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Phyto-Mitigation to Remove Neonicotinoids in Surface Runoff

Problem Statement

The transport of insecticides especially neonicotinoids from agricultural/urban settings to surface water represents a potential risk to aquatic ecosystems owing to their strong hydrophilicity, moderate to long persistence, and known non-target toxicity to aquatic invertebrates. Practical and implementable practices are urgently needed to mitigate offsite movement of neonicotinoid insecticides to surface waters. Given the strong systemic properties of neonicotinoids, assessing the neonicotinoid removal abilities of native vegetation will provide useful insights to develop natural and relatively inexpensive phyto-mitigation strategies to minimize the transport of neonicotinoids into surface water.

Background and Goals

Neonicotinoids are a relatively new class of insecticides that have found increasing uses in both urban and agricultural sectors. At present, imidacloprid from the neonicotinoid family is the most widely used insecticide in the world. Neonicotinoids are systemic insecticides and are

readily absorbed through plant roots and leaves. This characteristic underpins their flexible application methods, including as seed coatings, foliar spray, as well as application to soil. Plants readily absorb neonicotinoids and translocate them to different plant tissues, resulting protection against various herbivorous insects.

Unlike many other current-use insecticides, neonicotinoids are hydrophilic and do not strongly sorb to solids, which, when coupled with their moderate-to-long persistence, contribute to their high mobility in the environment and hence ubiquitous occurrence in surface water draining agricultural and urban areas. Monitoring studies in urban creeks in California have shown that imidacloprid often exceeds the EPA aquatic life benchmark of 10 ng/L. Effective and implementable mitigation practices are urgently needed to remove neonicotinoids in surface runoff water.

The high-water solubility and relatively long persistence suggest that conventional practices, such as erosion control and sediment trapping will be ineffective in minimizing neonicotinoids from runoff water. On the other hand, owing to the strong systemic property of neonicotinoids, plants may be used to absorb and remove such insecticides from runoff water in various settings, such as constructed and natural wetlands, vegetative strips, bioswales, and grassed waterways. Such phyto-mitigation practices are also more practical as they are relatively inexpensive, natural, and easy to maintain, and have a much larger treatment capacity than other practices. In addition, such phyto-mitigation practices may be used for treating runoff water originating from either agricultural fields or urban watersheds.

The primary objective of this project is to evaluate the potential of common wetland plant bulrush and common grass tall fescue for absorbing and attenuating neonicotinoids from runoff water. The obtained knowledge may be used for developing plants-based mitigation practices for

removing neonicotinoid residues in runoff water and protecting water quality.

Work to be Performed

- **Task 1.** Carry out hydroponic experiments to evaluate the capacity for hardstem bulrush and tall fescue to absorb and remove neonicotinoids from water, and understand the influence of plant density/biomass and contact time on the removal rate.
- **Task 2.** Construct field plots mimicking wetland cells and vegetated buffers at the Agricultural Operations at UC Riverside.
- **Task 3**. Using 4-6 neonicotinoid insecticides, carry out multiple experiments simulating runoff episodes and treatment events and monitor changes in pesticide concentrations before and after passing through the wetland cells and vegetated buffer strips.
- **Task 4.** Analyze variables influencing neonicotinoid removal, such as vegetation density, growth stages, soil properties, slope, and hydraulic residence time, and use models to consider different configurations and to optimize the mitigation efficacy of neonicotinoids using vegetated wetlands, vegetative filters, grassed waterways, and bio-swales.

Deliverables

Task 1: Hydroponic experiments

In the hydroponic experiments, we used tall fescue and bulrush, typical plants found in wetlands or vegetative buffers, to test their effectiveness to absorb neonicotinoids from water solution. The duration of the experiments was 28 days under greenhouse conditions, and samples were collected after 1, 3, 7, 14, 21, and 28 days. The different plant parts (shoots and roots) were collected, extracted, and analyzed separately using the QuEChERS method and LC-MS/MS. Six neonicotinoids were tested and used for spiking the nutrient solution, including acetamiprid, clothianidin, dinotefuran, imidacloprid, thiamethoxam, and thiacloprid. The results in Figure 1 illustrate that tall fescue and bulrush could absorb, accumulate, and translocate neonicotinoids to

various degrees. The six neonicotinoids were detected in the roots and shoots of all treated plants. The results also showed that neonicotinoid accumulation varied among plant species. Bulrush generally showed greater uptake, accumulation, and translocation than tall fescue (Figure 1). The removal efficiency of tall fescue increased over time, with over 95% of the initially spiked neonicotinoids removed by the end of 28-day experiments (Figure 2). Bulrush, likely due to their relatively large biomass, caused immediate removal of neonicotinoids from the system (Figure 2).

The results validated that grass and bulrush are highly efficient at absorbing neonicotinoid insecticides, and the rapid uptake and accumulation may be attributed to the systemic characteristics of these compounds. Therefore, using wetland and other plants (e.g., grass) may be expected to remove neonicotinoids from water column.

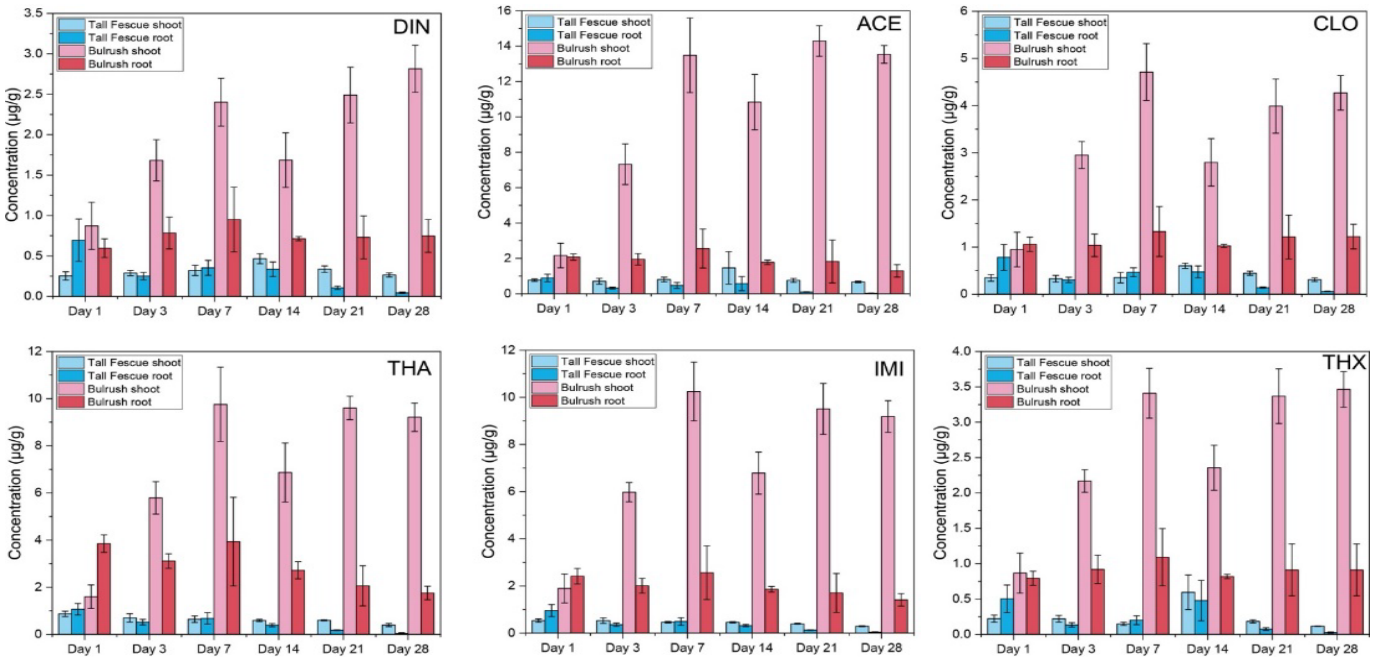

Figure 1. The distribution and accumulation of neonicotinoids in different plant tissues

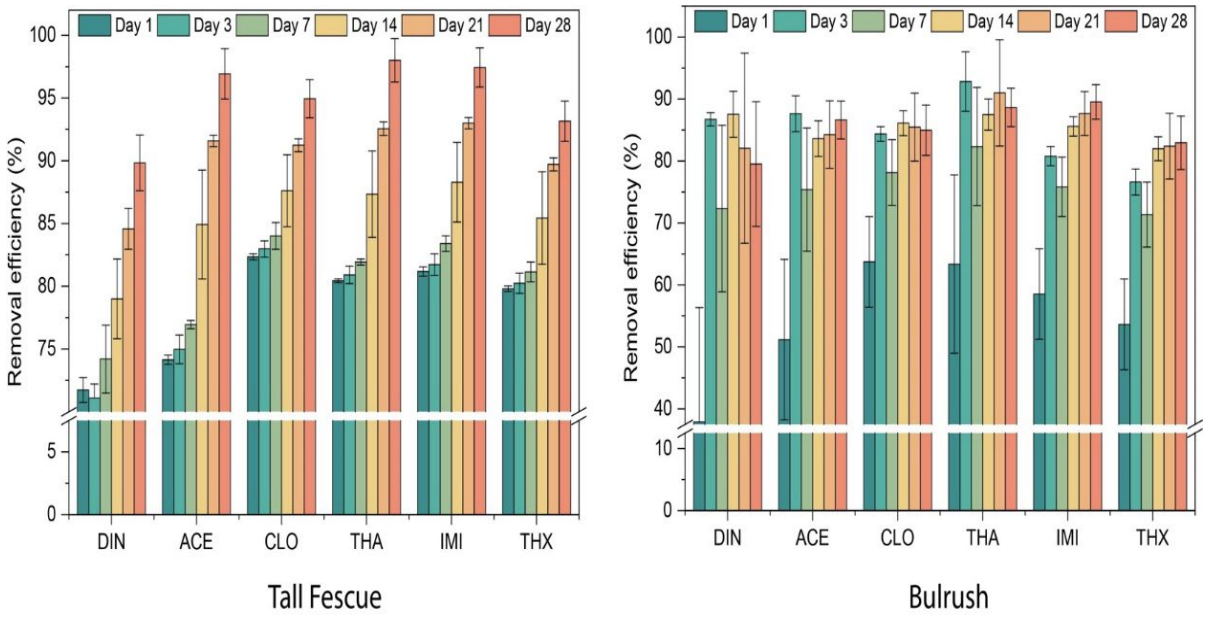

Figure 2. The Removal efficiencies of six neonicotinoids in different date points

Tasks 2-4. Constructed wetland experiments

We made modifications to Tasks 2-4. Instead of using small-scale, newly constructed systems, we collaborated with Orange County Water District to evaluate the effectiveness of wetlands in removing neonicotinoid residues in surface flow. The Prado wetlands are the largest constructed wetlands on the West Coast and consist of different wetland cells. We carried out monitoring at different locations within the Prado wetland system over multiple months. The experimental approach and findings were summarized in a manuscript submitted to Environmental Pollution for peer review and publication. The manuscript has been accepted for publication.

Below is a copy of the submitted manuscript.

Removal of neonicotinoid insecticides in a large-scale constructed wetland system

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Abstract

Neonicotinoid insecticides are among the most used insecticides and their residues are frequently found in surface water due to their persistence and mobility. Neonicotinoid insecticides exhibit toxicity to a wide range of aquatic invertebrates at environmentally relevant levels, and therefore their contamination in surface water is of significant concern. In this study, we investigated the spatiotemporal distribution of six neonicotinoids in a large wetland system, the Prado Wetlands, in Southern California, and further evaluated the wetlands' efficiency at removing these insecticides. Total neonicotinoid concentrations in water ranged from 3.17 to 46.9 ng L^{-1} at different locations within the wetlands, with imidacloprid and dinotefuran among the most detected. Removal was calculated based on concentrations as well as mass fluxes. The concentration-based removal values for a shallow pond (vegetation-free), moderately vegetated cells, densely vegetated cells, and the entire wetland train were 16.9%, 34.2%, 90.2%, and 61.3%, respectively. Principal component analysis revealed that pH and temperature were the primary factors affecting the removal of neonicotinoids. Results from this study demonstrated the ubiquitous presence of neonicotinoids in surface water impacted by urban runoff and wastewater effluent and highlighted the efficiency of wetlands in removing these trace contaminants due to concerted effects of uptake by wetland plants, photolysis, and microbial degradation.

Introduction

Surface water is the primary water source for direct human consumption, agriculture, industry, and biodiversity conservation, but is often impaired by contamination of man-made

chemicals (Gifford et al., 2018; Kolpin et al., 2002; Shi et al., 2019). Over the past two decades, neonicotinoids, which are broad-spectrum systemic insecticides (Simon-Delso et al., 2015), have been the most used insecticides in both agricultural and urban settings (Jeschke et al., 2011; Simon-Delso et al., 2015; Hladik and Kolpin, 2016; Gould et al., 2018; Douglas and Tooker, 2015; Jeschke et al., 2011; Simon-Delso et al., 2015). As water-soluble compounds, neonicotinoids are highly mobile and have been frequently detected in rivers and streams (Dijk et al., 2013; Hladik et al., 2014; Sánchez-Bayo and Hyne, 2014; Schaafsma et al., 2015; Starner and Goh, 2012). For example, a nationwide study of streams in the United States showed that at least one neonicotinoid compound was present in 63% of the 48 streams surveyed (Hladik and Kolpin, 2016). Neonicotinoids were found ubiquitously in all streams draining row-crop areas in the Midwest of the United States (Klarich et al., 2017), with maximal concentrations of 260, 43, and 190 ng L^{-1} for clothianidin, imidacloprid, and thiamethoxam, respectively. Another route for neonicotinoids to contaminate surface water is through wastewater treatment plant (WWTP) effluents (Sadaria et al., 2016), as they are not effectively removed via current WWTP systems (Iancu and Radu, 2018; Sadaria et al., 2016).

Recent studies highlighted the chronic toxicity of neonicotinoids, especially in aquatic invertebrates (Miles et al., 2017; Morrissey et al., 2015; Sánchez-bayo et al., 2016). The presence of neonicotinoids in surface water has been associated with observable impacts on invertebrates (Dijk et al., 2013; Prosser et al., 2016), as well as consequential effects on insect-eating birds (Hallmann et al., 2014) and fish (Gibbons et al., 2015). Research indicated the presence of neonicotinoid insecticides in surface water within urban areas (Buzby et al., 2020) at levels that hold toxicological significance for aquatic invertebrates (Tennekes, 2010), and similarly, in the

sediment, where residues have the potential to endure for prolonged periods following deposition (Kuechle et al., 2019).

Constructed wetlands (CWs) represent a potential option to remove neonicotinoid residues in surface water. Many studies have demonstrated that CWs can effectively remove nitrogen and phosphorous species (Vymazal, 2007), metals (Lima et al., 2013), antibiotic resistance genes (Du et al., 2022), and various organic compounds (Nguyen et al., 2019; Paz et al., 2019). Given that neonicotinoids are systemic insecticides (Simon-Delso et al., 2015), wetland plants such as macrophytes may be efficient at taking up neonicotinoids. The few studies to date have shown inconclusive results. In a greenhouse study, a variety of wetland plants were found to be capable of removing neonicotinoids when grown in hydroponic containers (Liu et al., 2021). However, in Sadaria et al. (2016), an engineered wetland did not show significant removal of imidacloprid or acetamiprid. In contrast, in Main et al. (2017), the presence of vegetation in prairie wetlands was found to attenuate contamination of clothianidin, and the reduction was attributed to accumulation by wetland macrophytes.

As neonicotinoid use and contamination of surface waters continue to grow in both frequency and spatial extent, it is important to evaluate management strategies to reduce neonicotinoid contamination of surface water. Therefore, the main objective of this study was to determine the ability of constructed wetlands to mitigate neonicotinoid water contamination. We specifically aimed to 1) explore the spatial-temporal variations of neonicotinoid insecticides in the Prado Wetlands, a large wetland system receiving both urban runoff and WWTP effluent; 2) assess the removal efficiencies of neonicotinoid insecticides of wetland cells with different vegetation densities; and 3) evaluate the reductions in aquatic toxicity achieved by the wetlands. This study provides information for ascertaining the effectiveness of constructed wetlands in

minimizing neonicotinoid contamination in surface flows under field and environmentally relevant conditions.

Materials and Methods

2.1 Study area

The Prado Wetlands is a managed constructed wetland system situated near the Prado Dam in Southern California. It is the largest constructed wetland (CW) on the west coast of the United States, covering an area of approximately 188 ha consisting of 50 shallow wetland ponds (OCWD, 2019). The primary use of the Prado Wetlands has been to remove nitrate from the wastewater-impacted Santa Ana River since 1992 (OCWD, 2019). During the dry months, approximately 50% of the Santa Ana River flow, which is dominated by discharge from twelve upstream WWTPs, is directed into the Prado Wetlands system for treatment (OCWD, 2019). During the rest of the year, stormwater runoff and snowmelt account for the majority of the river's flow.

The present study considered different wetland ponds, annotated as BB1, S7-S8, and S9-S10, as shown in Figure 1. BB1 covered 0.770 ha and was essentially absent of any vegetation; S7-S8 was 7.54 ha in size and consisted of two connected wetlands with moderate vegetation density; and S9-S10 was 9.41 ha in size and consisted of two connected wetlands with relatively high vegetation density. BB1 was located in the front section of the whole wetland system, where diverted flow entered the wetlands, while S7-S8 and S9-S10 were vegetated wetland cells located at the heart of the wetland system (Figure 1). From a rhodamine tracer experiment carried out at the Prado Wetlands (Lin et al., 2003), the hydraulic retention time of the entire Prado Wetlands was estimated to be 1.29 days. Samples and measurements were taken at the

inlet weir box (inlet) and the outlet weir box (outlet) of BB1, S7-S8, and S9-S10 wetland cells, as well as at the entry (Prado inlet) and W17 exit (Prado outlet) points of the entire wetland systems (Figure 1).

2.2 Chemicals and Materials

All analytical standards used in this study were procured with reported purities ≥ 98 %. Specifically, acetamiprid, clothianidin, dinotefuran, imidacloprid, thiamethoxam, and thiacloprid standards were purchased from Sigma-Aldrich (Saint Louis, MO). Methanol, acetone, and acetonitrile (HPLC grade) were purchased from Fisher Scientific (Fair Lawn, NJ). Ultrapure water was prepared using an in-house Milli-Q water purification system from Millipore (Carrigtwohill, Cork, Ireland).

2.3 Sample collection and water quality parameters

In order to investigate neonicotinoids removal in the Prado wetlands, a total of 54 surface water samples were collected on a monthly basis from June to November in 2022 at various locations, including Prado inlet, BB1 inlet, BB1 outlet, S7 inlet, S8 outlet, S9 inlet, S10 outlet, and Prado outlet (Figure 1). Grab samples were collected directly into 1-L amber glass bottles, kept at 4° C, and extracted within 24 h after collection. Additionally, plant samples including bulrush shoots (n = 5), bulrush roots (n = 5), duckweed (n = 5), hydrocotyle (n = 4) and sediment samples (n = 11) were collected in wetland cells BB1, S7-S8, and S9-S10. Sediment samples were collected by using a small hand shovel from a surface depth of 0 – 15 cm, and placed in 50 mL centrifuge tubes. Bulrush was collected along with the root, while only the shoot and leaves

were collected for hydrocotyle. Duckweed was collected by using a small hand fishing net. All the plant samples were wrapped in foil and stored in a -80°C freezer until analysis. All sediment and plant samples were freeze-dried under vacuum at -60°C for three days before analysis.

The water quality parameters, including temperature (T), pH, electric conductivity (EC), TDS, and dissolved oxygen (DO), were measured *in situ* using a YSI Pro20 meter (Yellow Spring, OH). Water samples (50 mL) were filtered through 0.45 μm-PTFE filters (ANPEL, Shanghai, China), and the filtrate was used for analysis of nutrients. The concentrations of nitrite (NO_2^--N), nitrate (NO_3^--N), and phosphorus (PO_4^{3-}-P) were measured by using a Dionex Aquion Ion Chromatography (Sunnyvale, CA), along with a Seal AQ2 Discrete Analyzer (Mequon WI) for ammonium (NH_4^+-N). Further information and details are given in Table S1.

2.4 Sample extraction and analysis

2.4.1 Extraction of water samples

A 1.0-L aliquot of water sample was filtered through glass fiber filters (GF/F, 0.7 mm, Whatman, England), followed by the addition of 500 mg Na4EDTA·2H2O. To address the matrix effects, the filtered samples were spiked with surrogate standard. Solid-phase extraction (SPE) was carried out using an Oasis HLB cartridge (500 mg 6mL, Waters) to extract and concentrate neonicotinoid compounds. The cartridges were sequentially activated with 18 mL methanol and 6 mL Milli-Q water. Subsequently, the water samples were loaded onto the cartridges at a flow rate of 5 mL min^{-1}, and the loaded cartridges were then dried under vacuum for approximately 10 min. The sample cartridges were then eluted with 12 mL methanol and 6 mL of acetone: methanol (1:1 v/v), sequentially. The eluate was evaporated to dryness under a gentle stream of nitrogen and reconstituted with 1.0 mL methanol: H_2O (1:1 v/v). The final

samples were filtered through a 0.22 μm-PTFE syringe filter into a glass HPLC vial and kept at -20 ℃ before further analysis by LC-MS/MS.

2.4.2 Extraction of sediment and plant samples

The freeze-dried plant tissue samples underwent a grinding process by a tissue grinder to achieve a finely powdered. A modified multi-step QuEChERS method (Sigma-Aldrich, n.d.) was employed to extract neonicotinoids from plant tissue samples. In brief, a plant tissue sample weighing 1.0 g was measured and introduced into a 50 mL centrifuge tube. Subsequently, 20 mL of acetonitrile (ACN) was added, and the mixture was vigorously vortexed for a duration of 1 min. To this mixture, 4.0 g of anhydrous MgSO4 and 1.0 g of NaCl were added, followed by vortexing for another 1 min, and sonication for 15 min. The sample tubes were centrifuged at 3500 rpm for 15 min, and a 9 mL aliquot of the supernatant was decanted into a 15 mL cleanup tube (Thermo Scientific product number 60105–205; 900 mg $MgSO_4$/400 mg PSA/400 mg GCB). The tubes were then shaken vigorously for approximately 1 min, followed by centrifugation at 3500 rpm for 15 min. A 6 mL portion of the ultimate supernatant was transferred into a test tube and subjected to evaporation until completely dry, using a gentle stream of nitrogen. The dried residue was reconstituted using 1.0 mL methanol: H2O (1:1 v/v) and subjected to sonication for 5 mins. The mixture was then filtered through a 0.22 μm-PTFE filter and transferred to an HPLC vial. The final extracts were stored at -20 ℃ before LC-MS/MS analysis.

2.4.3 Chemical Analysis

Analysis of sample extracts was carried out on a Waters ACQUITY ultra-performance liquid chromatography (UPLC) system coupled to a Waters triple quadrupole mass spectrometer (QqQ-MS/MS) (Waters, Milford, MA). An ACQUITY BEH C18 column (100 × 2.1 mm i.d., 1.7 μm; Waters, Milford, MA) was used for chromatographic separation. The LC conditions for the neonicotinoid analysis were as follows: injection volume, 5 μl; mobile phase flow rate, 0.3 mL min^{-1}; column temperature, 40 ℃; mobile phase A, 0.1% formic acid in Milli-Q water; mobile phase B, 100% methanol. The mobile phase gradient was programmed as follows (with regard to mobile phase B): 10% (0 min), 40% (1.5 min), 50% (4 min), 100% (6 min), 10% (8 min), and 10% B (9 min). The multiple reaction monitoring (MRM) transitions of all target compounds were optimized and are provided in Table S2. Data were processed using the TargetLynx XS software (Waters, Milford, MA).

The working solutions of the six neonicotinoids were prepared by diluting standard mixtures in methanol for UPLC-MS/MS analysis. The quantification of each neonicotinoid was conducted by the external standard method. For each sampling batch, and instrumental lank, procedural blank, sample repetition, blank spike, and matrix spike were applied. All instrumental and procedural blanks were below the method detection limits (MDLs). The blank recoveries, matrix recovery, MDLs, method quantification limits (MQLs), instrumental detection limits (IDLs), and instrumental quantification limits (IQLs) of the six neonicotinoids are shown in Table S3. The limit of quantification (LOQs) was estimated as a signal-to-noise ratio (S/N) of 10, which was given by TargetLynx XS software (Table S3).

2.5 Environmental risk assessment

The risk quotient (RQ) method was used to evaluate the potential ecological risk of individual neonicotinoids for freshwater species. The RQ values in the water were calculated as follows:

$$RQ = \frac{MEC}{PNEC} \quad (1)$$

where MEC and PNEC were the measured concentrations and predicted no-effect concentrations of neonicotinoids, respectively. The PNEC values for dinotefuran, thiamethoxam, clothianidin, imidacloprid, acetamiprid, and thiacloprid were reported to be 0.953, 0.4, 0.0024, 0.18, 0.1, and 0.017 mg L^{-1}, respectively (Mahai et al., 2019; Zhang et al., 2023). The ecological risks were classified into three levels: low risk, RQ < 0.1; medium risk, 0.1 ≤ RQ < 1; and high risk, RQ > 1 (Zhang et al., 2023).

3. Results and Discussion

3.1 Occurrence of neonicotinoid insecticides at the Prado Wetlands

3.1.1 Spatiotemporal trends of neonicotinoid insecticides in water

Neonicotinoid compounds were frequently detected in water samples collected within the Prado Wetland system, with dinotefuran (87.5%) and imidacloprid (100%) detected at a higher frequency than the other compounds (Figure S1). Figure 2A and Table 1 show the concentrations of the six neonicotinoids in water samples collected from the Prado Wetlands. The total concentrations of neonicotinoids varied from 3.17 to 46.9 ng L-1 at different sampling locations

within the wetland system. Compared to earlier studies, the concentrations of neonicotinoid insecticides in the water samples from the Prado Wetlands were relatively low. For example, previous studies reported a maximum total concentration of three neonicotinoids in the Maumee River to be 670 ng L-1 (Hladik et al., 2018), a maximum concentration of 0.13 μg L^{-1} of imidacloprid in the Kisco River (Phillips and Bode, 2004), a seasonal average concentration of 198.6 ng L^{-1} of four neonicotinoids (i.e., clothianidin, thiamethoxam, imidacloprid, and acetamiprid) in an intensive agricultural area in central Saskatchewan (Main et al., 2015), and a total neonicotinoid concentration up to 3290 ng L^{-1} in a river in California (Starner and Goh, 2012). The differences in the maximum concentrations between the Prado Wetlands and the surface streams in other areas could be attributed to the surrounding drainage areas, as the Prado Wetlands receive mostly treated wastewater and urban drainage water.

In this study, imidacloprid and dinotefuran were found to be the most prevalent neonicotinoid insecticides, accounting for an average of 54.82 ± 15.22% (10.8 ± 5.81 ng L^{-1}) and 39.42 ± 15.41% (9.03 ± 5.67 ng L^{-1}) of the total neonicotinoid concentrations in water, respectively. Imidacloprid was the most commonly detected, which was consistent with its widespread use in both agricultural (Jeschke et al., 2011) and urban areas (Sánchez-Bayo and Hyne, 2014; Simon-Delso et al., 2015). Previous research estimated that approximately 1.0 - 3.4 tons of imidacloprid was discharged into U.S. surface waters annually (Sadaria et al., 2016). Imidacloprid was the most frequently detected neonicotinoid insecticide in the Great Lakes, USA (Hladik et al., 2018), and concentrations of up to 10,400 ng L^{-1} were reported in Lake Erie and Lake Ontario (Struger et al., 2017). Globally, imidacloprid was detected at up to 4.56 μg L^{-1} in rivers near Sydney (Sánchez-Bayo and Hyne, 2014) and > 0.1 μg L^{-1} in New Brunswick, Canada (Anderson et al., 2015). Imidacloprid has relatively long persistence in aqueous environments,

with half-lives of 35.9-230 d in water (Pietrzak et al., 2020). Despite the low recovery of dinotefuran, leading to its exclusion from target list (Zhang et al., 2017), a small amount of studies have nonetheless reported the detection of dinotefuran in environmental waters. It was reported that only one sample had detectable dinotefuran (1.6 ng L-1) in Sope Creek, GA (Michelle L. Hladik, 2012), the concentrations of dinotefuran ranged from 9.4 - 100 ng L-1 in the rivers of Osaka City, Japan (Yamamoto et al., 2012), and dinotefuran was the most dominant neonicotinoids (200 ± 296 ng L-1) in Poyang Lake basin (Xiong et al., 2021). Dinotefuran has been used in residential and around commercial buildings, in professional turf management (USEPA, 2004), and also as a veterinary medicine for the prevention of fleas and ticks on dogs and cats (USEPA, 2004). The results of this study were also supported by the annual usage of imidacloprid and dinotefuran in the region; imidacloprid and dinotefuran are the most heavily used neonicotinoids in Riverside, CA, which drains into Santa Ana River that feeds the Prado Wetlands (Table S4). The transport of neonicotinoids to surface streams has been shown to be driven by both use and precipitation, with rainfall events increasing the potential for surface water contamination (Hladik et al., 2014). For example, a previous study suggested that dry weather conditions limited the offsite transport of neonicotinoids to streams (Chiovarou and Siewicki, 2007). In this study, the relatively low concentrations of neonicotinoids observed in the Prado Wetlands as compared to their detections in other studies may be also due to the fact that sampling was carried out during the dry season with little rainfall. To capture the full extent of neonicotinoid contamination in areas with distinct temporal patterns of precipitation, wet season and stormwater runoff monitoring should also be conducted.

During the sampling period, the concentrations of neonicotinoid insecticides in water samples exhibited a clear increasing trend (Figure 3). The total concentration of neonicotinoids at

each site increased steadily from June to October and then decreased from October to November. It is likely that the initial rain events in September and October mobilized some of the neonicotinoid residues, leading to their increases, while further rain events in November caused dilution, resulting in decreased concentrations (Table S5) (Hladik et al., 2014). A study of the Maumee River, a tributary of Lake Erie, showed an increase in neonicotinoid concentrations starting in May, with maximum concentrations frequently detected in July (Hladik et al., 2018). Rainfall-runoff was also found to play an important role in the offsite transport of neonicotinoids to streams in Struger et al. (2017), even during peak pesticide applications in summer (Main et al., 2014). Findings from this and earlier studies suggested that the management of neonicotinoid contamination in surface waters should take into consideration the effect of precipitation on their offsite movement, particularly during the rainy season.

3.1.2 Spatiotemporal variation of neonicotinoids in sediments and wetland plants

With the exception of imidacloprid, the other five neonicotinoids were below the detection limits in sediment and plant samples collected from the Prado Wetlands. The low occurrence or non-detection of these compounds in sediment and plant samples was consistent with their high water solubility, which would limit their partition into the sediment phase (Zhang et al., 2018). Figure 2B shows the imidacloprid concentrations in sediment and plant samples in the Prado Wetlands. The average imidacloprid concentrations in sediment, bulrush shoot, bulrush root, hydrocotyle, and duckweed were 0.770, 0.760, 0.700, 0.650, and 0.900 ng g^{-1}, respectively. The detection of imidacloprid in sediment and plant samples from the Prado Wetlands was likely due to the fact that it was present in the wetland system at higher levels and that imidacloprid is more persistent than the other neonicotinoids (Buzby et al., 2020; Maloney et al., 2017). The general

lack of detectable neonicotinoids in the wetland sediments was in line with that reported for the Walnut Creek Watershed in Jasper County (Hladik et al., 2017) and Sacramento and Orange County, CA (Ensminger et al., 2013), which also showed no or low levels of neonicotinoids in sediments. The lack of detectable systemic uptake of most neonicotinoids by plants may be attributed to the low concentrations of these compounds in the sediment, as well as to the potential effects of growth dilution and/or active metabolism of these insecticides in wetland plants (Hladik et al., 2017). Nevertheless, the finding of imidacloprid in various wetland plants underscored the potential importance of plants in contributing to the removal of neonicotinoids when the contaminated water passes through vegetated wetland systems. Despite of the infrequently detections of neonicotinoids of plants in this study, the bioaccumulation potential in plants cannot be overlooked for neonicotinoid removal. Neonicotinoids, as systemic insecticides, 2% - 20% of them can be accumulated in plant tissues due to the strong inhaling capacity of plants (Alsafran et al., 2022). It is usually frequently reported that neonicotinoids are readily accumulated by plant. Pecenka and Lundgren, (2015) found that clothianidin concentrations up to 4 μg kg-1 in milkweed plant, imidacloprid and thiamethoxam were the most commonly detected neonicotinoids in fruits and vegetables from USCC study and HZC study (Lu et al., 2018), Ge et al, (2017) found that imidacloprid accumulated in rice leaves and roots with 10 mg kg-1 and 1.37 mg kg-1 at a soil-treated experiment. Therefore, the bioaccumulation mechanisms of plants regarding neonicotinoids need further research.

3.2 Removal and mass fluxes of neonicotinoids

The concentration-based removal efficiencies of neonicotinoids in water as they passed through the Prado wetland system are given in Figure 4A. The removal factor (RF, in %) was calculated based on the differences in concentrations at the inlet and outlet of the system under consideration:

$$(\%)\ RF = \frac{C_{Inlet} - C_{Outlet}}{C_{Inlet}} \qquad (1)$$

where C_{in} and C_{out} are the neonicotinoid concentrations at the inlet and outlet of a wetland system. To estimate the removal factor for the entire Prado Wetland system, concentrations at the Prado inlet and Prado outlet (W17) were used for the calculation. Additionally, it is important to acknowledge that the 100% removal included outlet concentrations that were below the detection limit. Throughout the duration of this study, the average removal efficiencies of the Prado inlet-Prado outlet, BB1, S7-S8, and S9-S10 were 66.59%, 27.61%, 42.65%, and 79.18%, respectively. Among the systems under evaluation, S9-S10 exhibited the highest removal efficiency, followed by Prado inlet – Prado outlet and S7-S8, whereas BB1 displayed the lowest removal values. The lowest removal observed in BB1 could be attributed to its relatively small area (0.770 ha) as well as low vegetation density. In comparison, the higher vegetation density and the relatively large area of S9-S10 likely contributed to the greater removal efficiency. However, the removal efficiency of neonicotinoids for the entire wetland system was not the highest, likely due to the fact that many wetland cells of different configurations and with varying states of vegetation and hydraulic retention times were operated in parallel before the treated water converged and discharged (Figure 1). In addition, uncertainties caused by spot sampling and the associated flow and sediment resuspension conditions at the time of sampling could also contribute to variations

in chemical concentrations and hence the derived removal efficiencies. The generally efficient removal of neonicotinoids through vegetated wetlands was in agreement with previous studies showing that the systemic neonicotinoid insecticides were effectively eliminated from hydroponic planted systems, with removal rates ranging from 9.5% to 99.9% (Liu et al., 2021).

There were no discernible monthly or seasonal patterns observed in the removal of neonicotinoids (Figure S2A). However, the peak removal efficacy was observed in August, which may be due to the relatively elevated temperature during this month, as well as active vegetation growth. The observed variations in removal efficiencies among different wetland cells could be attributed to many factors, including differences in vegetation densities (Dabrowski et al., 2006), hydraulic retention time (Gregoire et al., 2009), and environmental parameters (Main et al., 2017). The upstream Santa Ana River supplies a sufficient amount of nutrients to the wetlands (Bear et al., 2017; Vitko, 1996), which facilitates the establishment and growth of macrophytes that act to take up and metabolize neonicotinoids. Moreover, microbial communities in wetlands in warm regions such as Southern California promote active biotic degradation in the sediment, especially in root zones of wetland plants (Cryder et al., 2021).

In addition to the concentration-based removal, another essential metric for ascertaining the effectiveness of wetlands in attenuating contaminants is the mass flux of chemicals (Figure 4B). In this study, the mass flux of neonicotinoids was calculated using the following equation:

$$MF = C_{water} * Water\ Flow\ Rate \quad (2)$$

where MF is the mass flux, C_{water} is the chemical concentration in water, and the water flow rate is estimated by the onsite weir boxes or flumes. It is important to note that the mass flux values obtained were discrete estimates at the time of sampling. Specifically, the mass influx, mass efflux, and changes in mass flux (Δ mass flux) were calculated for the inlet and outlet of the individual wetland systems under consideration. The median Δ mass flux of BB1, S7-S9, and S8-S10 were 137.89, 148.70, and 219.36 mg d^{-1}, respectively. Positive changes in mass flux indicate the removal of neonicotinoids in a system, while a negative value would indicate a net export from the system. The majority of Δ mass flux values were statistically significant (*Wilcoxon* test, $P < 0.05$).

Positive changes in mass flux values were observed for BB1 (with a median value, of 137.87 mg d^{-1}), S7-S8 (with a median value, of 148.70 mg d^{-1}), and S9-S10 (with a median value, of 219.36 mg d^{-1}), which provides further evidence that the wetland cells were effective in removing neonicotinoid insecticides. However, there were significant variations in Δ mass flux values based on specific sampling time points. The 5-95% ranges were 21.700 - 819.39, 0.61000 - 748.85, and 47.780 -1176.7 mg d^{-1} for BB1, S7-S8, and S9-S10, respectively. The large variations could be attributed to changes in flow rate and flow-induced resuspension of sediment particles when the flow rate was high. Overall, these findings suggest that wetlands, including both unvegetated and vegetated wetland systems, are effective at removing neonicotinoid insecticide residues from water (Braskerud and Haarstad, 2018; Chiovarou and Siewicki, 2007; Gregoire et al., 2009). Further research is needed to better understand factors contributing to enhanced removal of neonicotinoids from water, such as plant uptake and metabolism, wetland plant species, vegetation density, photolysis, and environmental conditions.

To discern the effect of environmental parameters on the removal of neonicotinoids in the Prado Wetlands, a PCA analysis was conducted. Figure 5 shows a negative correlation between pH and temperature (T) with neonicotinoid levels, suggesting that higher pH and temperature may lead to lower neonicotinoid concentrations. Liang et al. (2019) documented an increase in photo-degradation of all neonicotinoids with increasing pH, and Guzsvány et al. (2006) observed that imidacloprid and thiamethoxam degraded rapidly under alkaline conditions. There was no significant correlation between nutrient levels (i.e., NH_4^+, NO_2^-, NO_3^-, PO_4^{3-}) and neonicotinoid concentrations in water. However, the presence of nutrients could potentially stimulate plant growth and microbial activity, which could subsequently accelerate the removal of neonicotinoids through increased plant uptake and enhanced microbial degradation. The overall findings suggested that many factors worked in concert in influencing the fate of neonicotinoids in a wetland system, such as pesticide properties (e.g., DT_{50}, K_d), sediment resuspension, and plant uptake, as well as water characteristics (e.g., pH, temperature, conductivity). Aquatic plants may also influence the micro-environment through physical and chemical alterations, such as changing light intensity, pH, and nutrient distribution. Neonicotinoid compounds are highly water soluble and may co-exist with dissolved organic matter in water (Bonmatin et al., 2015), and could undergo indirect photolysis with dissolved organic matter as the photosensitizer (Roy et al., 1999; Zeng and Arnold, 2013). Other researchers also reported the role of photolysis in environmental degradation of neonicotinoids (Lavine et al., 2010; Wamhoff and Schneider, 1999). Photolysis may be especially pronounced in unvegetated wetlands, such as BB1 which was shallow and largely void of vegetation. Nevertheless, it is imperative not to disregard the filtration effects exerted by water and DOM on UV radiation (Lu et al., 2015).

3.3 Neonicotinoid insecticide toxicity and risk assessment

Based on previous studies, contamination of neonicotinoids in rivers can pose ecological risks to aquatic organisms, particularly aquatic animals, resulting in adverse impacts on the biodiversity and overall functions of the aquatic ecosystem (Chen et al., 2019; Naumann et al., 2022). The risk quotient (RQ) was calculated based on the detected concentrations of individual neonicotinoids in the Prado Wetland system during the sampling period (Figure 6A). The monitored neonicotinoids, except for clothianidin, presented a relatively low ecological risk to aquatic ecosystems with RQ < 0.1 (Sánchez-Bayo et al., 2002). The RQs in the Prado Wetlands were comparable to those in the Huai River (Zhang et al., 2023), the central Yangtze River (Mahai et al., 2019), and the Sousa Rivers (Sousa et al., 2019). For each sampling event, a slight reduction in RQs was observed as water passed through the wetland system, consistent with previous studies (Liu et al., 2021; Main et al., 2017).

The U.S. EPA established acute and chronic toxicity thresholds (i.e., 385 and 10 ng L^{-1}, respectively) for imidacloprid to further safeguard aquatic ecosystems (USEPA, 2017). According to the U.S. EPA aquatic life benchmark, no imidacloprid values detected in this study exceeded the current acute aquatic life benchmarks, but the chronic benchmarks were exceeded 29 times (57% of samples) (Figure 6B). In addition, previous research has demonstrated that neonicotinoid metabolites possess similar levels of toxicity as the parent compounds (Casida, 2011; Suchail et al., 2003; Jeschke et al., 2011). Therefore, it is probable that the overall ecological risks were underestimated in this study by neglecting neonicotinoid metabolites (Bonmatin et al., 2021; Chen et al., 2021; Nomura et al., 2013; Song et al., 2020).

4. Conclusions

This study provides a comprehensive characterization of the spatiotemporal variations and the removal of neonicotinoids in a large wetland system during the dry season in California. The detected neonicotinoid concentrations in the Prado Wetlands were relatively low, with imidacloprid and dinotefuran as the most frequently detected compounds. The changes in neonicotinoid concentrations and mass fluxes highlighted that constructed wetlands were effective at removing neonicotinoid insecticides, likely due to uptake into wetland plants, photolysis, and microbial degradation. These findings suggest that constructed wetlands may be used as a low-cost efficient option for removing neonicotinoid residues from surface water. Vegetation density and hydraulic retention time were among the main variables for optimizing the removal of neonicotinoids. However, long-term monitoring considering different precipitation conditions and parent compound-metabolite mixtures is necessary to obtain a holistic understanding of wetlands as a mitigation strategy for water contaminated by neonicotinoid insecticides. In addition, the potential release of neonicotinoids sequestered by plants or sediment overtime should also be understood when evaluating the overall functions of wetlands in attenuating man-made chemicals such as neonicotinoid insecticides.

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Tables:

Table 1. Concentrations of six neonicotinoid insecticides of different sampling sites at the Prado Wetlands.

	Dinotefuran	Acetamiprid	Clothianidin	Thiacloprid	Imidacloprid	Thiamethoxam
Prado in	10.0 ± 6.29	3.49 ± 5.30	1.30 ± 1.39	ND	15.5 ± 4.56	2.87 ± 4.27
BB1 in	9.43 ± 6.15	0.250 ± 0.140	1.01 ± 0.770	ND	13.8 ± 4.61	0.300 ± 0.190
BB1 out	8.93 ± 5.94	0.790 ± 0.300	0.760 ± 0.620	ND	12.8 ± 4.37	0.280 ± 0.0700
S7 in	10.9 ± 5.34	0.310 ± 0.290	1.13 ± 0.580	ND	12.5 ± 4.55	0.300 ± 0.0700
S8 out	9.25 ± 7.00	0.290 ± 0.240	0.930 ± 0.240	ND	7.08 ± 4.27	0.250 ± 0.0700
S9 in	8.84 ± 6.76	0.250 ± 0.350	0.940 ± 0.740	ND	11.0 ± 6.79	0.370 ± 0.260
S10 out	3.96 ± 3.41	0.250 ± 0.430	ND	ND	2.76 ± 3.07	ND
Prado out	9.11 ± 5.03	0.210 ± 0.130	0.770 ± 0.0500	ND	8.04 ± 3.70	0.200 ± 0

ND: Not detected (below detection limit)

Figures:

Figure 1. Schematic map of the Prado Wetlands in Corona, California. Red squares are sampling points for BB1, S7-S8, and S9-S10 wetland cells, and Prado inlet and Prado outlet of the whole wetland system (Figure credit: Orange County Water District).

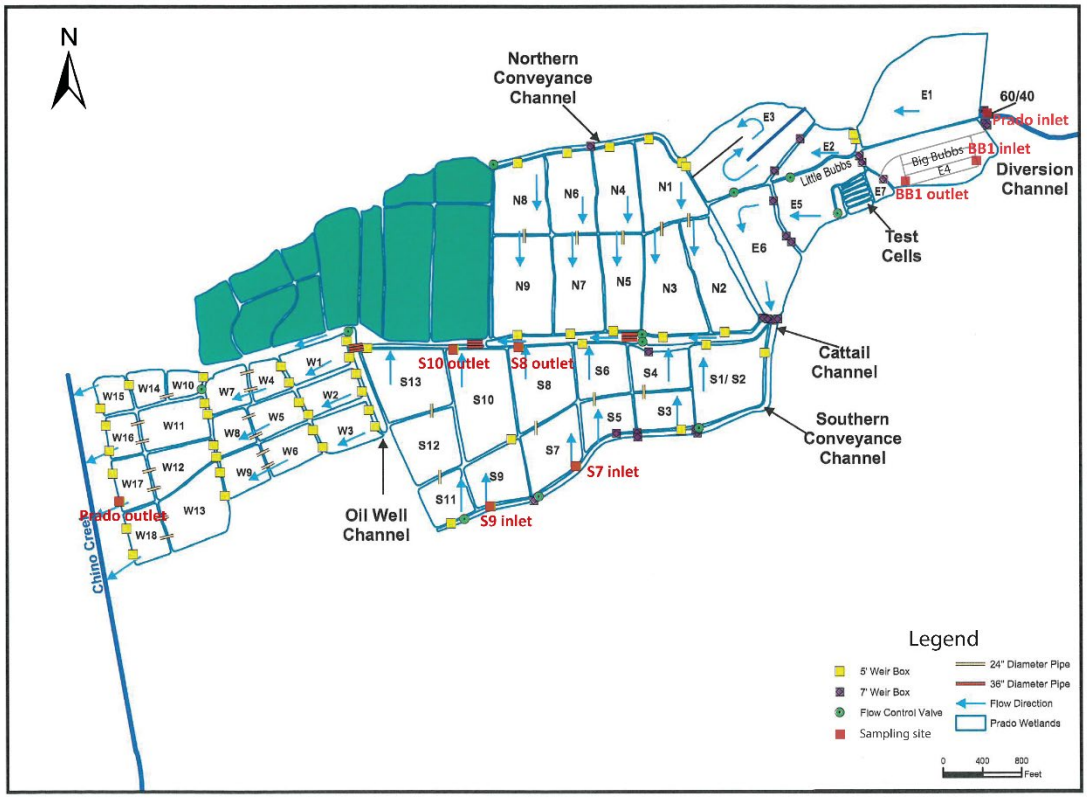

Figure 2. Total concentrations of six neonicotinoids in water samples **(A)**; Concentrations of imidacloprid in the sediment and plant tissue samples (**B**).

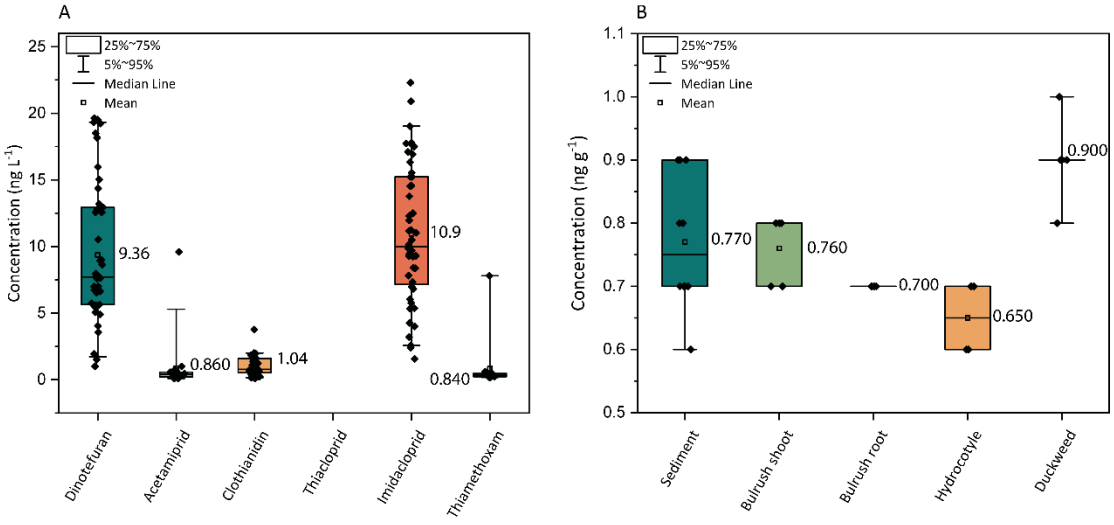

Figure 3. Temporal distribution and compositions of neonicotinoid insecticides in water samples from S7 inlet and Prado outlet sampling points in the Prado Wetlands.

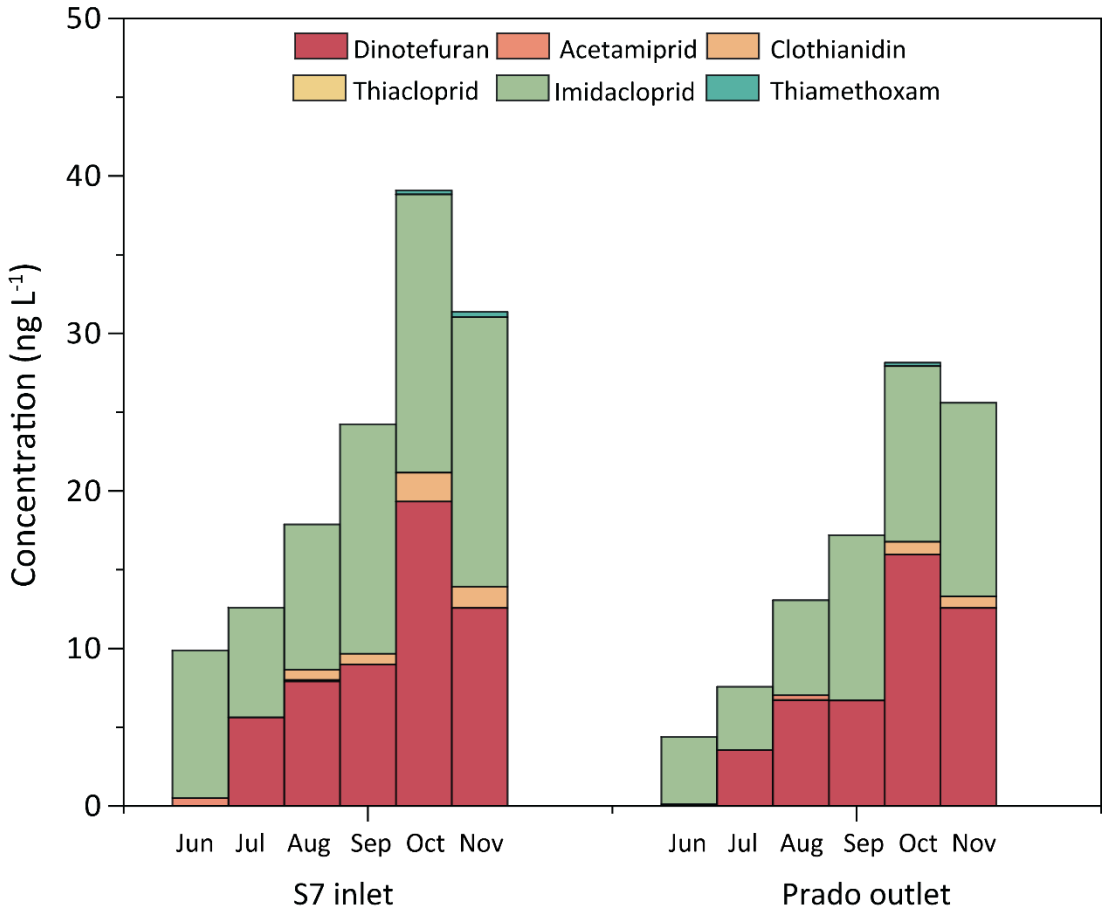

Figure 4. Removal efficiencies (A) and Δ mass flux (B) of six neonicotinoid insecticides in different cells at the Prado Wetlands. ***, P < 0.001; *, P < 0.05; NS, no significant difference.

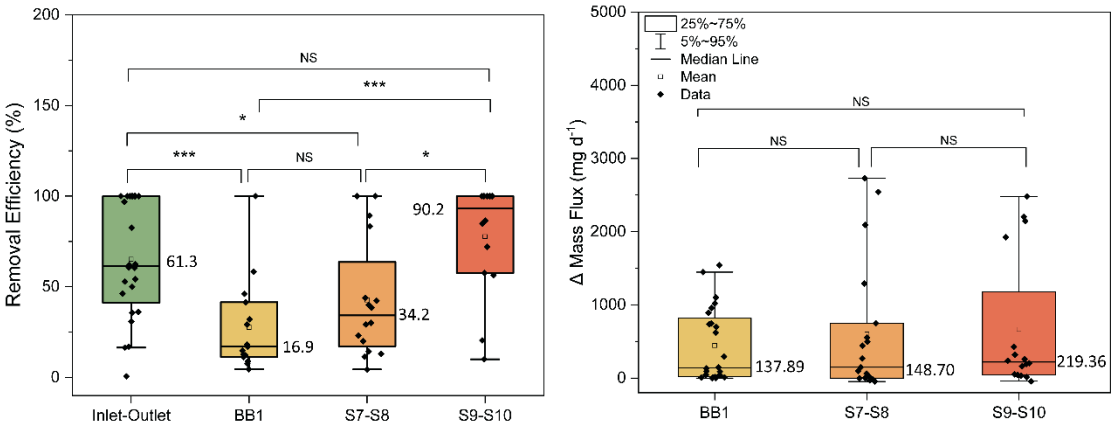

Figure 5. PCA biplots of 14 hydrogeochemical variables for the surface water of the Prado Wetlands. Arrows represent the PC1 and PC2 loading of each variable. The dots signify the PC1 and PC2 scores for each sampling site. The circles characterize the 95% confidence interval.

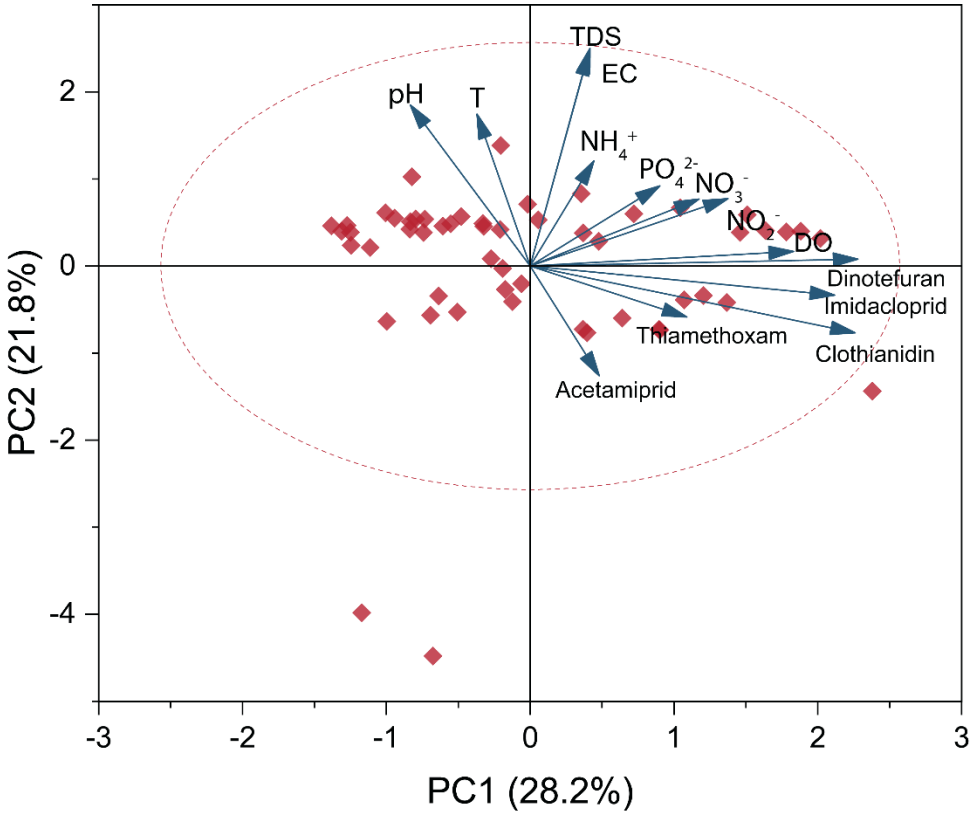

Figure 6. The ecological risk quotient of individual neonicotinoid (A); the ecological risk of imidacloprid in the water samples at Prado Wetlands (B).