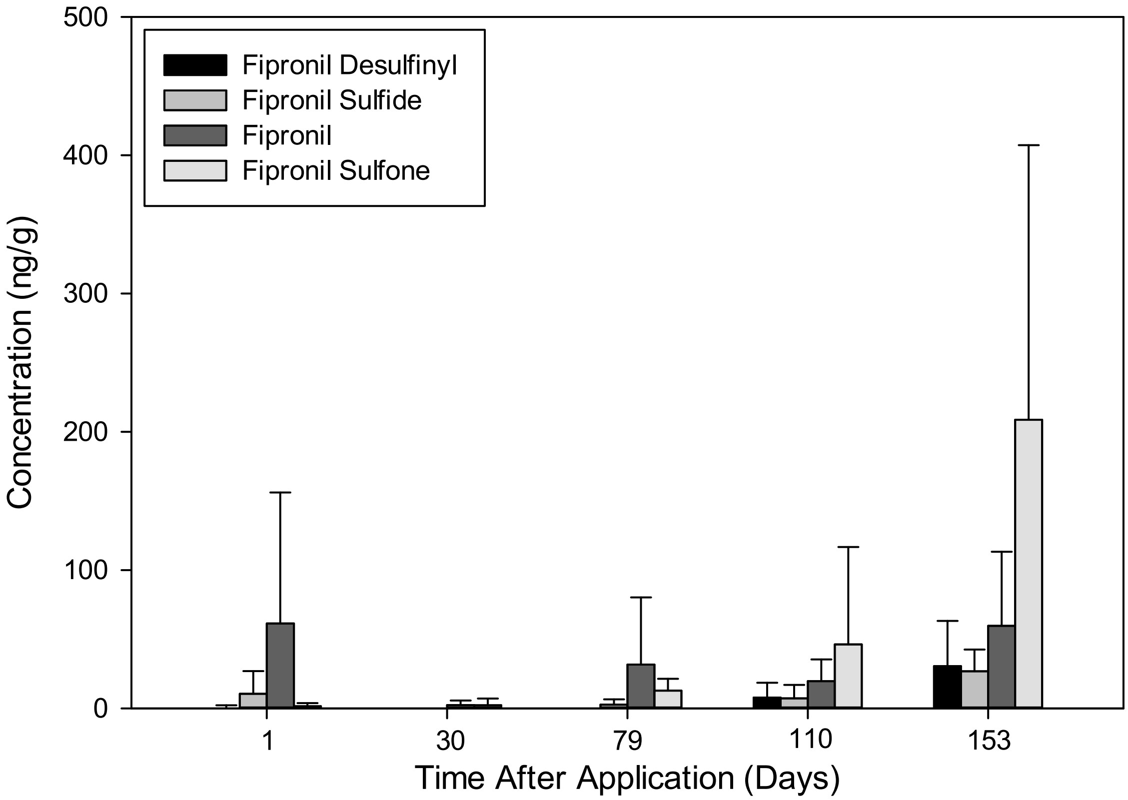

Figure 2.3: Concentrations of fiproles in soil collected from residential areas following fipronil application. Error bars are mean ± 1 SD.

<DL-30.5 ng g^{-1} and <DL-26.7 ng g^{-1}, respectively. Fipronil has an aerobic soil half-life of 188 d (Ying and Kookana, 2002), which supports the finding that mean fipronil concentrations were similar over the 153 d period considered in this study. In addition, the gradual formation and increasing soil concentrations of fipronil degradates was consistent with the relatively slow degradation rate of the parent compound. Fipronil (72%) and fipronil sulfone (64%) were detected with the greatest frequency and at the highest maximum concentrations (215 and 562 ng g^{-1}, respectively) (Table 2.2). Fipronil

desulfinyl (32%) and fipronil sulfide (56%) were detected less frequently and at substantially lower maximum concentrations (86.7 and 42.2 ng g^{-1}, respectively). Fiproles were measured in soil samples at detection frequencies similar to those measured in dust (32-72% in soil; 22-82% in dust), but maximum soil concentrations were much lower than maximum dust concentrations (42.2-562 ng g^{-1} for soil, and 140-6960 ng g^{-1} for dust). It is possible that soil concentrations were low relative to dust concentrations because soil samples were collected to a depth (0-3 cm) while dust particles partially originated from wind erosion of the surficial soil (Jiang et al., 2016b). Fiproles have been shown to be enriched in fine particles characteristic of urban dust (Richards et al., 2016), suggesting that residues initially present in the surrounding soil may have contributed to contamination of loose dust particles on impervious surfaces. Results summarized herein reveal that soil treated with fipronil-based pesticide formulations remains contaminated by fiproles for a significant amount of time following treatment and is a source of fipronil degradation products. These data collectively imply that soil has the potential to contribute fipronil and its degradation products to their loads in urban runoff. However, this contribution likely depends upon the entrance of soil particles into runoff, either by inundation of soil with a large runoff volume after a prolonged rainfall, an irrigation event, or by prior transport of soil particles onto urban impervious surfaces.

Mean concrete concentrations of fiproles were at their highest 1 d after application and decreased subsequently by 57-89% at the 30 d sampling point (Figure 2.4). Fipronil was rapidly transformed after application such that its degradation products were detected at high mean concentrations 1 d after application. This finding was

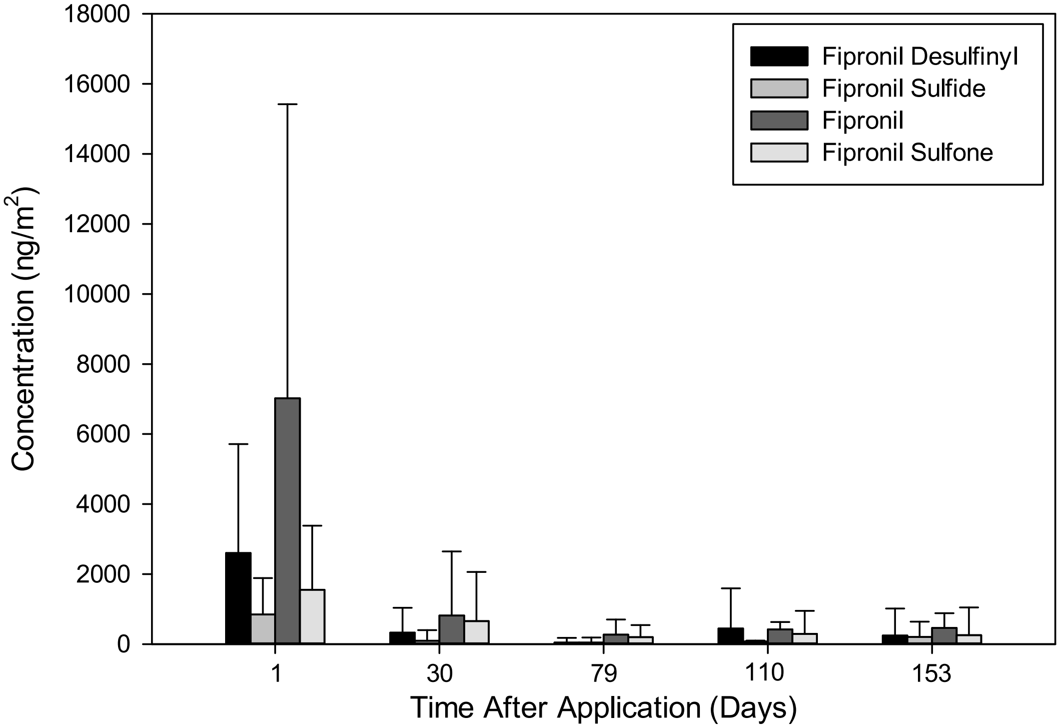

Figure 2.4: Concentrations of fiproles on residential concrete following fipronil application. Error bars are mean ± 1 SD.

consistent with results of a recent study focused on the degradation of pesticides on urban hard surfaces, where it was observed that fipronil was rapidly transformed to its biologically active degradation products on concrete in bench and field experiments (Jiang and Gan, 2016). Mean concentrations then remained relatively stable for the duration of the sampling campaign, with 30 d concentrations being similar to those at 79 d, 110 d, and 153 d. Detection frequencies of fiproles in concrete ranged from 27 to 92%, with maximum concentrations of 3.19-25.4 μg m^{-2} (Table 2.2). Fipronil was detected more often than its degradation products (92%). The most prevalent degradation product was fipronil sulfone (65%), while fipronil desulfinyl (27%) was detected at a higher maximum concentration (9.64 $\mu g\ m^{-2}$) than the other degradates, second only to the parent compound (25.4 $\mu g\ m^{-2}$). An investigation of the contribution of fine particles to the runoff loads of pyrethroid pesticides also revealed high concentrations of bifenthrin (approximately 100 $\mu g\ m^{-2}$) and permethrin (approximately 10,000 $\mu g\ m^{-2}$) on concrete following application of professional pesticide formulations (Jiang and Gan, 2012). Concrete data further showed that fiproles were present in the concrete at detectable concentrations for several months after initial application of fipronil for pest treatment. This suggests that concrete may act as a long-term source of these compounds in urban runoff.

2.3.3 Elucidation of Sources for Fiproles in Runoff

Several linear regression analyses were performed to assess the presence of statistically significant ($p < 0.05$) linear relationships between fiprole concentrations in different urban solid matrices and concentrations in runoff water (Table 2.3). The goal of these analyses was to identify primary sources for fiproles in urban runoff. A statistically significant relationship would indicate that a given component may be an important source for fiproles in runoff. It was observed that statistically significant relationships existed between the runoff and concrete concentrations of fipronil desulfinyl, fipronil sulfide, fipronil, and fipronil sulfone. A previous study similarly uncovered a highly significant linear relationship between runoff concentrations of pyrethroids and their concentrations on concrete surfaces measured using a surface wipe method (Jiang and Gan, 2012). In this study, significant relationships were also found between the runoff

Table 2.3: Linear regression analysis of mean runoff concentrations versus mean urban solid concentrations. Statistically significant regressions are indicated by *.

Matrix	Compound	Regression Equation	R^2	p-value
Dust	Desulfinyl	$C_{runoff}{}^{a} = -2680 + (114)\ (C_{dust}{}^{b})$	0.98	0.0015*
	Sulfide	$C_{runoff} = -1460 + (896)\ (C_{dust})$	0.20	0.454
	Fipronil	$C_{runoff} = -2170 + (32.6)\ (C_{dust})$	0.95	0.0054*
	Sulfone	$C_{runoff} = 8830 - (93.1)\ (C_{dust})$	0.083	0.639
Soil	Desulfinyl	$C_{runoff} = 22200 - (815)\ (C_{soil}{}^{c})$	0.092	0.619
	Sulfide	$C_{runoff} = 6140 + (81.3)\ (C_{soil})$	0.0031	0.929
	Fipronil	$C_{runoff} = -5130 + (349)\ (C_{soil})$	0.34	0.304
	Sulfone	$C_{runoff} = 9670 - (55.7)\ (C_{soil})$	0.11	0.579
Concrete	Desulfinyl	$C_{runoff} = -8340 + (33.0)\ (C_{concrete}{}^{d})$	0.98	0.0012*
	Sulfide	$C_{runoff} = -4400 + (44.8)\ (C_{concrete})$	0.97	0.0025*
	Fipronil	$C_{runoff} = -2320 + (5.23)\ (C_{concrete})$	0.99	0.0001*
	Sulfone	$C_{runoff} = -7860 + (24.5)\ (C_{concrete})$	0.90	0.0138*

[a] Runoff concentrations expressed in units of ng L^{-1}
[b,c] Dust and soil concentrations expressed in units of ng g^{-1}
[d] Concrete concentrations expressed in units of ng m^{-2}

and dust concentrations of fipronil desulfinyl and fipronil. Recent studies have also implicated dust particles in the offsite transport of hydrophobic organic contaminants, but the present study was the first to directly evaluate the connection between dust and runoff loads of fiproles (Jiang et al., 2016b; Richards et al., 2016). The significance of the concrete-runoff and dust-runoff relationships for fiproles together suggested that dust on impervious urban surfaces and residues on concrete are important sources of fiproles in runoff. Statistical analysis, however, did not show soil as a significant source for fiproles in runoff water. As discussed above, even though soil was not a direct source, it is possible that soil particles in the surface layer may be transported via wind and other mechanisms onto impervious surfaces, indirectly contributing to the contamination of runoff water by fiproles. Soil particles likely represent a major component of urban dust; other components may include concrete fragments generated from weathering and plant debris (Jiang et al., 2016b). Taken together, the most important finding of this analysis was that the effectiveness of mitigation efforts would be improved by focusing on reduction of dust particles on impervious surfaces and prevention of pesticide contact with concrete surfaces such as driveways. Moreover, the established regression equations may be used to predict fiprole loads in runoff using levels in urban dust and residues on impervious surfaces, before a runoff event occurs.

Contamination of surface water by fiproles poses a threat to many benthic invertebrate species (Key et al., 2003; Overmyer et al., 2005, 2007; United States Environmental Protection Agency, 1996; Weston and Lydy, 2014). Fiproles may therefore exert a significant effect on the benthic community structures of urban streams (Weston and Lydy, 2014). Mitigation efforts are essential in the prevention of such adverse ecological consequences. However, even though runoff from a given residential area enters downstream surface water as a point source, surface runoff from individual homes in a neighborhood resemble nonpoint sources and is technically challenging to control (Gan et al., 2012). Identification of concrete surfaces and urban dust as the major sources of fiprole contamination of surface runoff at the site of pesticide treatment (i.e., individual homes) highlights their importance in the effort to reduce fiprole residues around a homesite, especially on impervious surfaces.

2.4 Conclusions

The high use rate of fipronil, detection of fipronil and its biologically active degradates in surface water in urban streams, and evidence for adverse effects in non-target aquatic organisms warrant a better understanding of the distribution of these compounds among various urban compartments and their origins in runoff. Results of this study revealed that fiproles differed in their capacity to adsorb to concrete surfaces, soil, and urban dust. Therefore, these matrices likely contribute differently to contamination of runoff water by fiproles due to irrigation or rainfall events. In residential settings, fiproles were shown to persist for several months in concrete, soil, dust, and runoff water following a single pesticide treatment. Data presented in this study provided evidence that concrete and dust were likely the primary sources of fiproles for the contamination of urban runoff. Thus, mitigation efforts should focus on the reduction of fiproles in these matrices. Minimizing the occurrence of fiproles in urban dust and on concrete surfaces would serve to decrease their concentrations in runoff and their subsequent contamination of surface water following offsite transport. Modifying pesticide application practices among pest management professionals is the first step toward effective mitigation. For example, avoiding application on concrete surfaces should be enforced through regulations. In addition, removal of dust from urban surfaces (i.e., via vacuuming or sweeping) prior to pesticide application may be considered. Instituting such changes will contribute to the reduction of fiprole loading in urban runoff and a decrease in potential ecotoxicological impacts associated with their occurrence in surface water. It is important to note that the runoff concentrations reported in this study cannot be directly compared to established aquatic toxicity values since the dissolved and particulate phases were not separated via filtration and not all the fiproles present in runoff would be bioavailable. Furthermore, it is likely that substantial dilution of the surface runoff would take place in surface water, reducing the expected toxicological impact of the measurements provided here. However, the identification of runoff sources in this study was vital to prevent future toxicity to sensitive organisms. Additional research is needed to ascertain the efficacy of potential fiprole mitigation strategies at a watershed scale.

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2.6 Supporting Information

2.6.1 Florisil Clean-up of Solid Phase Sorption Isotherm Samples

The Florisil cartridges were conditioned with hexane, samples were redissolved in 98:2 hexane:acetone (v/v) before loading, and the cartridges were eluted with 10 mL of 8:2 hexane:acetone (v/v).

2.6.2 GC Oven Program

The following GC oven temperature program was used in this study: initial temperature of 120 °C and held for 1 min; ramped up at 25 °C min^{-1} to 205 °C and held for 1 min; ramped up at 1.5 °C min^{-1} to 220 °C and held for 1 min; ramped up at 3 °C min^{-1} to 225 °C and held for 1 min; and finally ramped up at 30 °C min^{-1} to a final temperature of 300 °C followed by a 5 min hold.

2.6.3 Matrix Spike Extraction Efficiency

For fipronil desulfinyl, fipronil sulfide, fipronil, and fipronil sulfone, respectively, extraction efficiencies were as follows: 172 ± 6%, 111 ± 5%, 125 ± 4%, and 109 ± 1% for aqueous samples from the sorption experiment; 145 ± 35%, 94 ± 23%, 105 ± 24%, and 90 ± 21% for solid phase samples from the sorption experiment; 104 ± 3%, 102 ± 1%, 103 ± 10%, and 109 ± 4% for residential runoff water samples; and 95 ± 10%, 92 ± 10%, 120 ± 24%, and 104 ± 12% for the residential urban solid samples.

2.6.4 Additional Procedural Controls

Glassware was baked prior to use at 400 °C for 4 h to reduce the risk of cross contamination. Sodium sulfate and sodium chloride were baked at 400 °C for 4 h prior to use. Florisil was activated at 130 °C before being packed into solid phase extraction cartridges. During urban dust samples collection, the vacuum apparatus was cleaned with 70% (v/v) isopropanol between each sample collection.

Chapter 3 Removal of Urban-Use Insecticides in a Large-Scale Constructed Wetland

3.1 Introduction

The combination of rampant urbanization, rapid population growth, and global climate change has resulted in an extraordinary reduction in the potable and non-potable water supply worldwide (Arnold and Gibbons, 1996; Kummu et al., 2010; McDonald et al., 2011; Seto et al., 2012). The deficiency of clean water supplies has led several nations, including the United States, to encourage a reduction in water use (Farré and Faci, 2009; Fielding et al., 2013; Tabbal et al., 2002) and pursue a myriad of water treatment and recycling initiatives (Adhikari et al., 2011; Anderson et al., 2016; Friedler, 2001; Greenway, 2005; Haile et al., 2016; Kadlec and Wallace, 2008; Yang et al., 2006). Water scarcity is exacerbated by pollution of surface water and ground water resources by anthropogenic contaminants such as pesticides. Indoor and outdoor use of insecticides in urban areas has been shown to cause contamination of urban surface water sources. Urban-use insecticides are incompletely removed at wastewater treatment plants (WWTPs) before the release of effluent into surface streams, and runoff after rain and irrigation events further exacerbates surface water contamination.

Fipronil and the synthetic pyrethroids are insecticides utilized at high rates in urban environments for professional and homeowner control of structural pest species such as ants, termites, spiders, and roaches, as well as for elimination of fleas and ticks in veterinary medications (Ensminger, 2014; Sadaria et al., 2017; Teerlink et al., 2017; United States Environmental Protection Agency, 2011; Weston et al., 2005, 2009).

Fipronil and its primary degradation products, fipronil desulfinyl, fipronil sulfide, and fipronil sulfone (abbreviated as fiproles hereafter) are moderately hydrophobic compounds (log K_{ow} = 3.9-4.1; Demcheck and Skrobialowski, 2003) while the pyrethroids are highly hydrophobic with log K_{ow} =5.7-7.6 (Laskowski, 2002). Numerous studies have shown occurrence of both insecticide classes in urban surface water (California Department of Pesticide Regulation, 2016; Gan et al., 2012; Weston et al., 2009; Weston and Lydy, 2012) at toxicologically relevant concentrations (Anderson et al., 2006; Maund et al., 1998; Weston and Jackson, 2009; Weston and Lydy, 2014) as well as in the sediment where residues may persist long after deposition (Lao et al., 2010; Lin et al., 2008). Furthermore, fipronil's major degradation products exhibit toxicity equal to or greater than that of the parent compound (Schlenk et al., 2001; Weston et al., 2014). There is also evidence of additive pyrethroid toxicity in sensitive organisms (Trimble et al., 2009). Fiproles and pyrethroids are easily transported in surface runoff (Jiang et al., 2010, 2012; Jorgenson and Young, 2010; Laskowski, 2002; Richards et al., 2016; Thuyet et al., 2012) and are present in WWTP effluents (Sadaria et al., 2017; Supowit et al., 2016; Weston et al., 2013), aggravating the risk of toxicity to non-target aquatic species.

In arid or semi-arid regions such as California, some urban streams are predominantly fed with urban runoff drainage and WWTP effluents. Constructed wetlands (CWs) are one potential solution to the shortcomings of WWTPs and the general lack of stormwater treatment. They have been shown to remove nitrogen and phosphorous species, metals, antibiotic resistance genes, and various organic compounds

(Chen et al., 2019; Dong et al., 2020; Kodituwakku and Yatawara, 2020). Existing data suggest that CWs are effective in reducing concentrations of fiproles and pyrethroids (Budd et al., 2009; Supowit et al., 2016). However, field data on the performance of urban wetlands are limited, and in-depth information on the role of various wetland compartments is scarce.

In this study, samples were collected from the Prado Wetlands, a 182 ha constructed treatment wetland system located in Southern California containing open water and vegetated cells, from June 2018-January 2019 and analyzed for fiproles and pyrethroids. The primary objectives were to determine the removal of these trace contaminants by the surface flow wetland, to understand the underlying processes most responsible for contaminant removal, and to estimate potential alleviations in invertebrate toxicity. It was hypothesized that sediment sorption and biodegradation would play a major role in the removal of fiproles and pyrethroids, resulting in reduced aquatic toxicity. Results from this study may be used to optimize the design of CWs and related water treatment systems to improve the quality of recycled water and to attenuate ecotoxicological and human health risks from potable and non-potable applications.

3.2 Materials and Methods

3.2.1 Chemicals and Materials

Fipronil (98.9%), fipronil desulfinyl (97.8%), fipronil sulfide (98.8%), and fipronil sulfone (99.7%) were obtained from the United States Environmental Protection Agency’s National Pesticide Standard Repository (Fort Meade, MD). Bifenthrin (98%) and deuterated (d5) bifenthrin (99%) were purchased from Toronto Research Chemicals

(Toronto, Ontario, Canada). Cyfluthrin (98%) was purchased from Santa Cruz Biotechnology (Dallas, TX). Ethiprole (97.4%) was obtained from the Shanghai Pesticide Research Institute (Shanghai, China). Decachlorobiphenyl (99.1%) was purchased from AccuStandard (New Haven, CT). Isotopically labeled fipronil ($^{13}C_4$-$^{15}N_2$-fipronil, 99.1%) was obtained from Cambridge Isotope Laboratories (Andover, MA). Solvents and other chemicals used were of pesticide or GC-MS grade.

3.2.2 Site Description

This study was undertaken at the Prado Wetlands in Corona, CA (Figure S3.1). This 182 ha complex of 45 surface flow wetland ponds was constructed in the 1990s and was initially established to remove NO_3^- from the Santa Ana River (Orange County Water District, 2018). Up to 50% of the Santa Ana River flow, which consists primarily of treated wastewater during non-storm seasons, is diverted into the wetland system for treatment (Orange County Water District, 2018). Additional details regarding the Prado Wetlands are provided in the Supporting Information (SI).

The ponds selected for use in this study were cells S5 and S6 (Figure S3.1; connected by an underground pipe), which together constitute a 4.45 ha vegetated CW complete with inlet and outlet weir boxes. In the context of this study, this vegetated CW will be referred to as the Prado Constructed Wetland (PCW). The hydraulic retention time of the PCW was estimated to be 1.29 d based on the results of a pilot-scale rhodamine WT tracer experiment conducted at the Prado Wetlands (Lin et al., 2003). Samples and measurements were taken at the inlet weir box (inlet), the interface between ponds S5 and S6 following the connection pipe (midpoint), and the outlet weir box (outlet).

3.2.3 Sediment and Water Properties

Sediment from the inlet, midpoint, and outlet of the PCW was collected from the 0-15 cm depth in 1 L amber glass jars using a small hand shovel and composited for determination of sediment physicochemical properties (Table S3.1). Sediment was air dried and sieved through a 2 mm mesh before analysis. Sediment particle size composition was determined to be 43% sand, 29% silt, and 28% clay using the 12-h hydrometer method (Klute, 1986). Sediment pH was measured to be 7.34 using a 1:2 (v/v) soil slurry (Donohue, 1992). The organic matter content was 2.35% (w/w), measured via loss-on-ignition (Gavlak et al., 2003). A total organic carbon content of 1.42% was determined using high temperature combustion on an Elementar Vario MAX C/N Analyzer (Elementar Americas, Mt. Laurel, NJ) after the addition of HCl for removal of carbonates (Schumacher, 2002).

The flow rate of the water entering and exiting the PCW at each time point was calculated based on an equation provided by Prado Wetlands staff:

$$CFS = 3.33\ L\ H^{3/2} \tag{1}$$

where CFS is the flow ($ft^3\ s^{-1}$), L is the length of the weir box (ft), and H is the height of the water flowing through the weir box (ft). The calculated flow rates were then converted into metric units (Table S3.2). Sedimentation rates were subsequently derived using these flow rates and the concentration of total suspended solids (TSS) in water samples collected from the PCW inlet and outlet (Table S3.2; Budd et al., 2009). Water temperatures were measured *in situ* at the PCW inlet, midpoint, and outlet (Table S3.3). After water sample collection, water pH measurements were performed *ex situ* in the laboratory prior to extraction (Table S3.3).

3.2.4 Collection and Extraction of Wetland Samples

Water, sediment, and plant samples were collected from the PCW monthly during the period of June 2018-January 2019, with the exception of September 2018 when there were ongoing maintenance activities. Triplicate 1 L water samples were collected in amber glass bottles at the inlet, midpoint, and outlet of the PCW. Inlet and outlet samples were collected from the water flowing into the corresponding weir boxes, while midpoint samples were collected by placing bottles below the surface of the water against the direction of flow. Sample bottles were transported to the laboratory on ice and stored at 4 °C until extraction. Before extraction, water samples were passed through 0.7 µm filters to separate the TSS from the water. Filtered TSS samples were then dried in preparation for extraction.

Wetland water samples were extracted using a method in Gan et al. (2012), with modifications. Briefly, 30 mL of NaCl was combined with each water sample and liquid-liquid extraction was performed with 60 mL aliquots of dichloromethane (x 3). Each extract was drained through a funnel containing anhydrous Na_2SO_4 to remove residual water, evaporated with a Büchi RE121 Rotovapor (Flawil, Switzerland), and solvent exchanged into 9:1 hexane:acetone (v/v). Samples were then evaporated to approximately 0.5 mL under a gentle nitrogen stream and reconstituted in 1.0 mL hexane for analysis.

Sediment and plant samples were also collected at the PCW inlet, midpoint, and outlet. Sediment samples were collected as described above. Whole samples of California bulrush (*Schoenoplectus californicus*) were collected by pulling the roots out of the sediment. All samples were transported on ice prior to laboratory storage at 4 °C. Sediment samples were dried and ground with a mortar and pestle before extraction. Plant samples were thoroughly washed with DI water to remove any attached sediment particles and biofilms. The cleaned plant samples were dried before pulverizing the tissue in the presence of liquid nitrogen using a mortar and pestle.

The TSS, sediment, and plant samples were extracted by pressurized fluid extraction on a Dionex ASE 350 (Thermo Fisher Scientific, Waltham, MA) using a method similar to Brennan et al. (2009) and Maul et al. (2008). Briefly, sample cells were filled with 1:1 dichloromethane:acetone (v/v), heated to 100 °C, and extracted at 1500 psi for two 5 min cycles before being flushed with 60% solvent for 1 min. For the TSS samples, the entirety of the solids from each water sample was extracted. Aliquots of the prepared sediment (25 g) and whole plant (5 g) samples were extracted and subjected to in-cell cleanup with Cu powder (Lin et al., 2008) packed between cellulose filters. All extracts were evaporated to approximately 2 mL under a gentle nitrogen stream. Each sample then underwent clean-up on a Florisil cartridge preconditioned with hexane and was eluted with 9:1 hexane:acetone (v/v). Cleaned extracts were again evaporated under a gentle stream of nitrogen to approximately 0.5 mL and reconstituted in 1.0 mL hexane for final analysis.

3.2.5 Chemical Analysis and Quality Control

Samples were analyzed using an Agilent 6890N/5973N GC/MSD (Santa Clara, CA) equipped with a 30 m x 0.25 mm x 0.25 μm DB-5MS column. Additional analytical information is provided in the SI. Following sample quantification, statistical analyses were performed using SAS® 9.4 (SAS Institute, Cary, NC). Numerous steps were taken to ensure the accuracy and quality of analysis. Instrumental controls during analysis included running a calibration standard every 10 samples, adding $^{13}C_4$-$^{15}N_2$-fipronil and d5-bifenthrin as internal standards to each extract, and determining method detection limits (MDLs) using EPA Method 40 CFR Part 136, Appendix B (United States Environmental Protection Agency, 2012). Calculated MDLs were 2 μg L^{-1} for all analytes except for fipronil desulfinyl, which had an MDL of 1 μg L^{-1}. In addition, several procedural controls were utilized. Reagent blanks were analyzed with every set of 7 liquid-liquid extraction samples and 10 pressurized fluid extraction samples. Reagent blanks revealed no presence of the target analytes. Matrix spike samples were analyzed to measure extraction efficiencies, which are listed in the SI. In addition, ethiprole and decachlorobiphenyl were added to all samples prior to extraction to assess surrogate recoveries, which were 105 ± 23% and 121 ± 18% for liquid-liquid extraction, respectively, and 108 ± 21% and 116 ± 18% for pressurized fluid extraction, respectively. Additional quality control measures are described in the SI.

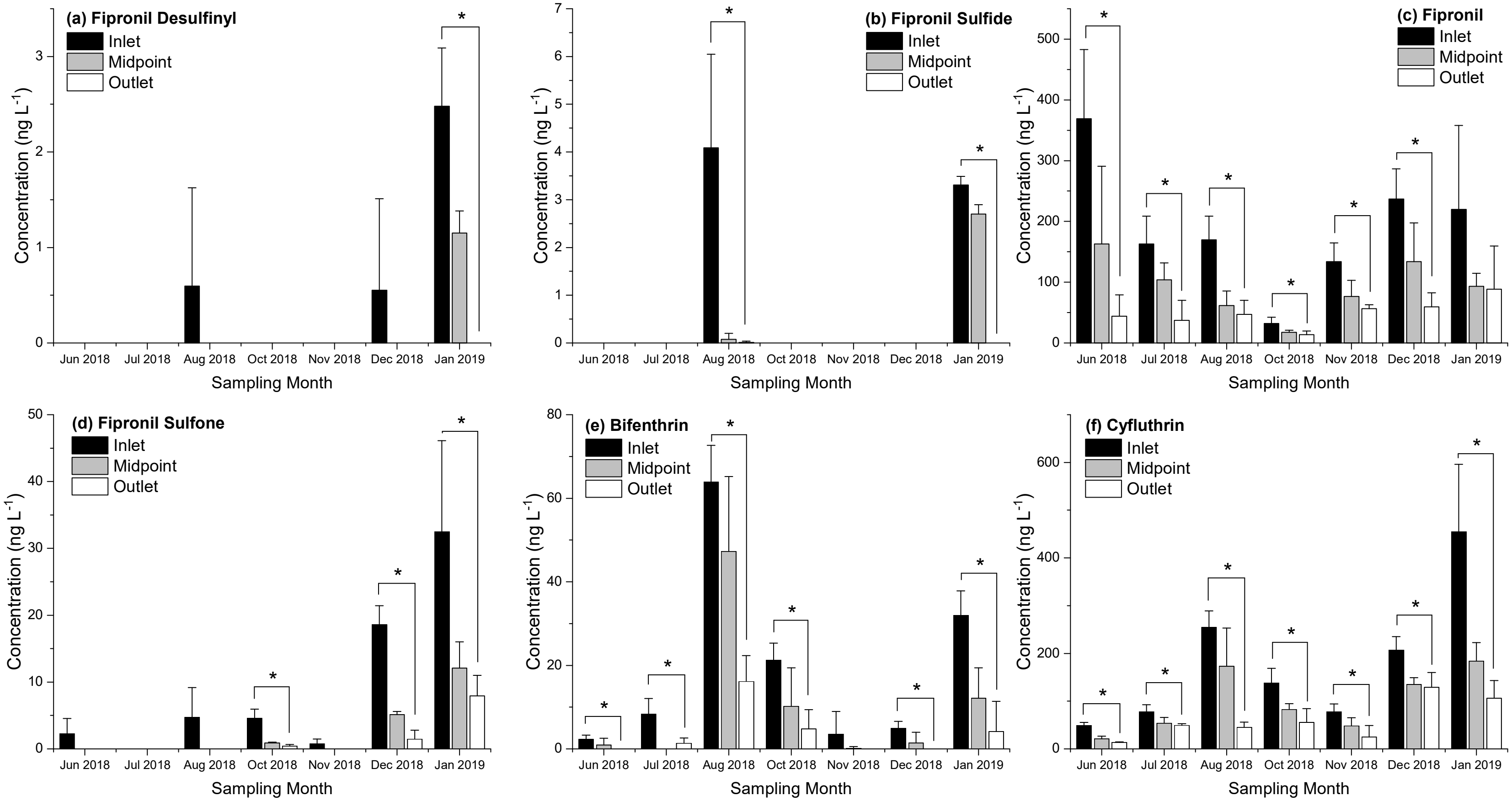
3.3 Results and Discussion

3.3.1 Spatiotemporal Trends of Fiproles and Pyrethroids

The concentrations of fiproles and pyrethroids in water samples collected from the PCW are depicted in Figure 3.1. In general, mean whole water concentrations progressively decreased on a spatial level when moving from the inlet to midpoint to outlet for all compounds of interest. In addition, mean outlet concentrations were always lower than mean inlet concentrations. Furthermore, statistically significant differences existed for the majority of inlet and outlet concentration comparisons. When the differences between inlet and outlet concentrations were not statistically significant, inlet concentrations were found at low levels and with relatively high variability. Since water and the associated TSS is all that enters and leaves the PCW, these findings provide evidence indicating that statistically significant removal of fiproles and pyrethroids occurred as a result of treatment in the PCW. Previous research by agricultural and WWTP effluent-polishing CWs has similarly shown removal of fiproles and pyrethroids (Budd et al., 2009; Supowit et al., 2016).

The mean whole water concentrations of fiproles and pyrethroids also exhibited distinct temporal trends during the sampling period (Figure 3.1). Mean fipronil concentrations peaked in June 2018, gradually decreased until October 2018, and then increased until January 2019. Mean whole water concentrations of fipronil desulfinyl, fipronil sulfide, and fipronil sulfone incrementally increased from June-August 2018, decreased, and then increased until January 2019. The peak mean whole water concentrations for fipronil desulfinyl and fipronil sulfone occurred in January 2019 while

Figure 3.1: Whole water concentrations of fipronil desulfinyl (a), fipronil sulfide (b), fipronil (c), fipronil sulfone (d), bifenthrin (e), and cyfluthrin (f) in samples collected from the Prado Constructed Wetland. Data are reported as mean ± 1 SD. Asterisks indicate a statistically significant ($p < 0.05$) difference between inlet and outlet concentrations.

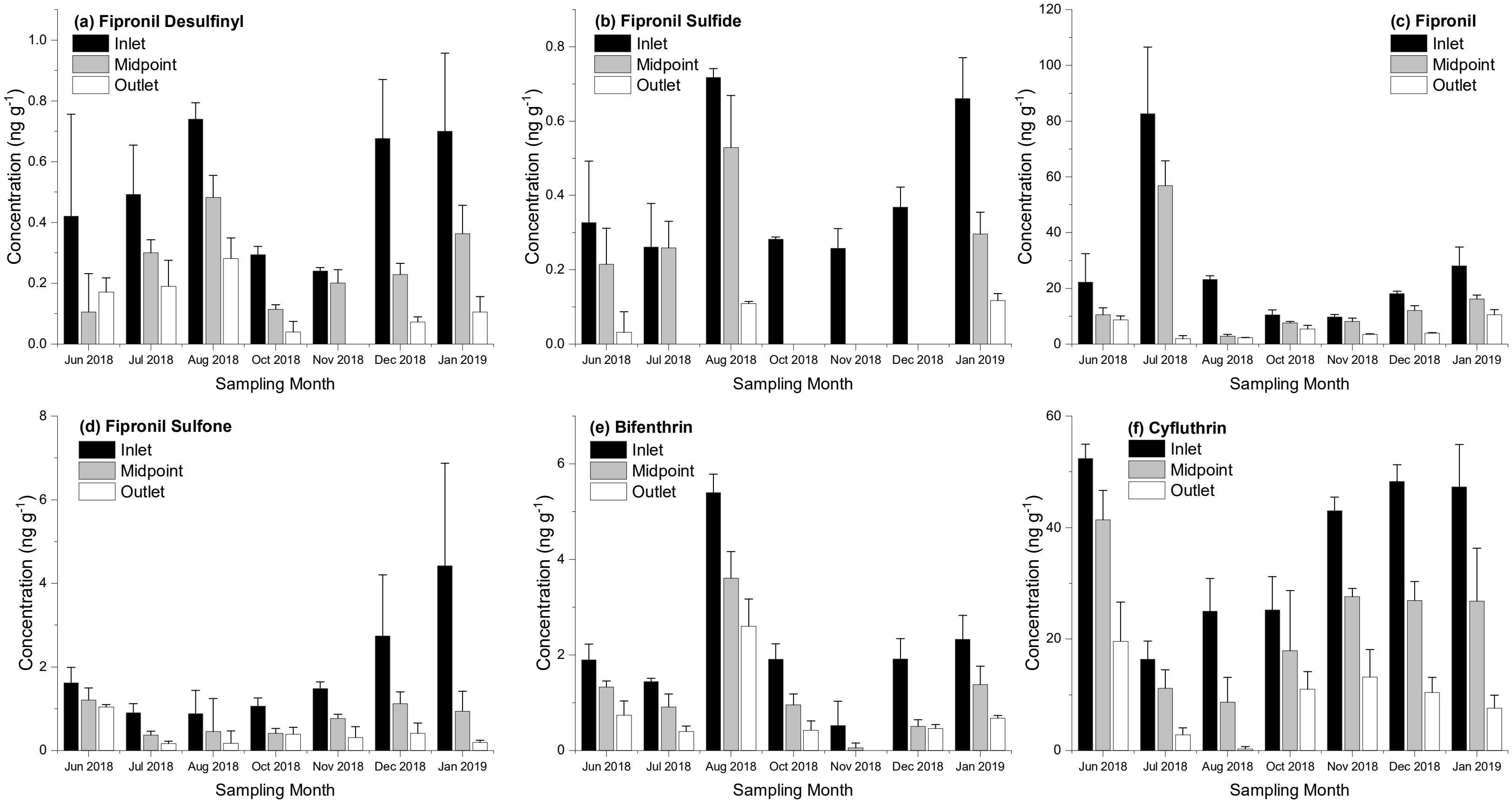


the peak for fipronil sulfide occurred in August 2018. Taken together, these trends correspond to high fipronil application rates in the summer months, gradual decrease via degradation of the parent compound and delayed formation of degradates, and increased transport of applied and degraded residues due to rainfall events in the winter months of 2018-2019. The mean whole water concentrations of bifenthrin and cyfluthrin, on the other hand, steadily increased from June-August 2018, gradually decreased until November 2018, and then increased in December 2018 and January 2019. The mean bifenthrin concentrations peaked in August 2018 while mean cyfluthrin concentrations peaked in January 2019. These trends indicate high use of pyrethroids in the summer months, followed by a period of reduced use and transport, and finally an increase due to runoff from the rain events in the winter that could have transported additional residues through the CW and/or caused resuspension of residues from the sediment bed.

Fipronil and cyfluthrin were detected at the highest levels in whole water PCW samples, with mean concentrations ranging from 13.5-369 and 13.8-455 ng L^{-1}, respectively (Figure 3.1). Fipronil sulfone and bifenthrin were detected at moderate mean concentrations of ND-32.5 and ND-63.9 ng L^{-1}, respectively. The compounds detected at the lowest levels, fipronil desulfinyl and fipronil sulfide, had mean whole water concentrations of ND-2.48 and ND-4.09 ng L^{-1}, respectively. The water concentrations of fiproles and pyrethroids measured in this study were similar to previous measurements in California in urban runoff (Gan et al., 2012; Weston et al., 2009).

Figure 3.2 shows the sediment concentrations (dry weight basis) of fiproles and pyrethroids in the PCW. As was the case with mean levels in whole water samples, mean

Figure 3.2: Sediment (dry weight) concentrations of fipronil desulfinyl (a), fipronil sulfide (b), fipronil (c), fipronil sulfone (d), bifenthrin (e), and cyfluthrin (f) in samples collected from the Prado Constructed Wetland. Data are reported as mean ± 1 SD.



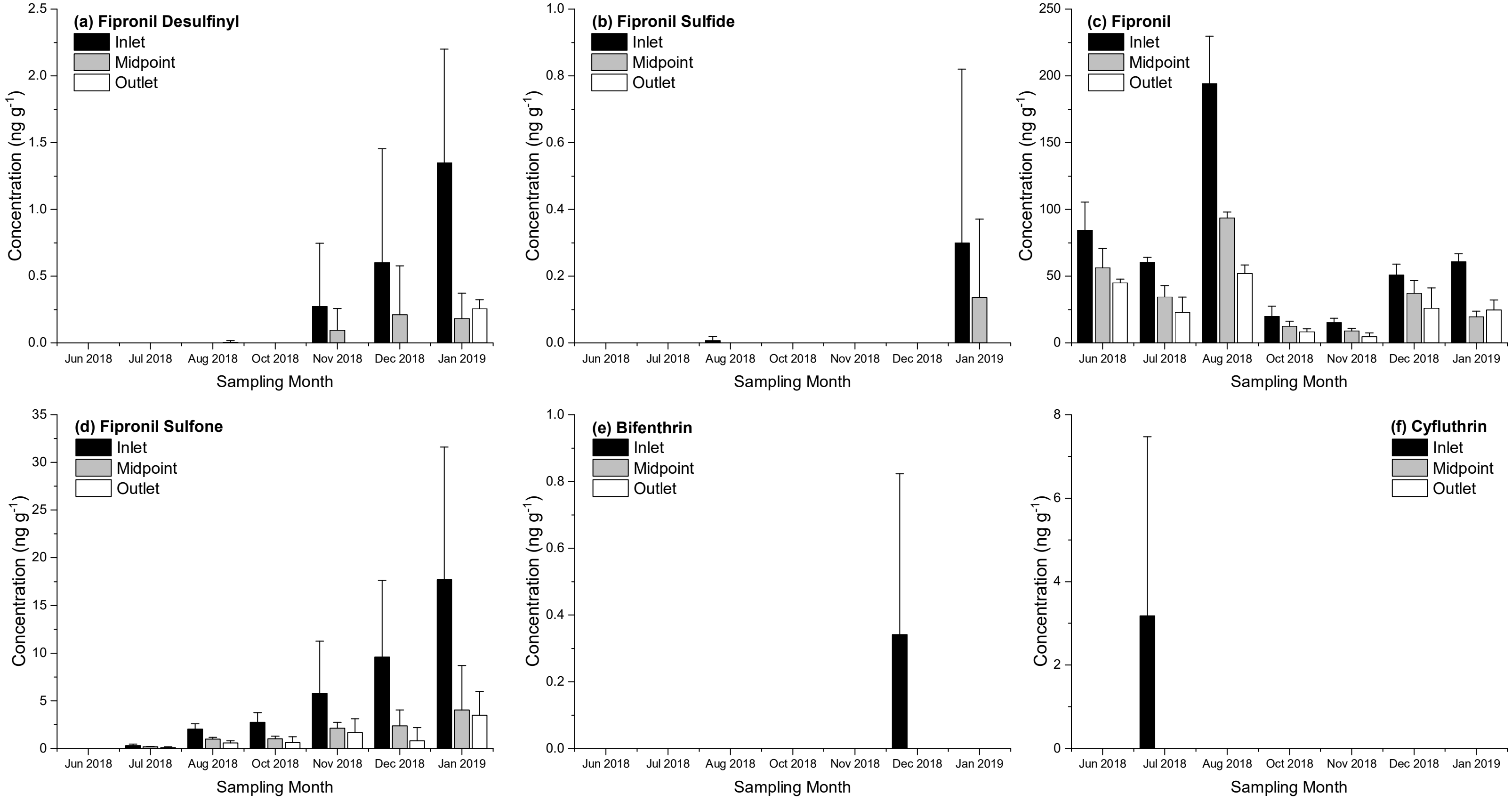
sediment concentrations for all compounds followed a decreasing spatial trend from the inlet to midpoint to outlet. Since the same spatial pattern was observed for mean water concentrations, this suggests that sediment binding was partially responsible for the dissipation of fiproles and pyrethroids from the water. This finding was to be expected due to the hydrophobicity and strong affinity of these two insecticide classes, particularly for pyrethroids. This was in agreement with previous studies where sediment binding was shown to be an important removal mechanism for pyrethroids in flow-through wetlands receiving agricultural drainage (Budd et al., 2009, 2011). However, based on this spatial trend alone, it was unclear whether the contaminants were temporarily retained by the sediment and available for partition back into the passing water or if they were subsequently degraded in the sediment.

The mean sediment concentrations of fipronil desulfinyl, fipronil sulfide, and bifenthrin followed a similar trend over time (Figure 3.2). These contaminants generally increased in concentration from June-August 2018, when they reached a peak for the entire study, followed by a decreasing trend until November 2018 before gradually increasing until January 2019. Fipronil in the sediment increased from June-July 2018 when it reached its peak level, decreased until November 2018, and then increased until January 2019. Fipronil sulfone and cyfluthrin peaked in January 2019 and June 2018, respectively, but they followed the same trend as the other compounds. An inspection of Figure 3.1 and Figure 3.2 reveals similar seasonal temporal trends for fiproles and pyrethroids in both whole water and sediment samples. In other words, when analytes were present at high levels in whole water samples, they tended to also be present at high levels in the sediment. This finding, combined with the fact that sediment concentrations (Figure 3.2) did not continuously increase over the duration of the study, indicates that fiproles and pyrethroids were likely actively degraded once partitioned into the sediment phase. Another possibility is that the contaminated sediment particles underwent resuspension and were carried out of the PCW; however, the generally lower whole water concentrations (Figure 3.1) at the outlet relative to the inlet suggested that the contribution of this process was likely negligible.

Among the six compounds, fipronil (1.93-82.7 ng g^{-1}) and cyfluthrin (0.263-52.4 ng g^{-1}) were detected at the highest mean sediment concentrations during the study period (Figure 3.2). Fipronil sulfone and bifenthrin were present at moderate levels ranging from 0.166-4.42 and ND-5.40 ng g^{-1}, respectively. The lowest mean levels were found for fipronil desulfinyl and fipronil sulfide, at ND-0.740 and ND-0.718 ng g^{-1}, respectively. This pattern was also reflected in the whole water concentrations (Figure 3.1). These results again suggest that fiprole and pyrethroid residues in PCW water partitioned into the sediment and underwent degradation on site, rather than accumulated over time.

Fiprole and pyrethroid concentrations in whole plant samples are shown in Figure 3.3. Mean plant concentrations follow the same spatial trend as whole water or sediment concentrations, with levels generally decreasing from inlet to midpoint to outlet for all compounds. Since the spatial trends of fiproles and pyrethroids are the same in whole water and plant samples, it appears that plant uptake played a role in the removal of these insecticides in the PCW. Fipronil is a systemic insecticide, so some degree of plant uptake of fiproles was to be expected (Simon-Delso et al., 2015). Detection of pyrethroid

Figure 3.3: Plant tissue concentrations of fipronil desulfinyl (a), fipronil sulfide (b), fipronil (c), fipronil sulfone (d), bifenthrin (e), and cyfluthrin (f) in samples collected from the Prado Constructed Wetland. Data are reported as mean ± 1 SD.



residues in whole plant samples was an unexpected finding due to the hydrophobicity of these insecticides and their consequent affinity for sediment. However, there are studies that have documented detection of pyrethroids in plant samples, either by uptake (Bouldin et al., 2006; Mikami et al., 1985) or by apparent irreversible sorption to plant tissues (Hand et al., 2001).

Different temporal trends were observed for fipronil, fipronil degradation products, and the pyrethroids in plant tissues (Figure 3.3). Fipronil mean plant concentrations initially decreased from June-July 2018, increased to peak levels in August 2018, gradually decreased until November 2018, and progressively increased until January 2019. This trend of fipronil concentrations over time coincided with the temporal trends of fipronil in whole water and sediment samples, providing more evidence that plant uptake contributed to the removal of fipronil in the PCW. Moreover, it is likely that fipronil initially adsorbed to wetland sediment and was then absorbed into macrophyte roots. The mean plant concentrations of fipronil desulfinyl, fipronil sulfide, and fipronil sulfone all gradually increased from June 2018-January 2019, indicating some degree of accumulation in plant tissues over time. However, since fipronil did not follow this temporal trend of accumulation in wetland macrophytes, it is likely that some of the parent compound was metabolized into these derivatives upon uptake. Fipronil sulfone was present at higher concentrations in plant samples than the other degradation products, which was in agreement with previous studies showing that *in vivo* plant oxidation is a major metabolic pathway for absorbed fipronil (Simon-Delso et al., 2015). Bifenthrin and cyfluthrin displayed no temporal trend in plant tissues since they were both only detected in inlet samples at one time point. This suggests that plant adsorption or absorption did not play a major role in the removal of pyrethroids by the PCW.

Fipronil was detected at the highest levels in PCW plants, with mean concentrations of 4.70-194 ng g^{-1} (Figure 3.3). Moderate mean concentrations of ND-17.7 ng g^{-1} were observed for fipronil sulfone. The lowest mean plant concentrations were measured for fipronil desulfinyl (ND-1.35 ng g^{-1}), fipronil sulfide (ND-0.300 ng g^{-1}), bifenthrin (ND-0.341 ng g^{-1}), and cyfluthrin (ND-3.18 ng g^{-1}). The results of plant tissue analysis reveal that plant uptake played an important role for the removal, degradation, and storage of fipronil, but did not contribute substantially to the removal of fipronil degradation products or pyrethroids. However, it must be noted that the dense vegetation was essentially slowing down the flow and filtering off suspended solids, contributing greatly to the removal through sedimentation. In addition, microbial activity in the rhizosphere of plant roots likely facilitated the degradation of these chemicals in the sediment, further contributing to the overall pesticide removal.

3.3.2 Removal and Mass Flux of Fiproles and Pyrethroids

The concentration-based removal values of fiproles and pyrethroids from water flowing through the PCW are given in Table 3.1. Over the entire course of the study, removal values for fipronil desulfinyl, fipronil sulfide, fipronil, fipronil sulfone, bifenthrin, and cyfluthrin were 100%, 99.7-100%, 57.8-88.1%, 75.6-100%, 74.7-100%, and 36.6-82.2%, respectively. The compounds with the highest removal values were fipronil desulfinyl, fipronil sulfide, fipronil sulfone, and bifenthrin, while fipronil and cyfluthrin showed the lowest removal. It is important to note that only fipronil, bifenthrin,

Table 3.1: Mass flux and concentration-based removal of fiproles and pyrethroids from the Prado Constructed Wetland (2018-2019). Data are reported as mean ± 1 SD where applicable.

		June	**July**	**August**	**October**	**November**	**December**	**January**
Fipronil Desulfinyl	**Mass Influx[a] (mg d^{-1})**	0	0	6.53 ± 11.3	0	0	0.268 ± 0.465	22.2 ± 5.46
	Mass Efflux[b] (mg d^{-1})	0	0	0	0	0	0	-[d]
	Δ Mass Flux (mg d^{-1})	0	0	6.53[c]	0	0	0.268	-[e]
	Removal (%)	-	-	100	-	-	100	100
Fipronil Sulfide	**Mass Influx (mg d^{-1})**	0	0	44.9 ± 21.5	0	0	0	29.7 ± 1.61
	Mass Efflux (mg d^{-1})	0	0	0.143 ± 0.247	0	0	0	-
	Δ Mass Flux (mg d^{-1})	0	0	44.8	0	0	0	-
	Removal (%)	-	-	99.7	-	-	-	100
Fipronil	**Mass Influx (mg d^{-1})**	4050 ± 1250	12700 ± 3560	1870 ± 427	566 ± 183	338 ± 77.0	115 ± 24.0	1980 ± 1240
	Mass Efflux (mg d^{-1})	413 ± 329	532 ± 478	518 ± 212	137 ± 61.9	765 ± 85.1	135 ± 51.9	-
	Δ Mass Flux (mg d^{-1})	3640*[f]	12100*	1350*	429	-426*[g]	-20.0	-
	Removal (%)	88.1	77.3	72.2	57.8	57.8	74.8	59.8
Fipronil Sulfone	**Mass Influx (mg d^{-1})**	25.1 ± 24.6	0	52.0 ± 48.9	81.0 ± 24.1	1.97 ± 1.72	9.02 ± 1.37	292 ± 122
	Mass Efflux (mg d^{-1})	0	0	0	4.53 ± 2.36	0	3.38 ± 2.93	-
	Δ Mass Flux (mg d^{-1})	25.1	0	52.0	76.5*	1.97	5.64	-
	Removal (%)	100	-	100	90.3	100	92.0	75.6

Bifenthrin	**Mass Influx (mg d^{-1})**	25.5 ± 10.8	646 ± 292	701 ± 96.0	376 ± 71.5	8.93 ± 13.6	2.37 ± 0.822	288 ± 52.4
	Mass Efflux (mg d^{-1})	0	19.8 ± 17.6	177 ± 67.2	48.7 ± 46.5	0	0	-
	Δ Mass Flux (mg d^{-1})	25.5	626	524*	328*	8.93	2.37*	-
	Removal (%)	100	83.5	74.7	77.4	100	100	87.0
Cyfluthrin	**Mass Influx (mg d^{-1})**	542 ± 65.3	6090 ± 1100	2790 ± 374	2440 ± 543	198 ± 40.1	100 ± 13.7	4090 ± 1270
	Mass Efflux (mg d^{-1})	129 ± 6.45	716 ± 40.9	497 ± 115	564 ± 291	338 ± 323	293 ± 69.8	-
	Δ Mass Flux (mg d^{-1})	413*	5380*	2300*	1880*	-140	-193*	-
	Removal (%)	72.0	36.6	82.2	59.7	68.1	37.6	76.7

[a] Influx is the flow of a given analyte into the wetland at the inlet weir box.

[b] Efflux is the flow of a given analyte out of the wetland at the outlet weir box.

[c] Positive values of Δ Mass Flux indicate import of a given analyte to the wetland cell.

[d] Due to rain-induced flooding of the outlet weir box in January, no flow calculation could be performed. As a result, no mass flux at the outlet weir box could be calculated.

[e] Since mass efflux could not be calculated for January, it was not possible to calculate Δ Mass Flux for this time point.

[f] Asterisks indicate a statistically significant ($p < 0.05$) difference in mass flux between inlet and outlet measurements.

[g] Negative values of Δ Mass Flux indicate export of a given analyte from the wetland cell.

and cyfluthrin were detected every month, and fipronil and cyfluthrin were detected at higher levels than all the other compounds. Previous studies have similarly shown that the average CW removal rates of fipronil and pyrethroids were 44% and 52-94%, respectively, for other CW systems (Budd et al., 2009; Supowit et al., 2016).

Removal of fiproles and pyrethroids followed no apparent monthly or seasonal trends (Table 3.1). Based on this finding, and on the conclusions drawn from the data presented in Figures 3.1-3.3, it is likely that removal of these compounds was not markedly influenced by seasonal fluctuations in temperature or weather patterns and was more dependent on availability of sediment bindings sites, degradation in the sediment compartment, and, in the case of fipronil, rate of plant uptake. Sediment microorganisms responsible for biotic degradation likely appeared to be unaffected by seasonal temperature variations because temperatures are relatively high year-round in Southern California. In addition, the emergent macrophytes present in the PCW experience rampant growth due to the constant availability of nutrients in the Santa Ana River water. Therefore, aquatic macrophytes were always available for plant uptake and subsequent transformation of fipronil.

In addition to concentration removal, another important metric for ascertaining the efficacy of the PCW is the mass flux of fiproles and pyrethroids (Table 3.1). In particular, mass influx, mass efflux, and change in mass flux were calculated from the chemical concentrations and water flow rates. Fipronil, bifenthrin, and cyfluthrin were imported into the PCW at the highest rates, with mean mass influxes of 115-12700 mg d^{-1}, 2.37-701 mg d^{-1}, and 100-6090 mg d^{-1}, respectively. Fipronil desulfinyl (0-22.2 mg d^{-1}), fipronil sulfide (0-44.9 mg d^{-1}), and fipronil sulfone (0-292 mg d^{-1}) exhibited much lower import rates into the wetland. Changes in mass flux, which represents the net import (positive value) or export (negative value) of chemicals to or from the PCW, were also the highest for fipronil (-426-12100 mg d^{-1}), bifenthrin (2.37-626 mg d^{-1}), and cyfluthrin (-193-5380 mg d^{-1}). The majority of changes in mass flux for these three compounds were statistically significant. In contrast, only one of the changes in mass flux values was statistically significant for fipronil desulfinyl (0-6.53 mg d^{-1}), fipronil sulfide (0-44.8 mg d^{-1}), and fipronil sulfone (0-76.5 mg d^{-1}) since the difference between the mass influxes and effluxes for these compounds was much smaller.

Negative changes in mass flux values were found for fipronil and cyfluthrin in the months of November and December 2018, though only two of these four measurements were statistically significant (Table 3.1). However, the export of these compounds during this time corresponded to higher outflow than inflow for the PCW (Table S3.2), which likely resulted in resuspension of sediment particles as evidenced by the negative sedimentation rates observed during these two months. The flow of water through the entire Prado wetlands is regulated to optimize water quality and quantity, which leads to occasional net outflow from certain wetland cells, as was the case for the PCW in November and December 2018. Therefore, although net mass export of fipronil and cyfluthrin occurred during this time, it was compensated for by the high volume of water exiting the PCW, as indicated by the positive concentration removal values (Table 3.1). Although no outflow, mass efflux, or change in mass flux values could be calculated for January 2019, it is important to mention that the rainfall that occurred during this month resulted in spikes of chemical mass influx and possibly additional resuspension of contaminated sediment particles. However, positive removal values again showed that dilution prevented an increase in outlet concentrations.

To further highlight the importance of adsorption in the removal of fiproles and pyrethroids by the PCW, the relative presence of each compound on TSS obtained from water samples was calculated (Table 3.2). With two exceptions, the % of each chemical on TSS at the wetland inlet was statistically similar to the outlet value. In August 2018, an average of 87.7% of the bifenthrin in the whole water sample was associated with TSS at the inlet, as compared to 100% at the outlet. In contrast, values decreased from 92.1% at the inlet to 71.9% at the outlet for cyfluthrin in December 2018. In addition, inlet, midpoint, and outlet values were anomalously low for fipronil and cyfluthrin in July 2018. It is likely that some cyfluthrin was associated with dissolved organic matter and included in the aqueous phase concentration (Gustafsson et al., 2001). The lower values for fiproles may be attributed to their moderate hydrophobicity as compared to the pyrethroids. The overall results suggested that the fractions of these compounds on TSS were similar throughout the PCW and were also similar over time, with inlet and outlet values ranging from 60-100%. Combined with the evidence for the importance of sediment binding in the removal of pyrethroids and fiproles, it may be concluded that adsorption to suspended particles and subsequent sedimentation was likely a dominant process governing the fate and transport of these contaminants in CW systems.

Linear regression was carried out to identify additional factors contributing to the removal of fiproles and pyrethroids in the PCW (Table 3.3). Two dependent variables,

Table 3.2: Relative presence of fiproles and pyrethroids on total suspended solids (TSS) present in water samples collected from the Prado Constructed Wetland. Data are reported as mean ± 1 SD.

		July[a]	**August**	**October**	**November**	**December**	**January**
Fipronil Desulfinyl on TSS (%)	**Inlet**	-[b]	100[c]	-	-	100	100 ± 0
	Midpoint	-	-	-	-	-	100 ± 0
	Outlet	-	-	-	-	-	-
Fipronil Sulfide on TSS (%)	**Inlet**	-	79.3 ± 2.19	-	-	-	100 ± 0
	Midpoint	-	98.1	-	-	-	100 ± 0
	Outlet	-	100	-	-	-	-
Fipronil on TSS (%)	**Inlet**	3.30 ± 5.72	84.7 ± 13.9	100 ± 0	71.9 ± 9.12	82.3 ± 1.51	95.2 ± 4.19
	Midpoint	0	100 ± 0	100 ± 0	76.8 ± 8.06	98.6 ± 2.50	98.4 ± 2.76
	Outlet	1.13 ± 1.60	93.6 ± 11.1	100 ± 0	61.6 ± 7.71	75.8 ± 8.16	100 ± 0
Fipronil Sulfone on TSS (%)	**Inlet**	-	94.9 ± 7.20	100 ± 0	100 ± 0	100 ± 0	100 ± 0
	Midpoint	-	-	100 ± 0	-	100 ± 0	100 ± 0
	Outlet	-	-	100 ± 0	-	100 ± 0	100 ± 0
Bifenthrin on TSS (%)	**Inlet**	100 ± 0	87.7 ± 3.08	95.2 ± 8.34	100 ± 0	92.5 ± 13.1	100 ± 0
	Midpoint	-	84.1 ± 3.07	100 ± 0	100	100	100 ± 0
	Outlet	66.7 ± 57.7	100 ± 0*[d,e]	100 ± 0	-	-	100 ± 0
Cyfluthrin on TSS (%)	**Inlet**	32.5 ± 9.64	93.5 ± 5.88	100 ± 0	84.6 ± 11.6	92.1 ± 5.44	100 ± 0
	Midpoint	25.7 ± 12.7	96.3 ± 6.45	100 ± 0	86.9 ± 12.1	100 ± 0	100 ± 0
	Outlet	21.7 ± 7.44	100 ± 0	100 ± 0	100 ± 0	71.9 ± 4.05*	100 ± 0

[a] Values for June 2018 are not included since the method used to separate the water and TSS phases for this initial time point resulted in incomplete filtration based on visual inspection of filtered samples. A more robust method was adopted for the remaining samples. Whole water concentrations were still obtained for these incompletely filtered samples.

[b] Hyphens indicate that the analyte was not detected in the aqueous or TSS phase.

[c] Data reported without a standard deviation indicate that only one sample contained analyte at a detectable level.

[d] Statistical tests were performed when there was mean inlet and outlet data available.

[e] Asterisks indicate a statistically significant ($p < 0.05$) difference in the percentage of analyte adsorbed to TSS between inlet and outlet measurements.

concentration-based removal and change in mass flux, and three independent variables, sedimentation rate, water pH, and water temperature, were considered. Statistically significant linear relationships were observed between fipronil removal and water pH ($p = 0.0248$, $R^2 = 0.67$), between change in fipronil mass flux and sedimentation rate ($p = 0.0001$, $R^2 = 1$), and between change in cyfluthrin mass flux and sedimentation rate ($p = 0.0175$, $R^2 = 0.88$). These results demonstrate that water pH and water temperature had minimal impact on the removal of fiproles and pyrethroids in the PCW, with the exception of the influence of water pH on the concentration-based removal of fipronil. This finding may emphasize the importance of pH in determining the ionization state of fipronil and hence its adsorption onto sediment particles under field conditions. In addition, the effect of sedimentation rate on the changes in mass flux for fipronil and cyfluthrin—the analytes detected at the highest concentrations and mass influxes—further supports the notion that settling of insecticide-laden particles played a major role in the removal of fiproles and pyrethroids. The evidence provided by this study therefore highlights that settling of contaminated particles and partition into the wetland sediment are crucial in achieving removal of these urban-use insecticides, which is in agreement with findings from an agricultural drainage wetland (Budd et al., 2009, 2011).

3.3.3 Invertebrate Toxicity Estimation

To understand the effect of PCW treatment in mitigating the potential toxicity of fiproles and pyrethroids to sensitive aquatic invertebrates, toxic units (TUs) were calculated using the following equation (Weston and Lydy, 2014):

Table 3.3: Linear regression analyses of fiprole and pyrethroid concentration-based removal and change in mass flux versus sedimentation rate, water pH, and water temperature.

	Fipronil	**Fipronil Sulfone**	**Bifenthrin**	**Cyfluthrin**
Removal (%) vs. Sedimentation Rate (kg d^{-1})	Removal = (Sedimentation Rate)*(0.00326) + 65.1	-[a]	Removal = (Sedimentation Rate)*(-0.00239) + 89.2	Removal = (Sedimentation Rate)*(-0.00555) + 61.8
R^2	0.35	-	0.11	0.23
p-value	0.294	-	0.580	0.410
Removal (%) vs. Water pH	Removal = (Water pH)*(67.3) – 436	Removal = (Water pH)*(49.5) – 278	Removal = (Water pH)*(21.4) – 71.8	Removal = (Water pH)*(-11.2) + 146
R^2	0.67	0.53	0.074	0.008
p-value	0.0248*[b]	0.101	0.555	0.854
Removal (%) vs. Water Temperature (°C)	Removal = (Water Temperature)*(1.22) + 52.2	Removal = (Water Temperature)*(1.08) + 77.6	Removal = (Water Temperature)*(-0.467) + 95.6	Removal = (Water Temperature)*(1.01) + 47.5
R^2	0.36	0.50	0.058	0.10
p-value	0.152	0.116	0.602	0.491
Δ Mass Flux (mg d^{-1}) vs. Sedimentation Rate (kg d^{-1})	Δ Mass Flux = (Sedimentation Rate)*(3.09) - 59.9	-	Δ Mass Flux = (Sedimentation Rate)*(0.128) + 183	Δ Mass Flux = (Sedimentation Rate)*(1.25) + 732
R^2	1	-	0.59	0.88
p-value	0.0001*	-	0.130	0.0175*
Δ Mass Flux (mg d^{-1}) vs. Water pH	Δ Mass Flux = (Water pH)*(22300) – 166000	Δ Mass Flux = (Water pH)*(-61.7) + 496	Δ Mass Flux = (Water pH)*(112) – 592	Δ Mass Flux = (Water pH)*(3970) – 28300
R^2	0.38	0.070	0.0028	0.061
p-value	0.192	0.668	0.921	0.637

Δ Mass Flux (mg d^{-1}) vs. Water Temperature (°C)	Δ Mass Flux = (Water Temperature)*(148) + 558	Δ Mass Flux = (Water Temperature)*(2.57) – 8.36	Δ Mass Flux = (Water Temperature)*(16.0) + 4.24	Δ Mass Flux = (Water Temperature)*(76.3) + 419
R^2	0.025	0.21	0.086	0.034
p-value	0.764	0.432	0.573	0.726

[a] Hyphens indicate that there was insufficient data to perform linear regression analysis.

[b] Asterisks indicate a statistically significant ($p < 0.05$) linear correlation.

$$TU = \frac{\text{Observed analyte concentration}}{\text{Species-specific analyte toxicity value}} \tag{2}$$

A TU value of 1 or greater indicates that toxicity would occur if the organism in question were exposed to an analyte at the observed concentration. The toxicity threshold values used in the calculations may be found in Table S3.4 (Weston and Jackson, 2009; Weston and Lydy, 2014).

Calculated TUs based on the concentrations measured at the PCW inlet and outlet are given in Table 3.4. Sublethal (EC_{50}) and lethal (LC_{50}) toxicity values for the amphipod *Hyalella azteca* were used to determine the change in potential pyrethroid toxicity between inlet and outlet measurements since previous research has demonstrated this organism's sensitivity to pyrethroids (Anderson et al., 2006; Maund et al., 1998; Weston and Jackson, 2009). Mean sublethal bifenthrin TUs decreased from 0.704-19.4 at the inlet to 0-4.91 at the outlet, while mean lethal TUs decreased from 0.302-8.30 to 0-2.10 at the inlet and outlet, respectively. All decreases were statistically significant except for the month of November 2018 when inlet TUs were relatively low with high variability and outlet TUs were 0 since no bifenthrin was detected. Cyfluthrin mean sublethal TUs were 26.0-240 at the inlet, and decreased to 7.27-68.0 at the outlet. The corresponding mean lethal TUs were 21.5-198 and 6.01-56.1 at the inlet and outlet, respectively. All decreases in cyfluthrin TUs were statistically significant.

The midge *Chironomus dilutus* was selected for the calculation of fiprole TUs (Table 3.4) since it has been shown to be extremely sensitive to these chemicals (Weston and Lydy, 2014). Mean sublethal TUs for fipronil sulfide decreased from 0-0.414 at the

Table 3.4: Toxic units (TU) of fiproles and pyrethroids in whole water samples collected from the Prado Constructed Wetland. Data are reported as mean ± 1 SD.

				June	**July**	**August**	**October**	**November**	**December**	**January**
***Hyalella azteca*[a,b]**	**Bifenthrin**	**TU EC_{50}**	**Inlet**	0.704 ± 0.297	2.52 ± 1.14	19.4 ± 2.65	6.44 ± 1.22	1.07 ± 1.63	1.48 ± 0.513	9.70 ± 1.77
			Outlet	0*[e]	0.417 ± 0.371*	4.91 ± 1.86*	1.46 ± 1.39*	0	0*	1.26 ± 2.19*
		TU LC_{50}	**Inlet**	0.302 ± 0.127	1.08 ± 0.488	8.30 ± 1.14	2.76 ± 0.525	0.460 ± 0.700	0.635 ± 0.220	4.16 ± 0.758
			Outlet	0*	0.179 ± 0.159*	2.10 ± 0.796*	0.624 ± 0.595*	0	0*	0.542 ± 0.938*
	Cyfluthrin	**TU EC_{50}**	**Inlet**	26.0 ± 3.13	41.3 ± 7.44	134 ± 17.9	72.6 ± 16.1	41.3 ± 8.37	109 ± 14.9	240 ± 74.3
			Outlet	7.27 ± 0.363*	26.2 ± 1.49*	23.9 ± 5.54*	29.3 ± 15.1*	13.2 ± 12.6*	68.0 ± 16.2*	55.8 ± 19.7*
		TU LC_{50}	**Inlet**	21.5 ± 2.59	34.1 ± 6.14	111 ± 14.8	60.0 ± 13.3	34.1 ± 6.91	89.9 ± 12.3	198 ± 61.4
			Outlet	6.01 ± 0.300*	21.6 ± 1.23*	19.7 ± 4.58*	24.2 ± 12.5*	10.9 ± 10.4*	56.1 ± 13.4*	46.1 ± 16.3*
***Chironomus dilutus*[c,d]**	**Fipronil Sulfide**	**TU EC_{50}**	**Inlet**	0	0	0.414 ± 0.198	0	0	0	0.334 ± 0.0181
			Outlet	0	0	0.00131 ± 0.00228*	0	0	0	0*
		TU LC_{50}	**Inlet**	0	0	0.0592 ± 0.0284	0	0	0	0.0479 ± 0.00260
			Outlet	0	0	1.88E-4 ± 3.26E-4*	0	0	0	0*
	Fipronil	**TU EC_{50}**	**Inlet**	11.4 ± 3.50	5.02 ± 1.41	5.24 ± 1.20	0.984 ± 0.318	4.13 ± 0.939	7.28 ± 1.52	6.77 ± 4.25
			Outlet	1.36 ± 1.08*	1.14 ± 1.02*	1.45 ± 0.700*	0.416 ± 0.188*	1.74 ± 0.194*	1.83 ± 0.703*	2.72 ± 2.19
		TU LC_{50}	**Inlet**	4.53 ± 1.40	2.00 ± 0.563	2.09 ± 0.477	0.392 ± 0.127	1.65 ± 0.375	2.90 ± 0.607	2.70 ± 1.69
			Outlet	0.540 ± 0.432*	0.454 ± 0.407*	0.580 ± 0.279*	0.166 ± 0.0749*	0.694 ± 0.0773*	0.730 ± 0.281*	1.08 ± 0.875
	Fipronil Sulfone	**TU EC_{50}**	**Inlet**	0.296 ± 0.292	0	0.615 ± 0.579	0.595 ± 0.177	0.102 ± 0.0886	2.41 ± 0.366	4.22 ± 1.76
			Outlet	0	0	0	0.0580 ± 0.0303*	0	0.193 ± 0.168*	1.03 ± 0.399*
		TU LC_{50}	**Inlet**	0.0219 ± 0.0216	0	0.0455 ± 0.0429	0.0440 ± 0.0131	0.00752 ± 0.00656	0.179 ± 0.0271	0.312 ± 0.131
			Outlet	0	0	0	0.00429 ± 0.00224*	0	0.0143 ± 0.0124*	0.0761 ± 0.0295*

[a] Toxicity values for *Hyalella azteca* sourced from Weston and Jackson, 2009.

[b] Sublethal end point for EC_{50}: ability to swim.

[c] Toxicity values for *Chironomus dilutus* sourced from Weston and Lydy, 2014.

[d] Sublethal end point for EC_{50}: ability to thrash when prodded.

[e] Asterisks indicate a statistically significant ($p < 0.05$) difference between the inlet and outlet TU values.

inlet to 0-0.00131 at the outlet in a statistically significant manner. Similarly, mean lethal fipronil sulfide TUs underwent statistically significant decreases from 0-0.0592 at the inlet to 0-1.88 x 10^{-4} at the outlet. Fipronil mean sublethal TUs decreased from 0.984-11.4 at the inlet to 0.416-2.72 at the outlet, while mean lethal TUs decreased from 0.392-4.53 at the inlet to 0.166-1.08 at the outlet. All inlet-outlet comparisons for fipronil were statistically significant except for TUs corresponding to the month of January 2019 when variability in concentrations at the inlet was high. Mean sublethal fipronil sulfone TUs were 0-4.22 at the inlet, decreasing to 0-1.03 at the outlet. Mean lethal TUs for fipronil sulfone were 0-0.312 and 0-0.0761 at the inlet and outlet, respectively. Statistically significant differences for fipronil sulfone were only observed in the months of October 2018, December 2018, and January 2019, but TU values for the other months were all <1 at the inlet and 0 at the outlet.

These results showed that removal of fiproles and pyrethroids by the PCW resulted in toxicity reductions for all urban-use insecticides, and the reductions were statistically significant in most instances (Table 3.4). The TU values reported in this study represent hypothetical worst-case single chemical exposure scenarios for the most sensitive aquatic invertebrates. Furthermore, the TU values were derived from whole water concentrations and did not take into account bioavailable concentrations. For pyrethroids, studies have shown that bioavailability in whole water and sediment is inhibited by DOM or organic matter (Cui et al., 2013; Xue et al., 2017). Therefore, it is likely that the TU values in this study overestimated the actual toxicity and would serve as a conservative assessment. The influence of bioavailability on fiproles should be less significant given their moderate hydrophobicity. Moreover, the TUs calculated from PCW data do not represent the Prado Wetlands as a whole, since it is composed of many interconnected ponds operating in series. The effluent from the PCW undergoes dilution as it recombines with additional treated water emanating from adjacent wetland cells, is subjected to further treatment, and is ultimately deposited into Chino Creek. As a result, the TU values for the entire treatment chain would very likely be further reduced.

3.4 Conclusions

The results of this study have revealed that a CW receiving water from an urban river was effective in removing the common urban-use insecticides considered in this study. It is clear that sedimentation of contaminated particles, adsorption of dissolved analytes to wetland sediment, uptake into or adsorption to macrophyte tissues, and subsequent degradation were the major mechanisms responsible for removal of these compounds. In addition, this removal coincided with reductions in potential toxicity to sensitive aquatic invertebrates. These findings indicate that CWs would be effective in removing these and similar contaminants from stormwater runoff or treated wastewater as a component of decentralized stormwater treatment or as a polishing step in WWTPs, respectively. CWs require a great deal of land to install and develop, but do not necessitate high operating costs relative to traditional wastewater treatment. Therefore, they could be utilized in conjunction with pollution mitigation efforts to reduce the overall contamination of surface water, reducing potential ecotoxicity and providing higher quality water for reuse initiatives. Future research should focus on further examining the mechanisms responsible and ideal conditions required for removal of hydrophobic organic contaminants in CWs to optimize the design and size of such systems.

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3.6 Supporting Information

Figure S3.1: Schematic map of the Prado Wetlands in Corona, California (Figure credit: Orange County Water District).

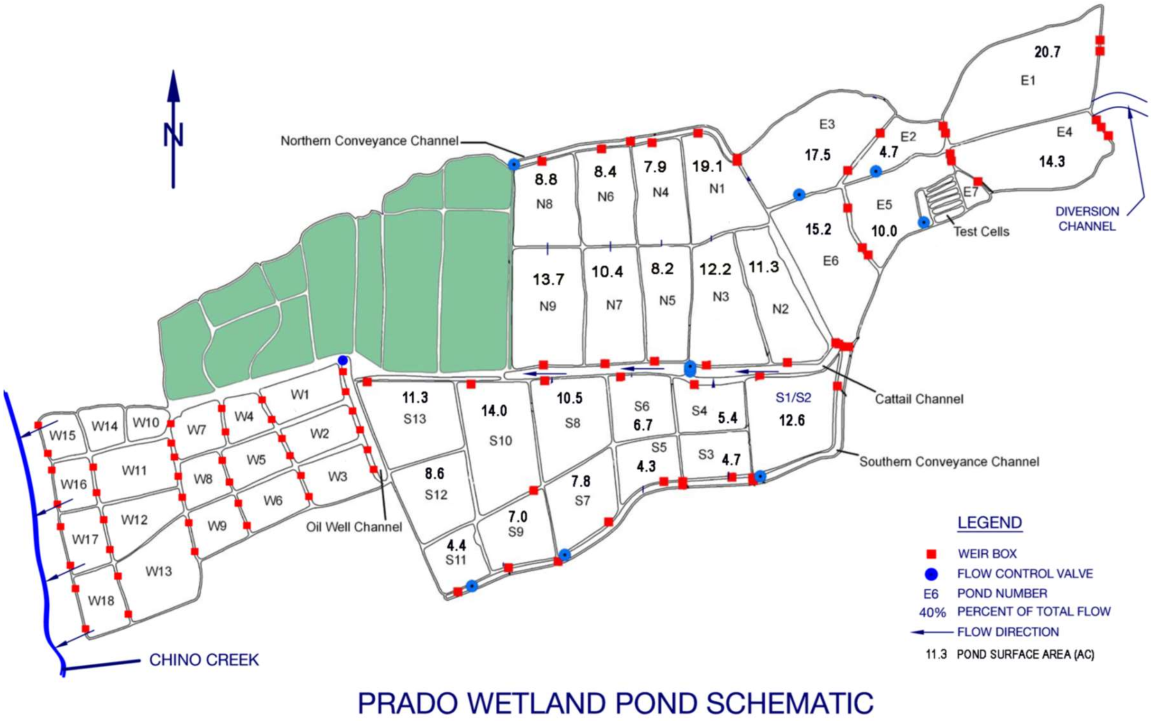


Table S3.1: Prado Constructed Wetland sediment properties.

Sand (%)	Silt (%)	Clay (%)	Texture Classification	pH	Organic Matter (%)	Organic Carbon (%)
43	29	28	Clay loam	7.34	2.35	1.42

Table S3.2: Prado Constructed Wetland flow and sedimentation rates during the study period.

Sampling Month	**Inlet Flow (ML d^{-1})**	**Outlet Flow (ML d^{-1})**	**Sedimentation Rate (kg d^{-1})[a]**
June 2018	11.0	9.35	-[b]
July 2018	77.7	14.4	3910[c]
August 2018	11.0	11.0	585
October 2018	17.7	10.1	278
November 2018	2.52	13.5	-265[d]
December 2018	0.485	2.27	-50.6
January 2019	8.98	-[e]	-[f]

[a] Sedimentation rates were calculated as the difference of the inlet and outlet total suspended solids (TSS) mass fluxes.

[b] The sedimentation rate for June 2018 could not be accurately calculated since the method used to separate the water and TSS phases for this initial time point resulted in incomplete filtration based on visual inspection of filtered samples. A more robust method was adopted for the remaining samples.

[c] Positive sedimentation rates indicate import of TSS into the wetland.

[d] Negative sedimentation rates indicate export of TSS from the wetland.

[e] Due to rain-induced flooding of the outlet weir box in January, no flow calculation could be performed.

[f] The sedimentation rate for January 2019 could not be accurately calculated since there was no outlet flow calculation available to calculate outlet TSS mass flux.

Table S3.3: Prado Constructed Wetland water temperature and pH during the study period. Data are reported as mean ± 1 SD.

Sampling Month	**Water Temperature (°C)[b]**	**Water pH[a]**		
		Inlet	**Midpoint**	**Outlet**
June 2018	22.2 ± 0.289	8.00 ± 0.0231	7.70 ± 0.0529	7.57 ± 0.0321
July 2018	14.4 ± 0.551	7.87 ± 0.0577	7.63 ± 0.0666	7.45 ± 0.0404
August 2018	21.0 ± 0.500	7.64 ± 0.0902	7.60 ± 0.0513	7.33 ± 0.135
October 2018	15.1 ± 0.777	7.54 ± 0.0839	7.43 ± 0.0961	7.23 ± 0.101
November 2018	11.1 ± 0.777	7.74 ± 0.0513	7.39 ± 0.0945	7.41 ± 0.110
December 2018	9.5 ± 1.3	7.59 ± 0.0200	7.40 ± 0.0794	7.33 ± 0.0153
January 2019	6.8 ± 1.5	7.51 ± 0.137	7.27 ± 0.163	7.29 ± 0.0458

[a] Water pH measurement was performed *ex situ* using whole water samples collected for extraction.

[b] Water temperature measurement was performed *in situ* at the locations where water, sediment, and plant samples were collected for extraction.

Table S3.4: Toxicity values used for toxic unit calculation.

Test Organism	Analyte	Toxicity Test	Toxicity Value ($ng\ L^{-1}$)	Source
Hyalella azteca[a]	Bifenthrin	EC_{50}	3.3	Weston and Jackson, 2009
		LC_{50}	7.7	
	Cyfluthrin	EC_{50}	1.9	
		LC_{50}	2.3	
Chironomus dilutus[b]	Fipronil	EC_{50}	32.5	Weston and Lydy, 2014
		LC_{50}	>81.5	
	Fipronil Sulfide	EC_{50}	9.9	
		LC_{50}	>69.1	
	Fipronil Sulfone	EC_{50}	7.7	
		LC_{50}	>104	

[a] Sublethal end point for EC_{50}: ability to swim.
[b] Sublethal end point for EC_{50}: ability to thrash when prodded.

3.6.1 Additional Prado Wetlands Details

The effluent from the Prado Wetlands enters Chino Creek, a tributary of the Santa Ana River, and later rejoins the main river system. Downstream of Prado Wetlands, a significant portion of the Santa Ana River flows are diverted into infiltration basins for groundwater recharge. The receiving aquifer, the Orange County Groundwater Basin, provides approximately 75% of the water for 2.5 million Orange County residents (Orange County Water District, 2018).

The Prado Wetlands are comprised of multiple wetland ponds operated in series (Figure S3.1). The diverted Santa Ana River water is initially separated into two treatment chains in the eastern ponds that are directed into the northern and southern pond complexes via their respective conveyance channels. The water from the two treatment chains is then recombined in a central cattail channel for treatment in the western pond complex prior to entering Chino Creek. The flow throughout the entire system is managed via a series of pumps and weir boxes depending on weather conditions and the volume of river water entering the wetlands. The combined hydraulic retention time of the eastern, northern, and southern ponds was determined to be 7.6 d by a rhodamine WT tracer study (Lin et al., 2003).

3.6.2 Additional GC-MS Analysis Information

The carrier gas was Helium at a flow rate of 1.2 mL min^{-1}. Sample aliquots of 2 μL were injected at 200 °C. The GC oven program was as follows: initial temperature of 120 °C and held for 1 min; ramped up at 25 °C min^{-1} to 205 °C and held for 1 min; ramped up at 1.5 °C min^{-1} to 220 °C and held for 1 min; ramped up at 3 °C min^{-1} to 225 °C and held for 1 min; and finally ramped up at 30 °C min^{-1} to a final temperature of 300 °C followed by a 5 min hold. Analyte quantification was performed using an internal standard-normalized calibration curve ranging from 1 to 500 μg L^{-1}. If necessary, samples were diluted to fall within the range of the calibration curve. Coefficients of determination all fulfilled the requirement of $R^2 \geq 0.99$.

3.6.3 Matrix Spike Extraction Efficiency

For fipronil desulfinyl, fipronil sulfide, fipronil, fipronil sulfone, bifenthrin, and cyfluthrin, respectively, matrix spike extraction efficiencies were as follows: 104 ± 3%, 102 ± 1%, 103 ± 10%, 109 ± 4%, 153 ± 24%, and 102 ± 7% for liquid-liquid extraction; and 110 ± 34%, 94 ± 20%, 112 ± 18%, 115 ± 35%, 101 ± 5%, and 120 ± 4% for pressurized fluid extraction.

3.6.4 Additional Quality Control Measures

All glassware was baked at 400 °C for 4 h to eliminate cross contamination. Na_2SO_4 and NaCl were baked at 400 °C for 4 h before use. Florisil was activated at 130 °C for 4 h before being packed into solid phase extraction cartridges. All amber glass bottles were rinsed with dichloromethane and acetone before sample collection.

Chapter 4 Removal of Urban Insecticides from a Unit Process Open Water Constructed Wetland

4.1 Introduction

Contemporary surface water is contaminated by a host of anthropogenic compounds that threaten aquatic organisms and the ecosystems in which they reside. This pollution exacerbates water scarcity in arid and semi-arid regions that seek to treat water for potable and non-potable reuse (Adhikari et al., 2011; Anderson et al., 2016; Greenway, 2005; Haile et al., 2016; Kadlec and Wallace, 2008; Yang et al., 2006). Climate change and urbanization will require the broad implementation of various water treatment technologies to supply sufficient, high-quality, recycled water for a growing global population (Arnold and Gibbons, 1996; Kummu et al., 2010; McDonald et al., 2011; Seto et al., 2012). Urban surface water is frequently contaminated by fipronil and its degradation products (*collectively*: fiproles) as well as the synthetic pyrethroids, which are hydrophobic insecticides (Demcheck and Skrobialowski, 2003; Laskowski, 2002) utilized at high rates in urban environments for a myriad of pest control applications (Ensminger, 2014; Sadaria et al., 2017; Teerlink et al., 2017; United States Environmental Protection Agency, 2011; Weston et al., 2005, 2009). These contaminants are known to be present in surface water at concentrations exceeding toxicity thresholds for sensitive aquatic organisms (Anderson et al., 2006; California Department of Pesticide Regulation, 2016; Gan et al., 2012; Maund et al., 1998; Weston et al., 2009; Weston and Jackson, 2009; Weston and Lydy, 2012, 2014). In addition, the potential human health consequences of fiprole and pyrethroid exposure via contaminated recycled water are unknown.

Constructed wetlands (CWs) are a promising option for the treatment of water containing hydrophobic organic contaminants (HOCs) such as fiproles and pyrethroids. There is a great deal of evidence in the literature demonstrating the efficacy of CWs in the removal of nitrogen species, phosphorous species, metals, antibiotic resistance genes, total suspended solids (TSS), biological oxygen demand, and chemical oxygen demand (Ashraf et al., 2020; Chen et al., 2019; Dong et al., 2020; Kodituwakku and Yatawara, 2020; Moogouei and Chen, 2020; Zhang et al., 2020). CWs of various designs have been studied, and it is clear that treatment is typically facilitated by some combination of abiotic degradation, microbial degradation, sorption, and phytoremediation, depending on the treatment endpoint (Kadlec and Wallace, 2008). Existing data regarding select organic contaminants have demonstrated that CWs are effective in their removal (Budd et al., 2009; Cryder et al., in review; Jasper et al., 2013, 2014; Liu et al., 2020; Supowit et al., 2016), but detailed mechanistic information is largely lacking for many important classes of organic pollutants. There is evidence that sediment sorption and subsequent degradation is vital for removal of HOCs (Budd et al., 2009, 2011; Cryder et al., in review), and plant uptake appears to play a role as well (Cryder et al., in review). However, differences in HOC removal efficiency and the mechanisms responsible among CW types and configurations is not well understood, which prevents comprehension of the optimal conditions for their treatment.

Unit process CWs are designed to facilitate treatment of a particular endpoint or set of endpoints by encouraging specific physical, chemical, and/or biological processes (Jasper et al., 2013). Unit processes include open water cells, macrophyte-dominated wetland cells, and bivalve filtration wetland cells (Jasper et al., 2013). These unit processes encourage degradation of their target contaminants via the following mechanisms: direct photolysis, indirect photolysis, sorption, and biotransformation in open water cells; denitrification, metal sulfide precipitation, plant-derived carbon biotransformation, and peat sorption in macrophyte-dominated wetland cells; and pathogen ingestion, particle-associated contaminant ingestion, and inactivation or transformation of ingested contaminants in bivalve filtration wetland cells (Jasper et al., 2013). Some water treatment initiatives seek to leverage the unit process concept by installing distinct unit process cells in series to remove several classes of contaminants, allowing for optimization of CW size and isolation of necessary physical and chemical conditions (Jasper et al., 2013). Previous work has demonstrated that unit process open water (UPOW) cells are effective for the removal of pharmaceuticals via photolysis and microbial biodegradation (Jasper et al., 2014). It is unknown if UPOW cells are similarly effective for removal of HOCs such as fiproles and pyrethroids.

To investigate the efficacy of UPOW cells in the removal of HOCs and begin to understand the mechanisms underlying said removal, water and sediment samples were collected from the Prado Wetlands from June 2018-January 2019 and analyzed for fipronil desulfinyl, fipronil sulfide, fipronil, and fipronil sulfone as well as the pyrethroids bifenthrin and cyfluthrin. The primary objectives of this study were to examine the spatial and temporal trends of fiproles and pyrethroids in the water and sediment compartments of a UPOW wetland cell, to calculate the percent removal of each analyte by the wetland as well as their mass fluxes through it, and to measure changes in invertebrate toxic units (TUs) caused by UPOW treatment. It was hypothesized that sedimentation of contaminated particles and sediment binding would play a major role in the removal of fiproles and pyrethroids by the UPOW cell, causing significant reductions in sensitive aquatic invertebrate toxicity. The results presented herein may be used to identify the CW type and associated conditions that are optimal for HOC removal and thus reduce the potential risk to aquatic organisms and human health caused by reuse of water containing such pollutants.

4.2 Materials and Methods

4.2.1 Chemicals

Fipronil (98.9%), fipronil desulfinyl (97.8%), fipronil sulfide (98.8%), and fipronil sulfone (99.7%) were obtained from the United States Environmental Protection Agency's National Pesticide Standard Repository (Fort Meade, MD). Bifenthrin (98%) and deuterated (d5) bifenthrin (99%) were purchased from Toronto Research Chemicals (Toronto, Ontario, Canada) while cyfluthrin (98%) was purchased from Santa Cruz Biotechnology, Inc. (Dallas, TX). Ethiprole (97.4%) was provided by the Shanghai Pesticide Research Institute (Shanghai, China), and decachlorobiphenyl (99.1%) was purchased from AccuStandard, Inc. (New Haven, CT). Isotopically labeled fipronil ($^{13}C_4$-$^{15}N_2$-fipronil, 99.1%) was obtained from Cambridge Isotope Laboratories (Andover, MA). Solvents and other chemicals used were of pesticide or GC-MS grade.

4.2.2 Site Description

This study was conducted at the Prado Wetlands (Corona, CA), a large complex of CW ponds operated in series (Figure S4.1). Owned and operated by the Orange County Water District (OCWD), the Prado Wetlands were initially designed to remove NO_3^- from the Santa Ana River. Up to 50% of the river flow is diverted into the wetland system for treatment. Treated effluent from the wetlands enters Chino Creek and flows to Orange County for groundwater recharge. Additional information regarding the Prado Wetlands system is provided elsewhere (Cryder et al., in review).

The pond selected for use in this study was one of the UPOW cells located in region E4 of the Prado Wetlands (Figure S4.1). Three cells, each containing a 1 ft (0.30 m) water column, with distinct hydraulic retention times were constructed in E4 with the initial purpose of facilitating photolysis of resident contaminants (Bear et al., 2017). A geotextile liner was installed at the bottom of the cells to prevent wetland macrophyte growth. Over time, a unique biomat layer composed of sediment particles as well as autotrophic and heterotrophic microorganisms formed on the geotextile liner in the UPOW cells. Details regarding the biomat composition are provided elsewhere (Jones et al., 2018) and additional information concerning the Prado UPOW cells has been reported in previous work (Bear et al., 2017; Jones et al., 2018). The middle pond, with a hydraulic retention time of 4 d, was chosen for sampling. In the context of this study, this cell will be referred to as the Unit Process Open Water Constructed Wetland (UPOW-CW). Samples and measurements were taken at the inlet weir box, the midpoint of the pond, and the outlet weir box. These sampling locations will be designated the inlet, the midpoint, and the outlet, respectively.

4.2.3 Sediment and Water Properties

UPOW-CW sediment was collected from the 0-10 cm depth of the inlet, midpoint, and outlet, and was composited for measurement of sediment physicochemical properties (Table S4.1). Prior to analysis, sediment was air dried and sieved through a 2 mm mesh. UPOW-CW sediment was composed of 34% sand, 39% silt, and 27% clay, as determined using the 12-h hydrometer method (Klute, 1986). Sediment pH was 7.18, measured via the 1:2 (v/v) soil slurry method (Donohue, 1992). Loss-on-ignition (Gavlak et al., 2003) revealed an organic matter content of 3.66% (w/w). A total organic carbon content of 2.08% (w/w) was measured using high temperature combustion on an Elementar Vario MAX C/N Analyzer (Elementar Americas, Mt. Laurel, NJ) after the addition of HCl for removal of carbonates (Schumacher, 2002).

The flow rate of the water entering and exiting the UPOW-CW at each time point was calculated using equations provided by Prado Wetlands staff. Inlet flow rates at the v-notch weir box were calculated using the following equation:

$$CMS = 1.37\ H^{5/2} \tag{1}$$

where CMS is the flow ($m^3\ s^{-1}$) and H is the height of the water flowing through the weir box (m). Outlet flow rates were similarly calculated using the following equation:

$$CMS = 0.0942\ L\ H^{3/2} \tag{2}$$

where *CMS* is the flow (m^3 s^{-1}), *L* is the length of the weir box (ft), and *H* is the height of the water flowing through the weir box (ft). The calculated flow rates were then converted into appropriate units and are reported in Table S4.2. Sedimentation rates were subsequently derived using these flow rates and the concentrations of TSS measured in water samples collected from the UPOW-CW inlet and outlet (Table S4.2; Budd et al., 2009). Water temperatures were measured *in situ* at the UPOW-CW inlet, midpoint, and outlet (Table S4.3). After water sample collection, pH measurements were performed *ex situ* prior to extraction (Table S4.3).

4.2.4 Collection and Extraction of Wetland Samples

Water and sediment samples were collected from the UPOW-CW on a monthly basis from June 2018-January 2019, with the exception of September 2018 when maintenance activities were being performed on the southern pond complex. All samples were monitored for fipronil desulfinyl, fipronil sulfide, fipronil, fipronil sulfone, bifenthrin, and cyfluthrin. Water samples (1 L) were collected in triplicate in amber glass bottles at the inlet, midpoint, and outlet of the UPOW-CW. Inlet and outlet samples were collected from the water flowing into the corresponding weir boxes, while midpoint samples were collected by placing bottles beneath the surface of the water against the direction of flow. Sample bottles were transported to the laboratory on ice and stored at 4 °C until extraction. Water samples were passed through 0.7 μm filters to separate TSS from the water for independent extraction, and filtered TSS samples were then dried. UPOW-CW water samples were extracted according to a modified version of the methods described in Gan et al., 2012. Briefly, liquid-liquid extraction was performed with three 60 mL aliquots of dichloromethane. Extracts were then evaporated with a Büchi RE121 Rotovapor (Flawil, Switzerland) and solvent exchanged into 9:1 hexane:acetone (v/v). Samples were evaporated to approximately 0.5 mL under a gentle nitrogen stream and reconstituted to 1.0 mL in hexane.

Sediment samples were also collected at the UPOW-CW inlet, midpoint, and outlet. Sediment samples were collected from the 0-10 cm depth in 1 L amber glass jars using a small hand shovel. Samples were transported on ice prior to laboratory storage at 4 °C. Sediment samples were dried and pulverized with a mortar and pestle to prepare for extraction. TSS and sediment samples were extracted by pressurized fluid extraction with a Dionex ASE 350 (Thermo Fisher Scientific, Waltham, MA) using a modified version of previously described methods (Brennan et al., 2009; Maul et al., 2008). Sample cells were filled with 1:1 dichloromethane:acetone (v/v), heated to 100 °C, and extracted at 1500 psi for two 5 min cycles before being flushed with 60% solvent for 1 min. The entirety of the TSS filtered from each water sample was extracted, and the extracts passed through cellulose filters within the ASE cells. Aliquots of the prepared sediment (25 g) samples were extracted and subjected to in-cell cleanup with Cu powder (Lin et al., 2008) packed between cellulose filters. All extracts were evaporated to approximately 2 mL under a gentle nitrogen stream, cleaned up on a Florisil cartridge preconditioned with hexane and eluted with 9:1 hexane:acetone (v/v), evaporated with nitrogen again to an approximate volume of 0.5 mL, and reconstituted to 1.0 mL in hexane.

4.2.5 Analysis and Quality Control

An Agilent 6890N/5973N GC/MSD (Santa Clara, CA) equipped with a 30 m x 0.25 mm x 0.25 μm DB-5MS column was utilized for sample analysis. Helium was used as the carrier gas at a flow rate of 1.2 mL min^{-1}, and 2 μL sample aliquots were injected at 200 °C. The GC oven program utilized in this study is as follows: initial temperature of 120 °C and held for 1 min; ramped up at 25 °C min^{-1} to 205 °C and held for 1 min; ramped up at 1.5 °C min^{-1} to 220 °C and held for 1 min; ramped up at 3 °C min^{-1} to 225 °C and held for 1 min; and finally ramped up at 30 °C min^{-1} to a final temperature of 300 °C followed by a 5 min hold. Additional analytical information is provided in the supporting information (SI). Following sample quantification, statistical analyses were performed using SAS® 9.4 (SAS Institute, Cary, NC).

Several steps were taken to ensure the accuracy and quality of data produced in this study. Instrumental controls used during GC/MSD analysis included injecting a duplicate calibration standard every 10 samples, adding $^{13}C_4$-$^{15}N_2$-fipronil and d5-bifenthrin as internal standards to each extract before analysis, and determining method detection limits (MDLs) using EPA Method 40 CFR Part 136, Appendix B (United States Environmental Protection Agency, 2012). All calculated analyte MDLs were 2 μg L^{-1} except for fipronil desulfinyl, which had an MDL of 1 μg L^{-1}. In addition, several procedural controls were utilized. Reagent blanks were analyzed with every set of 7 liquid-liquid extraction samples and 10 pressurized fluid extraction samples, none of which revealed detection of the analytes of interest. Matrix spike samples were analyzed to measure extraction efficiencies, which are listed in the SI. In addition, ethiprole and decachlorobiphenyl were added to all samples prior to extraction to measure the following surrogate recoveries: 103 ± 22% and 123 ± 25% for liquid-liquid extraction, respectively, and 108 ± 20% and 118 ± 18% for pressurized fluid extraction, respectively. Additional quality control measures are described in the SI.

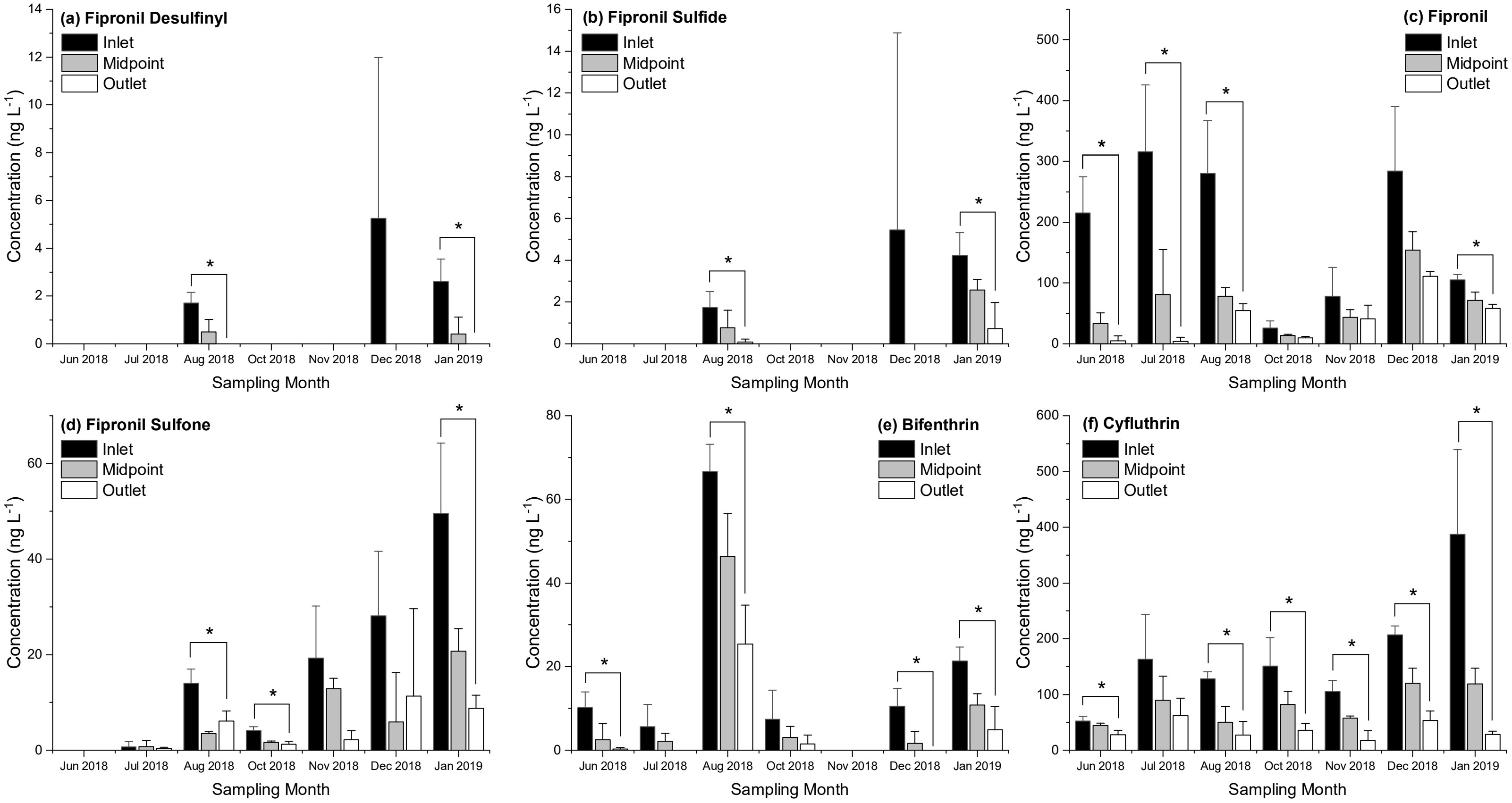
4.3 Results and Discussion

4.3.1 Spatial and Temporal Concentration Trends

Mean fiprole and pyrethroid concentrations in whole water samples collected from the UPOW-CW are reported in Figure 4.1. All mean analyte concentrations followed a general decreasing spatial trend when moving from inlet to midpoint to outlet. In addition, all mean outlet concentrations were lower than corresponding mean inlet concentrations. The majority of mean outlet concentrations showed statistically significant reductions compared with inlet measurements, and each analyte was significantly reduced in at least two of the sampling months. When the comparisons were not statistically significant, mean values were either relatively low or exhibited high variability. Since water and the solids it contains are all that enters and exits the treatment system, these results indicate that the UPOW-CW successfully reduced the concentrations of fiproles and pyrethroids, often in a statistically significant manner. Previous research has also revealed the efficacy of CWs in the concentration reduction of fiproles and pyrethroids (Budd et al., 2009, 2011; Cryder et al., in review; Supowit et al., 2016).

The fiproles and pyrethroids analyzed in this study exhibited distinct temporal trends in water samples collected from the UPOW-CW (Figure 4.1). Fipronil desulfinyl

Figure 4.1: Whole water concentrations of fipronil desulfinyl (a), fipronil sulfide (b), fipronil (c), fipronil sulfone (d), bifenthrin (e), and cyfluthrin (f) in samples collected from the Unit Process Open Water Constructed Wetland. Data are reported as mean ± 1 SD. Asterisks indicate a statistically significant ($p < 0.05$) difference between inlet and outlet concentrations.



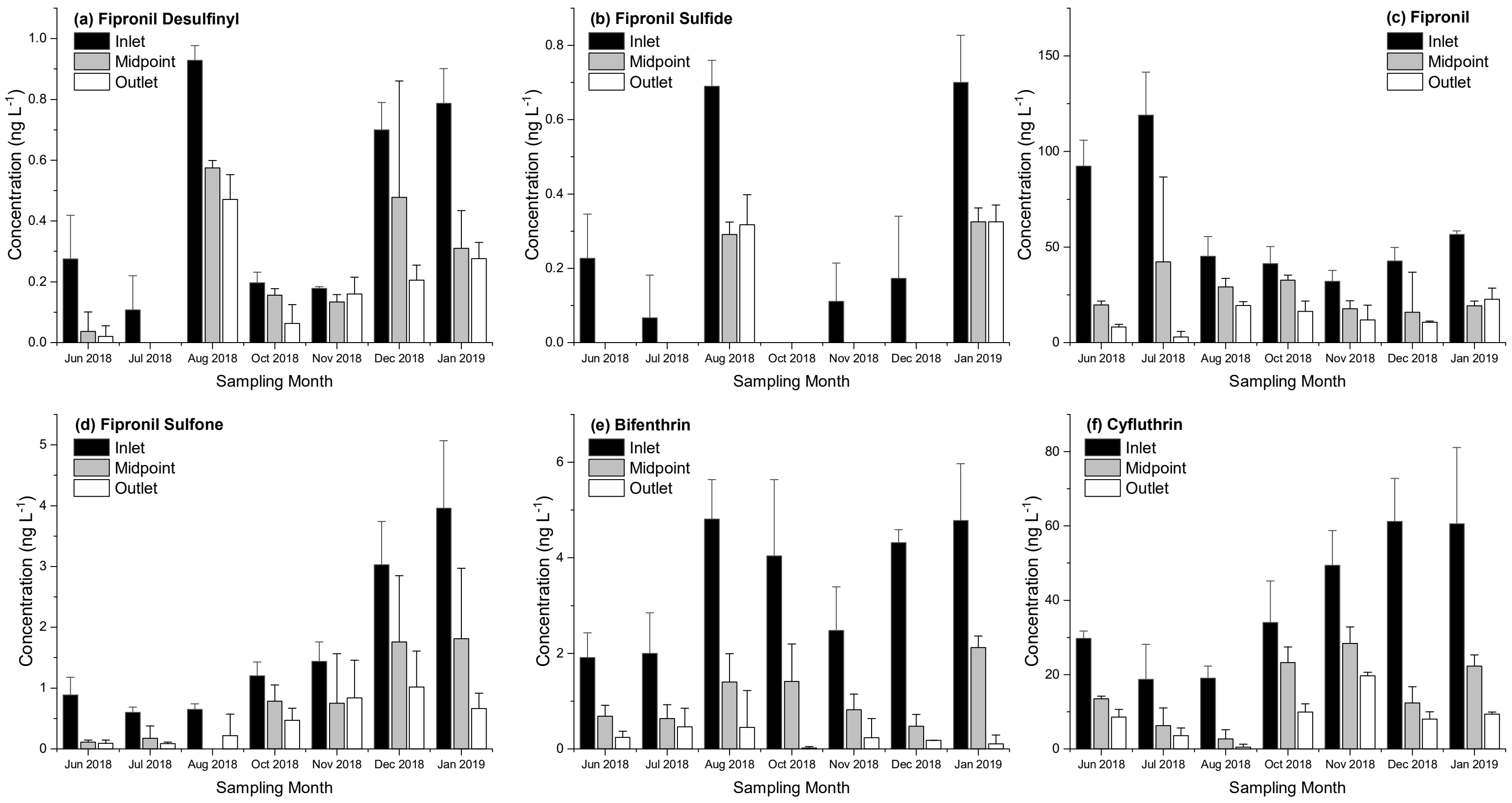
and fipronil sulfide were only detected in August 2018, December 2018, and January 2019. Both compounds followed the same mean concentrations trends over time: low levels in August 2018, a peak in December 2018, and a subsequent decrease in January 2019. Fipronil mean levels showed a unique temporal trend: mean values increased from June-July 2018 when peak detection occurred, decreased until October 2018, increased until December 2018, and once again decreased in January 2019. Fipronil sulfone and cyfluthrin mean concentrations showed a generally increasing trend throughout the study and peaked in January 2019. Bifenthrin was detected at peak levels in August 2018, decreased until November 2018, and steadily increased until January 2019. Collectively, these results suggest high rates of application and subsequent wash off of fipronil, bifenthrin, and cyfluthrin in the summer months and sustained transport of persistent urban residues in the fall and winter exacerbated by the relatively high rainfall that took place in winter 2018-2019. In addition, fipronil desulfinyl, fipronil sulfide, and fipronil sulfone demonstrated a temporal delay in detections relative to the parent compound, indicating transformation of fipronil residues at sites of application and latent transport. These temporal trends are in line with those observed for fiproles and pyrethroids during this time frame in a vegetated CW at the Prado Wetlands (Cryder et al., in review).

Fipronil and cyfluthrin were the analytes detected at the highest levels in UPOW-CW whole water samples, with mean concentrations ranging from 3.93-316 ng L^{-1} and 17.5-387 ng L^{-1}, respectively (Figure 4.1). Moderate mean concentrations were observed for bifenthrin (0-66.6 ng L^{-1}) and fipronil sulfone (0-49.5 ng L^{-1}). The compounds detected at the lowest levels were fipronil desulfinyl and fipronil sulfide. Their mean concentrations ranged from 0-5.25 ng L^{-1} and 0-5.45 ng L^{-1}, respectively. These analyte groupings are identical to those detected in a vegetated CW at the Prado wetlands and the mean levels were very similar (Cryder et al., in review).

Fiprole and pyrethroid mean sediment (dry weight) concentrations are summarized in Figure 4.2. Similar to the results for whole water concentrations, mean sediment levels generally decreased spatially from inlet to outlet. In addition, mean outlet concentrations were always lower than mean inlet concentrations. Combined with the water results from Figure 4.1, these spatial results suggest that, as contaminated water traveled through the UPOW-CW, insecticide laden particles settled in the pond and dissolved residues sorbed to wetland sediment particles. This highlights the importance of sedimentation and sediment binding in the CW treatment of HOCs such as fiproles and pyrethroids. The vital role of sediment binding has been reported in previous CW studies (Budd et al., 2009, 2011; Cryder et al., in review).

Distinct temporal trends of fiprole and pyrethroid levels in UPOW-CW sediment can also be gleaned from Figure 4.2. All of the analytes were detected in sediment at every time point except for fipronil sulfide, which was not detected in October 2018. Fipronil desulfinyl, fipronil sulfide, and bifenthrin were observed at initial high concentrations in August 2018, decreased in October and November 2018, and finally increased until January 2019. Peak levels of these compounds were measured in August 2018, except for fipronil sulfide which peaked in January 2019. Fipronil was detected at high mean levels in June 2018, and those levels increased in July 2018 before decreasing and stabilizing for the remainder of the sampling campaign. Mean sediment

Figure 4.2: Sediment (dry weight) concentrations of fipronil desulfinyl (a), fipronil sulfide (b), fipronil (c), fipronil sulfone (d), bifenthrin (e), and cyfluthrin (f) in samples collected from the Unit Process Open Water Constructed Wetland. Data are reported as mean ± 1 SD.



concentrations of fipronil sulfone and cyfluthrin steadily decreased from June to August 2018 before gradually increasing until January 2019. Fipronil sulfone was measured at the highest levels in January 2019 while the highest cyfluthrin levels were reported in December 2018. Overall, these trends seem to indicate higher rates of application and subsequent wash off of fipronil and bifenthrin in the summer months followed by continuous transport of residual applied product. However, the trend of cyfluthrin levels throughout the study suggests moderate use in the summer of 2018 followed by apparent increased use in the fall and likely exaggerated wash off in winter 2018-2019 due to the relatively heavy rain season. Elevated concentrations of fipronil desulfinyl, fipronil sulfide, and fipronil sulfone were temporally offset from the parent compound, suggesting that residual fipronil in urban environments was transformed and transported or transformation of sediment-bound fipronil took place after initially entering the UPOW-CW. Peaks in analyte sediment concentrations tended to correspond to peaks in whole water concentrations (Figure 4.1), further reinforcing the importance of sediment binding and settling of contaminated solids in the removal of fiproles and pyrethroids. Since analyte sediment concentrations did not steadily increase from June 2018-January 2019, it is clear that fiproles and pyrethroids were not simply being stored in the sediment compartment of the UPOW-CW. Therefore, the results indicate that these pollutants were removed from the water entering the pond by sedimentation and sediment sorption followed by degradation in the sediment compartment, which was also observed at a vegetated CW at the Prado Wetlands (Cryder et al., in review). It is also possible that resuspension and loss of contaminated sediment particles from the CW was occurring, but the contribution of this pathway was likely negligible since outlet whole water concentrations of the analytes of interest were still lower than inlet concentrations (Figure 4.1).

Fipronil and cyfluthrin were detected at the highest mean sediment concentrations in the UPOW-CW (Figure 4.2). Their mean levels ranged from 2.89-119 ng g^{-1} and 0.457-61.2 ng g^{-1}, respectively. Moderate levels of 0-3.96 ng g^{-1} and 0.0182-4.81 ng g^{-1} were observed for fipronil sulfone and bifenthrin, respectively. The lowest levels were observed for fipronil desulfinyl (0-0.928 ng g^{-1}) and fipronil sulfide (0-0.700 ng g^{-1}). These sediment concentrations and relative analyte levels are very similar to what has previously been measured at the Prado Wetlands in a vegetated pond (Cryder et al., in review). In addition, these analyte magnitude groupings are identical to those observed in water samples collected from the UPOW-CW (Figure 4.1). This supports the finding that sediment binding and degradation of fiproles and pyrethroids plays a major role in the removal of fiproles and pyrethroids in this pond, since any deviation from the relative magnitudes reported in water would suggest gradual accumulation. This phenomenon was also observed for fiproles and pyrethroids measured in a vegetated CW at the Prado Wetlands (Cryder et al., in review).

4.3.2 Insecticide Removal and Mass Flux

Concentration-based removal values of fiproles and pyrethroids from the UPOW-CW are reported in Table 4.1. Removal values for fipronil desulfinyl, fipronil sulfide, fipronil, fipronil sulfone, bifenthrin, and cyfluthrin were 100%, 82.9-100%, 44.2-98.8%, 55.7-88.7%, 61.8-100%, and 47.3-92.7%, respectively. Fipronil desulfinyl, fipronil

Table 4.1: Mass flux and concentration-based removal of fiproles and pyrethroids from the Unit Process Open Water Constructed Wetland. Data are reported as mean ± 1 SD where applicable.

		June	July	August	October	November	December	January
Fipronil Desulfinyl	**Mass Influx[a] (mg d^{-1})**	0	0	1.16 ± 0.300	0	0	0.997 ± 1.28	1.01 ± 0.366
	Mass Efflux[b] (mg d^{-1})	0	0	0	0	0	0	0
	Δ Mass Flux (mg d^{-1})	0	0	1.16*[c,d]	0	0	0.997	1.01*
	Removal (%)	-	-	100	-	-	100	100
Fipronil Sulfide	**Mass Influx (mg d^{-1})**	0	0	1.18 ± 0.521	0	0	1.03 ± 1.79	1.65 ± 0.428
	Mass Efflux (mg d^{-1})	0	0	0.0481 ± 0.0832	0	0	0	0.425 ± 0.736
	Δ Mass Flux (mg d^{-1})	0	0	1.13	0	0	1.03	1.22
	Removal (%)	-	-	95.3	-	-	100	82.9
Fipronil	**Mass Influx (mg d^{-1})**	83.6 ± 23.2	123 ± 42.8	190 ± 59.4	37.6 ± 16.4	67.5 ± 40.8	53.8 ± 20.1	40.8 ± 3.45
	Mass Efflux (mg d^{-1})	2.85 ± 4.93	6.53 ± 11.3	32.3 ± 6.38	19.6 ± 4.84	33.9 ± 18.1	42.3 ± 2.95	34.3 ± 3.99
	Δ Mass Flux (mg d^{-1})	80.7*	117*	158*	18.0	33.6	11.5	6.45
	Removal (%)	97.7	98.8	80.4	62.1	47.2	60.9	44.2
Fipronil Sulfone	**Mass Influx (mg d^{-1})**	0	0.258 ± 0.448	9.50 ± 2.05	5.92 ± 1.12	16.7 ± 9.42	5.33 ± 2.55	19.3 ± 5.75
	Mass Efflux (mg d^{-1})	0	0.489 ± 0.543	3.58 ± 1.23	2.45 ± 1.19	1.80 ± 1.56	4.30 ± 6.97	5.14 ± 1.63
	Δ Mass Flux (mg d^{-1})	0	-0.230[e]	5.92*	3.47*	14.9	1.03	14.1
	Removal (%)	-	55.7	56.4	69.9	88.7	59.9	82.3

Bifenthrin	**Mass Influx (mg d^{-1})**	3.97 ± 1.46	2.20 ± 2.06	45.3 ± 4.49	10.6 ± 10.1	0	2.00 ± 0.813	8.29 ± 1.33
	Mass Efflux (mg d^{-1})	0.163 ± 0.194	0	14.9 ± 5.46	2.90 ± 4.29	0	0	2.88 ± 3.26
	Δ Mass Flux (mg d^{-1})	3.81*	2.20	30.4*	7.72	0	2.00	5.42
	Removal (%)	97.3	100	61.8	80.1	-	100	77.0
Cyfluthrin	**Mass Influx (mg d^{-1})**	20.3 ± 3.28	63.4 ± 31.2	87.4 ± 8.69	218 ± 73.7	91.0 ± 17.7	39.2 ± 2.98	151 ± 59.3
	Mass Efflux (mg d^{-1})	16.2 ± 4.89	103 ± 52.7	16.0 ± 14.3	70.4 ± 25.1	14.4 ± 14.4	20.4 ± 6.57	16.7 ± 3.34
	Δ Mass Flux (mg d^{-1})	4.15	-39.2	71.4*	148	76.7*	18.9*	134
	Removal (%)	47.3	62.1	78.8	76.5	83.4	74.2	92.7

[a] Influx is the flow of a given analyte into the wetland at the inlet weir box.
[b] Efflux is the flow of a given analyte out of the wetland at the outlet weir box.
[c] Positive values of Δ Mass Flux indicate net import of a given analyte to the wetland cell.
[d] Asterisks indicate a statistically significant ($p < 0.05$) difference in mass flux between inlet and outlet measurements.
[e] Negative values of Δ Mass Flux indicate net export of a given analyte from the wetland cell.

sulfide, fipronil, and bifenthrin tended to be removed at higher rates while fipronil sulfone and cyfluthrin were removed at lower rates. It is important to note that fipronil desulfinyl and fipronil sulfide, despite having the highest concentration-based removal values, were detected at the lowest levels in whole water samples (Figure 4.1). Fipronil and cyfluthrin, being present at higher concentrations than the other analytes of interest, were still removed at substantial rates during several of the sampling months. Similar removal values of 100%, 99.7-100%, 57.8-88.1%, 75.6-100%, 74.7-100%, and 36.6-82.2% were calculated for fipronil desulfinyl, fipronil sulfide, fipronil, fipronil sulfone, bifenthrin, and cyfluthrin, respectively, in a vegetated CW at the Prado Wetlands (Cryder et al., in review).

Concentration-based removal of fipronil was highest in the summer months of June-August 2018 (Table 4.1). Conversely, cyfluthrin removal values were highest from August 2018-January 2019, with lower removal occurring in June and July 2018. The remaining analytes displayed no apparent trends in removal values. These removal value trends for fipronil and cyfluthrin correspond to their seasonal concentration values in water and sediment (Figure 4.1, Figure 4.2). This suggests that the highest removal of these two analytes took place when their mass inputs were at their peak. Removal of the remaining compounds was likely less influenced by this phenomenon since they were present at much lower levels. Importantly, none of the compounds displayed a clear removal trend based on seasonality and weather. Southern California is relatively warm year-round, with cooler temperatures occurring during the rainy winter seasons. If temperature and weather patterns contributed significantly to the removal of fiproles and pyrethroids, we would have expected to observe higher removal of all analytes of interest in the summer and lower values in the fall and winter. Overall, these results suggest that removal was more dependent on the availability of sediment binding sites and microbial degradation, which was also observed for a Prado Wetlands vegetated CW (Cryder et al., in review).

The mass influx, mass efflux, and change in mass flux of each analyte of interest through the UPOW-CW are also reported in Table 4.1 to provide additional vital information regarding removal of fiproles and pyrethroids. Fipronil, bifenthrin, and cyfluthrin were imported into the cell at the greatest rates, with mean mass influx values of 37.6-123 mg d^{-1}, 0-45.3 mg d^{-1}, and 20.3-218 mg d^{-1}, respectively. Lower mean mass influx values were observed for fipronil desulfinyl (0-1.16 mg d^{-1}), fipronil sulfide (0-1.65 mg d^{-1}), and fipronil sulfone (0-19.3 mg d^{-1}). Changes in mass flux represent the net import (positive value; storage in UPOW-CW) or net export (negative value; release from UPOW-CW) of a given analyte to or from the UPOW-CW. Fipronil (6.45-158 mg d^{-1}), bifenthrin (0-30.4 mg d^{-1}), and cyfluthrin (-39.2-148 mg d^{-1}) experienced the greatest changes in mass flux, several of which were statistically significant. The lowest changes in mass flux were reported for fipronil desulfinyl, fipronil sulfide, and fipronil sulfone, with values of 0-1.16 mg d^{-1}, 0-1.22 mg d^{-1}, and -0.230-14.9 mg d^{-1}, respectively. Several statistically significant differences were also reported for these analytes. These changes in mass flux were much lower than those reported in a vegetated cell at the Prado Wetlands, but this seems to be a result of much lower mass influxes of fiproles and pyrethroids in the UPOW-CW (Cryder et al., in review).

Negative changes in mass flux were reported for fipronil sulfone and cyfluthrin in July 2018 (Table 4.1). However, neither of these changes in mass flux was statistically significant. Furthermore, concentration-based removal values were positive despite net mass export. It is likely that the higher flow values at the UPOW-CW outlet relative to the inlet (Table S4.2) that were calculated for most of the sampling months resulted in resuspension of some contaminated sediment particles and led to release of transiently stored residues in these two instances. This is supported by the finding that all sedimentation rates calculated during the study were negative (Table S4.2), indicating net export of TSS. Predominantly positive changes in mass flux and positive concentration-based removal values indicate that fiproles and pyrethroids were effectively treated by the UPOW-CW despite this loss of TSS. As discussed elsewhere (Cryder et al., in review), flow through the Prado wetlands is regulated to optimize water quality and quantity throughout the entire pond complex, which results in occasional net outflow from isolated wetland cells. Furthermore, a small number of negative changes in mass flux occurred at the Prado Wetlands in a vegetated cell when water flow was higher at the outlet than the inlet (Cryder et al., in review).

To further emphasize the importance of fiprole and pyrethroid sorption during treatment, the percent of analytes detected on TSS in UPOW-CW whole water samples was calculated (Table 4.2). Where applicable, values are reported for inlet, midpoint, and outlet samples for comparison. Statistically significant differences were measured between inlet and outlet values for fipronil in June, August, and December 2018; for fipronil sulfone in December 2018; and for cyfluthrin in December 2018. Every

Table 4.2: Relative presence of fiproles and pyrethroids on total suspended solids (TSS) present in water samples collected from the Unit Process Open Water Constructed Wetland. Data are reported as mean ± 1 SD.

		June	July	August	October	November	December	January
Fipronil Desulfinyl on TSS (%)	**Inlet**	-[a]	-	91.6 ± 7.30	-	-	85.2 ± 20.9	100 ± 0
	Midpoint	-	-	90.2 ± 13.9	-	-	-	100[b]
	Outlet	-	-	-	-	-	-	-
Fipronil Sulfide on TSS (%)	**Inlet**	-	-	96.0 ± 6.85	-	-	63.2	100 ± 0
	Midpoint	-	-	81.5 ± 16.2	-	-	-	100 ± 0
	Outlet	-	-	100	-	-	-	100
Fipronil on TSS (%)	**Inlet**	39.1 ± 2.22	29.8 ± 14.7	64.2 ± 2.69	100 ± 0	93.2 ± 11.9	69.4 ± 5.29	100 ± 0
	Midpoint	100 ± 0	32.0 ± 7.32	92.7 ± 12.7	100 ± 0	87.3 ± 21.9	87.6 ± 16.9	100 ± 0
	Outlet	100 ± 0*[c,d]	0	100 ± 0*	100 ± 0	100 ± 0	98.5 ± 2.53*	100 ± 0
Fipronil Sulfone on TSS (%)	**Inlet**	-	0	89.1 ± 2.31	100 ± 0	76.5 ± 20.6	77.3 ± 1.69	100 ± 0
	Midpoint	-	0	100 ± 0	100 ± 0	70.3 ± 2.28	100	100 ± 0
	Outlet	-	0	85.9 ± 16.8	100 ± 0	100 ± 0	100 ± 0*	100 ± 0
Bifenthrin on TSS (%)	**Inlet**	100 ± 0	90.5 ± 13.4	88.1 ± 0.716	100 ± 0	-	100 ± 0	92.2 ± 13.5
	Midpoint	100 ± 0	100 ± 0	93.6 ± 8.74	100 ± 0	-	100	100 ± 0
	Outlet	100 ± 0	-	94.7 ± 9.17	100 ± 0	-	-	100 ± 0
Cyfluthrin on TSS (%)	**Inlet**	61.5 ± 5.80	36.3 ± 33.4	100 ± 0	100 ± 0	71.7 ± 7.60	67.9 ± 6.19	94.9 ± 8.83
	Midpoint	50.2 ± 7.63	6.41 ± 10.6	100 ± 0	100 ± 0	92.4 ± 13.2	79.1 ± 7.80	100 ± 0
	Outlet	52.5 ± 3.34	0	100 ± 0	100 ± 0	90.2 ± 16.9	100 ± 0*	100 ± 0

[a] Hyphens indicate that the analyte was not detected in the aqueous or TSS phase.

[b] Data reported without a standard deviation indicate that only one sample contained analyte at a detectable level.

[c] Statistical tests were performed when there was mean inlet and outlet data available.

[d] Asterisks indicate a statistically significant ($p < 0.05$) difference in the percentage of analyte adsorbed to TSS between inlet and outlet measurements.

one of these statistically significant differences represents an increase in the relative amount of analyte adsorbed to TSS from inlet to outlet. This further supports the consensus of the data detailed thus far: that contaminants entering the UPOW-CW become enriched in the sediment compartment via settling of previously contaminated solids and sorption of dissolved fiproles and pyrethroids. Since whole water and sediment concentrations were lower at the outlet than the inlet (Figure 4.1, Figure 4.2), it is clear that degradation of these sediment-bound residues is taking place which offsets the loss of analyte via particle resuspension. All other inlet-outlet comparisons were statistically similar, meaning that there was insufficient evidence to conclude that there were any differences between the reported mean values (Table 4.2). Some of the % on TSS values for fipronil and cyfluthrin in June and July 2018 were anomalously low. It is possible that conditions in the wetland water (e.g. pH, presence of co-contaminants) altered the phase partitioning of these insecticides. Alternatively, residues that were apparently dissolved in the aqueous phase may have been present in suspended organic colloids, distorting analyte partitioning (Gustafsson et al., 2001). Previous research at the Prado Wetlands has also suggested that this occurs (Cryder et al., in review). The remainder of the values ranged from 63.2-100%, which is more in line with the expected partitioning of these HOCs.

Several linear regression analyses were performed in an effort to identify additional conditions that facilitated fiprole and pyrethroid removal from the UPOW-CW (Table 4.3). Two dependent variables—concentration-based removal and change in mass flux—were each analyzed with three independent variables—sedimentation rate, water

Table 4.3: Linear regression analyses of fiprole and pyrethroid concentration-based removal and change in mass flux versus sedimentation rate, water pH, and water temperature.

	Fipronil Desulfinyl	**Fipronil Sulfide**	**Fipronil**	**Fipronil Sulfone**	**Bifenthrin**	**Cyfluthrin**
Removal (%) vs. Sedimentation Rate (kg d^{-1})	-[a]	-	y = 0.210x + 72.4	y = -0.0576x + 68.2	y = -0.0558x + 85.4	y = -0.193x + 71.6
R^2	-	-	0.0089	0.0020	0.0013	0.0175
p-value	-	-	0.8402	0.9332	0.9461	0.7776
Removal (%) vs. Water pH	-	-	y = 29.7x – 167	y = -7.20x + 125	y = -4.17x + 119	y = -17.1x + 210
R^2	-	-	0.3315	0.0262	0.0152	0.2527
p-value	-	-	0.1762	0.7592	0.8162	0.2502
Removal (%) vs. Water Temperature (°C)	-	-	y = 2.95x + 27.5	y = -1.58x + 89.9	y = -0.743x + 97.2	y = -1.65x + 97.4
R^2	-	-	0.4972	0.3051	0.0733	0.3568
p-value	-	-	0.0768	0.2557	0.6037	0.1567
Δ Mass Flux (mg d^{-1}) vs. Sedimentation Rate (kg d^{-1})	y = 0.0140x + 0.597	y = 0.0107x + 0.594	y = 2.10x + 82.6	y = 0.0559x + 6.18	y = 0.257x + 10.0	y = -2.58x + 32.4
R^2	0.0625	0.0325	0.1307	0.0077	0.0620	0.1459
p-value	0.5887	0.6988	0.4255	0.8512	0.5903	0.3979
Δ Mass Flux (mg d^{-1}) vs. Water pH	y = 0.0248x + 0.255	y = 0.0236x + 0.295	y = 76.8x – 553	y = -1.98x + 21.4	y = 7.71x – 54.2	y = -46.0x + 427
R^2	0.0004	0.0003	0.3204	0.0179	0.1030	0.0854
p-value	0.9677	0.9712	0.1853	0.7751	0.4828	0.5249

Δ Mass Flux (mg d^{-1}) vs. Water Temperature (°C)	y = -0.0149x + 0.668	y = -0.0250x + 0.845	y = 8.18x – 57.7	y = -0.561x + 13.7	y = 1.20x – 10.1	y = -2.81x + 99.8
R^2	0.0199	0.0495	0.5534	0.2188	0.3828	0.0484
p-value	0.7628	0.6315	0.0552	0.2899	0.1386	0.6353

[a] Hyphens indicate that there was insufficient data to perform linear regression analysis.

pH, and water temperature. None of the performed regressions revealed any statistically significant linear relationships, indicating that sedimentation rate, water pH, and water temperature did not significantly contribute to the concentration-based removal values or changes in mass flux that are reported in this study. Sedimentation rate was expected to share a significant relationship with at least one of the dependent variables due to the importance of analyte sorption in wetland removal and based on past results at the Prado Wetlands (Cryder et al., in review). However, the negative sedimentation rates calculated in this study (Table S4.2) already seemed to contradict this hypothesis. Therefore, it is likely that the net loss of TSS from the UPOW-CW caused by efforts to regulate the flow of the Prado Wetlands complex precluded additional confirmation of the importance of sedimentation in the removal of fiproles and pyrethroids in the UPOW-CW based on the measurements taken. Future research should consider laboratory experiments to identify the contribution of other mechanisms (e.g. microbial degradation, photolysis, hydrolysis, etc.) to the removal of these compounds.

4.3.3 Invertebrate Toxic Units

In order to estimate the change in fiprole and pyrethroid invertebrate toxicity caused by UPOW-CW treatment, TUs were calculated using the following equation (Weston and Lydy, 2014):

$$TU = \frac{Observed\ analyte\ concentration}{Species\text{-}specific\ analyte\ toxicity\ value} \quad (3)$$

A TU value greater than or equal to 1 indicates that the given environmental concentration is sufficient to elicit the toxicity endpoint used to derive the given benchmark value. In other words, a TU value less than 1 suggests that the risk of toxicity has been mitigated. Table S4.4 contains the toxicity values utilized for TU calculation in this study (Weston and Jackson, 2009; Weston and Lydy, 2014).

Sublethal and lethal toxicity values derived for the amphipod *Hyalella azteca* were used to calculate TUs for bifenthrin and cyfluthrin (Table 4.4). Previous research has identified this organism as being particularly sensitive to pyrethroids (Anderson et al., 2006; Maund et al., 1998; Weston and Jackson, 2009). Sublethal and lethal outlet TUs were always lower than corresponding inlet TUs for bifenthrin and cyfluthrin, and the majority of these reductions were statistically significant ($p < 0.05$). Sublethal bifenthrin TUs were reduced from 0-20.2 at the inlet to 0-7.70 at the outlet while lethal values decreased from 0-8.65 to 0-3.30. Cyfluthrin sublethal TUs changed from 27.5-204 to 9.21-32.5 at the inlet and outlet, respectively. Lethal TUs decreased from 22.7-168 at the inlet to 7.61-26.8 at the outlet for cyfluthrin. These sublethal and lethal TU values are similar to those reported in a previous Prado Wetlands study (Cryder et al., in review).

TUs for fipronil sulfide, fipronil, and fipronil sulfone were calculated for the midge *Chironomus dilutus* since it is the most sensitive organism reported in the literature (Weston and Lydy, 2014). As was the case for pyrethroids, UPOW-CW treatment reduced fiprole TU values in every instance, and these decreases were statistically significant in the majority of comparisons Table 4.4). Fipronil sulfide sublethal TUs were reduced from 0-0.550 at the inlet to 0-0.0731 at the outlet. Lethal fipronil sulfide TUs were 0-0.0788 and 0-0.0105 at the inlet and outlet, respectively. Fipronil TUs decreased from 0.804-9.74 to 0.121-3.41 (sublethal) and from

Table 4.4: Toxic units (TU) of fiproles and pyrethroids in whole water samples collected from the Unit Process Open Water Constructed Wetland. Data are reported as mean ± 1 SD.

				June	July	August	October	November	December	January
***Hyalella azteca*[a,b]**	**Bifenthrin**	**TU EC_{50}**	**Inlet**	3.09 ± 1.14	1.71 ± 1.61	20.2 ± 2.00	2.23 ± 2.13	0	3.19 ± 1.30	6.45 ± 1.03
			Outlet	0.0842 ± 0.100*[e]	0	7.70 ± 2.82*	0.443 ± 0.655	0	0*	1.48 ± 1.68*
		TU LC_{50}	**Inlet**	1.33 ± 0.488	0.733 ± 0.688	8.65 ± 0.857	0.957 ± 0.912	0	1.37 ± 0.557	2.77 ± 0.443
			Outlet	0.0361 ± 0.0430*	0	3.30 ± 1.21*	0.190 ± 0.281	0	0*	0.636 ± 0.720*
	Cyfluthrin	**TU EC_{50}**	**Inlet**	27.5 ± 4.43	85.6 ± 42.2	67.6 ± 6.72	79.6 ± 26.9	55.5 ± 10.8	109 ± 8.28	204 ± 80.1
			Outlet	14.5 ± 4.38*	32.5 ± 16.7	14.3 ± 12.8*	18.7 ± 6.66*	9.21 ± 9.25*	28.1 ± 9.05*	14.9 ± 2.99*
		TU LC_{50}	**Inlet**	22.7 ± 3.66	70.8 ± 34.9	55.8 ± 5.55	65.7 ± 22.2	45.8 ± 8.91	89.9 ± 6.84	168 ± 66.2
			Outlet	12.0 ± 3.62*	26.8 ± 13.8	11.8 ± 10.6*	15.4 ± 5.50*	7.61 ± 7.64*	23.2 ± 7.48*	12.3 ± 2.47*
***Chironomus dilutus*[c,d]**	**Fipronil Sulfide**	**TU EC_{50}**	**Inlet**	0	0	0.175 ± 0.0773	0	0	0.550 ± 0.953	0.427 ± 0.111
			Outlet	0	0	0.00826 ± 0.0143*	0	0	0	0.0731 ± 0.127*
		TU LC_{50}	**Inlet**	0	0	0.0251 ± 0.0111	0	0	0.0788 ± 0.137	0.0611 ± 0.0159
			Outlet	0	0	0.00118 ± 0.00205*	0	0	0	0.0105 ± 0.0181*
	Fipronil	**TU EC_{50}**	**Inlet**	6.60 ± 1.83	9.74 ± 3.38	8.60 ± 2.69	0.804 ± 0.351	2.41 ± 1.46	8.73 ± 3.26	3.22 ± 0.272
			Outlet	0.149 ± 0.258*	0.121 ± 0.209*	1.69 ± 0.337*	0.304 ± 0.0751	1.27 ± 0.680	3.41 ± 0.238	1.80 ± 0.209*
		TU LC_{50}	**Inlet**	2.63 ± 0.731	3.88 ± 1.35	3.43 ± 1.07	0.320 ± 0.140	0.959 ± 0.580	3.48 ± 1.30	1.28 ± 0.109
			Outlet	0.0595 ± 0.103*	0.0482 ± 0.0835*	0.674 ± 0.134*	0.121 ± 0.0300	0.507 ± 0.271	1.36 ± 0.0950	0.717 ± 0.0834*
	Fipronil Sulfone	**TU EC_{50}**	**Inlet**	0	0.0862 ± 0.149	1.81 ± 0.390	0.534 ± 0.101	2.51 ± 1.42	3.65 ± 1.75	6.43 ± 1.92
			Outlet	0	0.0382 ± 0.0425	0.791 ± 0.272*	0.161 ± 0.0780*	0.284 ± 0.247	1.46 ± 2.37	1.14 ± 0.359*
		TU LC_{50}	**Inlet**	0	0.00638 ± 0.0110	0.134 ± 0.0289	0.0395 ± 0.00748	0.186 ± 0.105	0.270 ± 0.129	0.476 ± 0.142
			Outlet	0	0.00283 ± 0.00314	0.0586 ± 0.0202*	0.0119 ± 0.00578*	0.0210 ± 0.0183	0.108 ± 0.176	0.0841 ± 0.0266*

[a] Toxicity values for *Hyalella azteca* sourced from Weston and Jackson, 2009.

[b] Sublethal end point for EC_{50}: ability to swim.

[c] Toxicity values for *Chironomus dilutus* sourced from Weston and Lydy, 2014.

[d] Sublethal end point for EC_{50}: ability to thrash when prodded.

[e] Asterisks indicate a statistically significant ($p < 0.05$) difference between the inlet and outlet TU values.

0.320-3.88 to 0.0482-1.36 (lethal) as a result of CW treatment. Sublethal TUs for fipronil sulfone were reduced from 0-6.43 at the inlet to 0-1.46 at the outlet. Similarly, fipronil sulfone lethal TUs were 0-0.476 at the inlet and decreased to 0-0.108 at the outlet. Similar TU values were observed in a vegetated CW at the Prado Wetlands (Cryder et al., in review).

The results of the TU analysis reveal that the UPOW-CW always reduced fiprole and pyrethroid TUs for sensitive aquatic invertebrates (Table 4.4). It is important to note that the TU values calculated in this study represent worst-case scenarios, given that they utilize the most sensitive known organisms and whole water concentrations. If bioavailability were considered, actual TUs would be much lower since these analytes would be predominantly sorbed to solids in the water column (Table 4.2). In addition, the UPOW-CW cell studied does not represent the treatment efficacy and toxicity reduction of the entire Prado Wetlands complex. However, the values reported in this study are useful to demonstrate effective mitigation of toxicity via comparison of the inlet and outlet values. Similarly effective TU reductions have been reported in previous research at the Prado Wetlands (Cryder et al., in review).

4.4 Conclusions

In this study, a UPOW-CW was found to be effective in treating surface water containing fiproles and pyrethroids. The results presented herein provide strong evidence that partitioning into the sediment compartment and subsequent degradation were responsible for the observed removal of these HOCs and the consequent reductions in aquatic invertebrate toxicity. There is also evidence to suggest that movement into sediment was a result of settling of contaminated particles and sorption of dissolved residues. This indicates that the presence of wetland macrophytes is not necessarily required for the effective removal of HOCs and that UPOW cells are adequate to significantly reduce their concentrations in water. However, it is likely that the presence of wetland plants would have partially mitigated resuspension of contaminated sediment particles and contributed to analyte removal via plant uptake and sediment modification, thereby increasing the overall treatment efficacy of fiproles and pyrethroids. Future research should focus on disentangling the precise chemical and biochemical pathways responsible for removal of HOCs to minimize the costs and optimize the benefits of CW construction and operation while seeking to identify the type of CW that is ideal for HOC treatment.

4.5 References

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4.6 Supporting Information

Figure S4.1: Schematic map of the Prado Wetlands in Corona, California (from Orange County Water District).

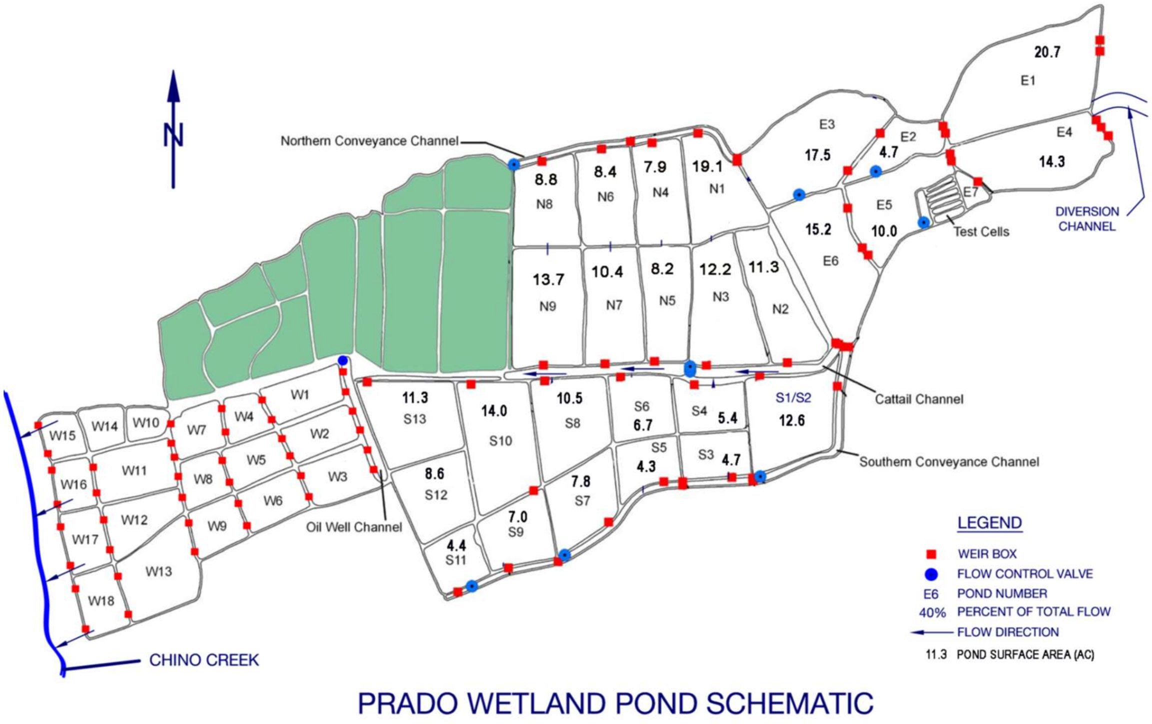

Table S4.1: Unit Process Open Water Constructed Wetland sediment properties.

Sand (%)	Silt (%)	Clay (%)	Texture Classification	pH	Organic Matter (%)	Organic Carbon (%)
34	39	27	Clay Loam	7.18	3.66	2.08

Table S4.2: Unit Process Open Water Constructed Wetland flow and sedimentation rates during the study period.

Sampling Month	**Inlet Flow (ML d^{-1})**	**Outlet Flow (ML d^{-1})**	**Sedimentation Rate (kg d^{-1})[a]**
June 2018	0.389	0.588	-5.31[b]
July 2018	0.389	1.66	-17.0
August 2018	0.680	0.588	-0.202
October 2018	1.44	1.98	-25.7
November 2018	0.863	0.821	-0.571
December 2018	0.190	0.382	-4.49
January 2019	0.389	0.588	-19.3

[a] Sedimentation rates were calculated as the difference of the inlet and outlet total suspended solids (TSS) mass fluxes.

[b] Negative sedimentation rates indicate export of TSS from the wetland.

Table S4.3: Unit Process Open Water Constructed Wetland water temperature and pH during the study period. Data are reported as mean ± 1 SD.

Sampling Month	Water Temperature (°C)[b]	Water pH[a]		
		Inlet	Midpoint	Outlet
June 2018	21.2 ± 0.764	8.38 ± 0.0551	8.68 ± 0.174	9.14 ± 0.0100
July 2018	13.6 ± 0.346	8.10 ± 0.0265	7.94 ± 0.147	7.86 ± 0.00577
August 2018	21.8 ± 0.289	8.04 ± 0.0173	8.12 ± 0.179	8.76 ± 0.0265
October 2018	15.4 ± 0.709	7.60 ± 0.0300	7.41 ± 0.138	7.61 ± 0.0100
November 2018	11.4 ± 1.25	7.79 ± 0.0603	7.77 ± 0.0321	7.66 ± 0.0872
December 2018	10.1 ± 2.35	7.72 ± 0.0764	7.46 ± 0.218	7.42 ± 0.200
January 2019	7.8 ± 3.2	7.82 ± 0.0950	8.11 ± 0.305	8.37 ± 0.194

[a] Water pH measurement was performed *ex situ* using whole water samples collected for extraction.

[b] Water temperature measurement was performed *in situ* at the locations where water and sediment samples were collected for extraction.

Table S4.4: Toxicity values used for toxic unit calculation.

Test Organism	Analyte	Toxicity Test	Toxicity Value (ng L^{-1})	Source
Hyalella azteca[a]	Bifenthrin	EC_{50}	3.3	Weston and Jackson, 2009
		LC_{50}	7.7	
	Cyfluthrin	EC_{50}	1.9	
		LC_{50}	2.3	
Chironomus dilutus[b]	Fipronil	EC_{50}	32.5	Weston and Lydy, 2014
		LC_{50}	>81.5	
	Fipronil Sulfide	EC_{50}	9.9	
		LC_{50}	>69.1	
	Fipronil Sulfone	EC_{50}	7.7	
		LC_{50}	>104	

[a] Sublethal end point for EC_{50}: ability to swim.
[b] Sublethal end point for EC_{50}: ability to thrash when prodded.

4.6.1 Additional GC-MS Analysis Information

Analyte quantification was performed using an internal standard-normalized calibration curve ranging from 1 to 500 μg L^{-1}. If necessary, samples were diluted to fall within the range of the calibration curve. Coefficients of determination all fulfilled the requirement of $R^2 \geq 0.99$.

4.6.2 Matrix Spike Extraction Efficiency

For fipronil desulfinyl, fipronil sulfide, fipronil, fipronil sulfone, bifenthrin, and cyfluthrin, respectively, matrix spike extraction efficiencies were as follows: 104 ± 3%, 102 ± 1%, 103 ± 10%, 109 ± 4%, 153 ± 24%, and 102 ± 7% for liquid-liquid extraction; and 110 ± 34%, 94 ± 20%, 112 ± 18%, 115 ± 35%, 101 ± 5%, and 120 ± 4% for pressurized fluid extraction.

4.6.3 Additional Quality Control Measures

All glassware was baked at 400 °C for 4 h to eliminate cross contamination. Na_2SO_4 and NaCl were baked at 400 °C for 4 h before use. Florisil was activated at 130 °C for 4 h before being packed into solid phase extraction cartridges. All amber glass bottles were rinsed with dichloromethane and acetone before sample collection.

Chapter 5 Conclusions and Future Work

5.1 Conclusions

The primary goal of this work was to examine strategies to reduce contamination of surface water by urban insecticides. The specific strategies investigated were mitigation of surface water pollution via identification of fiprole runoff sources and constructed wetland treatment of surface water contaminated with fiproles and pyrethroids. Conclusions for the specific objectives of this dissertation are given below:

Objective 1: Identify the contribution of concrete surfaces, soil, and urban dust to surface runoff of fiproles in residential environments following treatment with a professional fipronil formulation.

Urban-use pesticides present a unique risk to non-target organisms in surface aquatic systems because impervious pavement facilitates runoff that may lead to serious contamination and ensuing aquatic toxicity. Fipronil is an insecticide used at high rates in urban environments, especially in regions such as California. This compound and its biologically active degradation products have been detected in urban runoff drainage and downstream surface water bodies at concentrations exceeding toxicity thresholds for sensitive aquatic invertebrates, necessitating a better understanding of the runoff sources and causes of this contamination at sites of application. Sorption of fipronil, fipronil desulfinyl, fipronil sulfide, and fipronil sulfone was evaluated in urban dust, soil, and concrete, matrices commonly associated with the perimeter of a residential home. Samples were also collected from five single family homes treated with fipronil in Riverside, California, for five months to determine the occurrence of fipronil and its degradates in runoff water, urban dust, soil, and on concrete surfaces. Statistical analysis was performed to determine which urban matrices contributed more significantly to the contaminant levels in runoff water. Freundlich sorption coefficients for fipronil and its degradation products in dust were 3- to 9-fold greater than their values in soil. Fipronil and its degradates were detected in 100% of runoff samples and their presence was observed in dust, soil, and concrete wipe samples for 153 d after the treatment. Linear regression analysis showed that concrete surfaces were a primary source of all four compounds to runoff, and loose dust on concrete pavement also served as an important contributor. This research represents the first comprehensive investigation of the sources and causes for surface runoff contamination by fipronil and its degradation products. Findings highlight the importance of reducing fipronil residues on concrete surfaces through improved application methods and other mitigation practices.

Objective 2: Evaluate the effect of a large-scale vegetated surface flow constructed wetland on concentrations and mass loadings of fiproles and pyrethroids. Toxic unit values were also calculated to determine the change in adverse effects presented to sensitive aquatic invertebrates.

Water treatment and reuse initiatives are essential to combat declining water supplies in a changing climate, especially in arid and semi-arid regions. Pollution of water resources intensifies the search for strategies to provide water for potable and non-potable reuse that mitigates detrimental ecological and human health effects. Fipronil and synthetic pyrethroids are common urban-use insecticides that exert aquatic toxicity at trace levels and have been often found in urban surface streams. Samples were collected from the 182 ha Prado Wetlands in Southern California for seven months to assess the occurrence of fipronil and its degradation products as well as pyrethroids (bifenthrin and cyfluthrin) in water, sediment, and plants in a 4.45 ha vegetated surface flow constructed wetland. Concentration-based removal values and changes in mass flux were calculated to determine the efficacy of constructed wetland treatment. Observed water concentrations were further used to calculate toxic units for the invertebrates *Hyalella azteca* and *Chironomus dilutus*. Pesticide concentrations in water, sediment, and plant samples consistently decreased during passage through the constructed wetland at all time points. Removal values for fipronil desulfinyl, fipronil sulfide, fipronil, fipronil sulfone, bifenthrin, and cyfluthrin were 100%, 99.7-100%, 57.8-88.1%, 75.6-100%, 74.7-100%, and 36.6-82.2%, respectively, and there was a general net deposition of pesticides into constructed wetland compartments. Toxic unit values decreased in every instance for both aquatic invertebrates. Settling of contaminated particles, adsorption to sediment, plant uptake or adsorption, and subsequent degradation contributed to the effective removal of these urban-use insecticides, which highlights the potential of constructed wetlands for protecting urban water quality.

Objective 3: Assess the fiprole and pyrethroid treatment efficacy of a unit process open water constructed wetland and compare results to those generated in the study of the vegetated surface flow constructed wetland. Toxic unit values were calculated to demonstrate the effect of insecticide treatment on potential ecotoxicity.

Fresh water scarcity has required the development of treatment technologies to eliminate chemical and biological contaminants. Such treatment allows for recycling of existing water resources for potable and non-potable applications. Frequently detected, toxic urban contaminants such as fiproles and pyrethroids represent a subset of such harmful pollutants that must be removed from surface water to prevent ecological and human health risks posed by water reuse. Water and sediment samples were collected from a unit process open water constructed wetland and analyzed for fipronil desulfinyl, fipronil sulfide, fipronil, fipronil sulfone, bifenthrin, and cyfluthrin. Concentration-based removal and changes in analyte mass flux were calculated to determine the efficacy of unit process open water constructed wetland treatment. Aquatic invertebrate toxic units were calculated pre- and post-treatment to determine the effect of wetland treatment on toxicity endpoints in sensitive organisms. Water and sediment concentrations decreased during transport through the wetland, mean outlet concentrations were always lower than mean inlet concentrations, and wetland treatment always reduced mean toxic units. Concentration-based removal values for fipronil desulfinyl, fipronil sulfide, fipronil, fipronil sulfone, bifenthrin, and cyfluthrin were 100%, 82.9-100%, 44.2-98.8%, 55.7-88.7%, 61.8-100%, and 47.3-92.7%, respectively, and they generally exhibited net mass import into the wetland. These results demonstrate that unit process open water constructed wetlands are effective in removing fiproles and pyrethroids via sediment binding and subsequent degradation, and thus represent an important component of future water treatment and mitigation initiatives.

5.2 Recommendations for Future Work

Based upon literature evidence and the research detailed in this dissertation, it is clear that contamination of surface water by fiproles and pyrethroids can be combated by mitigating pollution at sites of application and treating polluted water with constructed wetlands or similar treatment processes. However, to understand the exact impact of urban mitigation strategies and to minimize the cost of water treatment while maximizing removal efficacy, more information is required. Future investigations of urban insecticide runoff mitigation should determine how runoff loads and pest treatment efficacy are affected by eliminating pesticide application on concrete surfaces and urban dust. In addition, more information is needed to optimize constructed wetland treatment of fiproles and pyrethroids. Laboratory experiments should be conducted to isolate the precise mechanisms responsible for their effective treatment, and varied constructed wetland designs must be evaluated to identify ideal designs and materials. This combined data may be used to direct water treatment initiatives focused on urban insecticides. The findings of such work will also lay the foundation for the reduction of surface water pollution by multiple classes of anthropogenic compounds.