Monitoring risk from contaminant mixtures in stormwater with water quality measurements, bioassays, and bioassessment

by

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The following served on the Examining Committee for this thesis. The decision of the Examining Committee is by majority vote.

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Author's Declaration

This thesis consists of material all of which I authored or co-authored: see Statement of Contributions included in the thesis. This is a true copy of the thesis, including any required final revisions, as accepted by my examiners.

I understand that my thesis may be made electronically available to the public.

Statement of Contributions

I, Gab Izma, am the sole author for Chapters 1 and 7, which were written under the supervision of Dr. Rebecca Rooney and are not intended for publication.

This thesis consists in part of five independent manuscripts written for publication. As the principal author of these chapters, I conceived study designs, conducted experiments and data collection, performed data analysis, and wrote the manuscripts. I use the pronoun "we" instead of "I" to reflect the collaborative aspect of this research, which included research partners who provided resources and guidance, lab technicians who assisted in field work, and my supervisor who oversaw all stages of my work.

Chapter 2 was published in February 2025 and was co-authored by Dr. Melanie Raby, Dr. Justin Renaud, Dr. Mark Sumarah, Dr. Paul Helm, Daniel McIsaac, Dr. Ryan Prosser, and Dr. Rebecca Rooney. **Citation**: Izma, G., Raby, M., Renaud, J. B., Sumarah, M., Helm, P., McIsaac, D., Prosser, R., & Rooney, R. (2025). Three complementary sampling approaches provide comprehensive characterization of pesticide contamination in urban stormwater. *Urban Science*, *9*(2). https://doi.org/10.3390/urbansci9020043

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Chapter 6 has been prepared for submission and is co-authored by Daniel McIsaac, Dr. Adam Yates, Dr. Melanie Raby, Dr. Ryan Prosser, and Dr. Rebecca Rooney.

Abstract

Urban stormwater management ponds (SWPs) are increasingly valued not only for their role in mitigating runoff but also for the biodiversity they support in densely developed environments. However, these systems receive complex contaminant mixtures from urban runoff, including pesticides, pharmaceuticals, industrial chemicals, and metals. These pollutants can accumulate in biologically active compartments like biofilms, posing risks that are not always captured by traditional water-based monitoring. My thesis investigates the nature, accumulation, and ecological effects of contaminants in SWPs using a combination of chemical, biological, and toxicological approaches.

The objectives of my research were to: (1) characterize pesticide contamination in SWPs using water, biofilm, and passive samplers; (2) quantify pesticide accumulation in biofilms and identify influencing factors; (3) assess the toxicity of contaminated biofilms through dietary exposure; (4) survey the broader suite of urban contaminants in SWPs to develop a stormwater contaminant signature; and (5) examine relationships between environmental conditions and aquatic community composition.

In **Chapter 2**, I surveyed 21 SWPs in Brampton, Ontario for pesticide contamination. I compared three monitoring approaches across the ponds - time-integrated water sampling, biofilm cultured on artificial substrates, and organic-diffusive gradients in thin films (o-DGT) passive samplers - finding that o-DGTs had the highest pesticide detection rates. However, issues with reproducibility in passive sampler data highlighted the challenges of using them for quantitative risk assessment. Despite generally low concentrations in water and biofilm samples, the widespread detection of diverse pesticide classes across all three matrices emphasized the chronic, mixture-based exposures in these ponds and informed recommendations for future monitoring strategies. In **Chapter 3**, I further investigated the use of biofilms as a sensitive and ecologically relevant matrix for contaminant monitoring. Examining a wider set of pesticide analytes, I found that over half of the pesticides detected in biofilm samples were not detected in water, suggesting that conventional sampling approaches may overlook important alternative exposure routes. Calculated bioconcentration factors (BCFs) varied widely and were not well explained by pesticide properties or water quality variables, pointing to the complexity of contaminant uptake mechanisms. To test the potential toxicity of these contaminated biofilm samples, in **Chapter 4** I conducted a series of dietary exposure assays with two invertebrate grazers. Mayfly nymphs (*Neocloeon triangulifer*) and juvenile freshwater snails (*Planorbella pilsbryi*) fed with contaminated biofilms from the SWPs showed reduced survival and growth compared to controls. Although the test results did not

always correlate with measured pesticide levels, these results support the ecological relevance of biofilm-mediated exposure and suggest the presence of additional stressors not captured in targeted chemical analyses. I further expanded the chemical scope in **Chapter 5** by analyzing over 700 unique urban contaminants across water, biofilm, and o-DGT samples. In total, 200 organic compounds were detected, including personal care products and traffic-related pollutants, as well as persistent elevated levels of fecal indicators and chloride. From these data, I developed the Urban Stormwater Contaminant Signature (USCS): a proposed list of common, environmentally relevant compounds to guide future monitoring and toxicity testing in urban aquatic systems. Finally, in **Chapter 6** I examine how environmental variables shape aquatic community composition. Diatom and macroinvertebrate assemblages sampled from the SWPs were dominated by pollution-tolerant taxa, with diatoms responding primarily to water quality (e.g., nutrients, chloride, herbicides) and macroinvertebrates more sensitive to habitat features associated with pond naturalization. Landscape-scale metrics (e.g., impervious cover) calculated from buffer zones had limited predictive power, suggesting that local conditions and upstream drainage characteristics play a stronger role in shaping biological communities.

This research highlights the need to expand contaminant monitoring in stormwater systems beyond conventional water sampling, incorporating matrices like biofilm and tools such as passive samplers to better reflect the complexities of exposures in urban environments. The detection of numerous unmonitored or rarely assessed compounds suggests that current regulatory frameworks may underestimate the complexity and risk of urban chemical mixtures. Recognizing stormwater ponds as both infrastructure and ecosystems calls for more ecologically grounded approaches to design, management, and risk assessment; ones that support biodiversity alongside water quality improvement and flood protection.

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Dedication

To the water that flows through the cities. One day you will be free once more.

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Chapter 1
Introduction

1.1 Overview of urban stormwater

"Stormwater is not a mechanical system. It is an environmental process, joining the atmosphere, the soil, vegetation, land use, and streams, and sustaining landscapes. In every landscape the falling of the rain, the shining of the sun, and the blowing of the wind are the beginning of all life."

- Bruce K. Ferguson

Urban stormwater represents a transformed expression of the hydrologic cycle: precipitation altered by the built environment and redirected through networks of impervious surfaces. In undeveloped landscapes, precipitation is intercepted by vegetation, infiltrates into soils, and gradually returns to the atmosphere through evapotranspiration, or flows slowly into surface and groundwater systems (Dingman, 2015). This process regulates the timing and volume of runoff, maintains water quality, and supports ecological integrity by preserving the connectivity and function of terrestrial and aquatic systems (Poff et al., 1997).

Contemporary urban development profoundly disrupts this balance. The expansion of impervious surfaces (e.g., pavement, rooftops, compacted soils) drastically reduces infiltration, increases surface runoff, and accelerates the delivery of water to drainage networks (Paul & Meyer, 2001; Barbosa et al., 2012). Storm drains replace root networks, and engineered conveyance systems short-circuit the slow, absorptive functions of the landscape (Roy et al., 2008). As a result, urban hydrology is defined by altered flow regimes: runoff volumes are higher, peak flows are sharper, and baseflows decline (Meyer et al., 2005; Walsh et al., 2005). These hydrologic shifts exacerbate erosion, destabilize stream channels, and impair aquatic habitat (Walsh et al., 2005; Booth et al., 2016). What was once a slow-release system has become a rapid-conveyance network that discharges stormwater, and the contaminants it carries, into receiving environments with little attenuation.

The water that flows off urban surfaces is far from clean. Stormwater runoff carries a complex and variable mixture of contaminants that reflects the land uses, activities, and materials present across the urban landscape (Burant et al., 2018; Masoner et al., 2019; Tran et al., 2019). Roads and parking lots contribute hydrocarbons (Smith et al., 2000; Brown & Peake, 2006), metals (e.g., zinc, copper, lead; Brown & Peake, 2006; Lundy et al., 2012; Hwang et al., 2016), salt (Marsalek, 2003), tire-wear particles

(Lindfors et al., 2025), and microplastics (Werbowski et al., 2021); lawns and parks shed nutrients (Yang & Lusk, 2018; Janke et al., 2017) and pesticides (Haith & Duffany, 2007; Meftaul et al., 2020); residential areas can generate pathogens from pet waste (Ram et al., 2007); commercial and industrial sites can release a wide spectrum of synthetic organics, including flame retardants and plasticizers (Burant et al., 2018; Zhang et al., 2024), and heavy metals (Van Metre & Mahler, 2003; De Buyck et al., 2021; Xiao et al., 2012; Brown & Peake, 2006); and construction sites contribute sediments and associated contaminants through soil erosion and runoff (Burkhardt et al., 2011). These pollutants are mobilized during precipitation events and are conveyed rapidly into storm drains, bypassing the natural filtration processes of soil and vegetation (Maniquiz-Redillas et al., 2022). The result is a mixture of suspended solids (Surbeck et al., 2006), nutrients (Yang & Lusk, 2018), pathogens (Ahmed et al., 2019), heavy metals (Brown & Peake, 2006), hydrocarbons (Masoner et al., 2020), and emerging contaminants (Tran et al., 2019), each with its own set of potential ecological consequences.

As stormwater travels, it becomes a mobile vector of urban contamination. Receiving waterbodies experience not only chemical degradation but also thermal pollution (Herb et al., 2008), altered sediment regimes (Russell et al., 2020), and flow pulses (Walsh et al., 2012) that disrupt aquatic life (Walsh et al., 2005). The ecological consequences are often severe: declines in biodiversity (Smucker & Detenbeck, 2014), promotion of eutrophication and algal blooms (Lewitus et al., 2008; Grogan et al., 2023), and the degradation of habitat complexity (Wang et al., 2001; Walsh et al., 2005). Stormwater pollution thus represents one of the most diffuse yet pervasive environmental stressors in urban watersheds; a by-product of the built environment that flows visibly and invisibly through our towns and cities.

1.2 Fate of contaminants in urban stormwater

Contaminants in urban stormwater originate from a diverse array of sources (Müller et al., 2020) and are transported through interconnected hydrological and biogeochemical pathways that vary across spatial (Mikkelsen et al., 1997) and temporal scales (Fairbairn et al., 2018). These dynamics shape both the environmental distribution of pollutants and their ecological risk.

Urban runoff is generated through a combination of surface and subsurface hydrological pathways. Precipitation that falls on impervious surfaces, such as asphalt, concrete, and rooftops, cannot infiltrate and instead becomes overland flow, rapidly collecting and concentrating contaminants as it moves toward storm drains and conveyance infrastructure (Leopold 1968). Some water infiltrates into pervious urban areas or vegetated features, where it may contribute to shallow subsurface flow or be

temporarily stored in engineered bioretention systems (USEPA, 2008). The movement of water is closely tied to contaminant mobilization, influencing not only the quantity but also the composition of pollutants that ultimately reach receiving waters (Peter et al., 2020; Christian et al., 2020).

Once mobilized, contaminants undergo a range of transformations and interactions that influence their fate. Many pollutants exhibit strong affinity for particulates and are transported primarily with the solid phase, becoming concentrated in stormwater sediments (Gromaire-Mertz et al., 1999; Zhao et al., 2016; Flanagan et al., 2021). Others remain dissolved in the water column, particularly salts, nutrients, and some organics, and are thus more readily bioavailable (Bressy et al., 2012; Kayhanian et al., 2012; Hopkins & Blaney, 2016). Chemical partitioning between solid and aqueous phases depends on environmental factors such as organic matter content (Yuan et al., 2019; Zhao et al., 2019) and flow rate (Maniquiz-Redilla & Kim, 2014), as well as the physicochemical properties of the contaminants (Saifur & Gardner, 2021; Yu et al., 2024). These interactions determine not only how far contaminants are transported, but also their potential to be taken up by organisms or accumulate in sediments.

Temporal variability plays a critical role in shaping stormwater contamination dynamics. Seasonal patterns, such as the use of de-icing salts in winter (Marsalek, 2003) or increased pesticide application in spring and summer (Chen et al., 2019), can lead to temporally distinct contaminant profiles (Fairbairn et al., 2018). Storm events are key drivers of episodic loading, with the "first flush" phenomenon often concentrating pollutants in early runoff (Perera et al., 2019). The intensity, frequency, and duration of rainfall events determine both the magnitude and timing of pollutant delivery (Lee et al., 2002; Li et al., 2007; Charters et al., 2016), as well as the capacity of stormwater infrastructure to contain or attenuate them (Kayhanian & Stenstrom, 2005).

The fate of contaminants in urban stormwater is thus governed by a confluence of sources, transport mechanisms, and environmental conditions (USEPA, 2008). Understanding these processes is critical for designing effective mitigation strategies, assessing ecological risk, and developing regulatory frameworks that reflect the true complexity of urban pollutant dynamics (Yu et al., 2024).

1.3 The evolution of stormwater management: From drainage to ecological function

Stormwater management has undergone a profound transformation over the past five millennia, evolving from early civil engineering feats designed to drain cities, to contemporary frameworks that integrate

ecological health, water quality, and urban sustainability (Burian & Edwards, 2002). This shift reflects both technological advancement and a growing recognition of the environmental consequences associated with unmanaged urban runoff.

Historically, the management of stormwater was rooted in the imperative to protect urban infrastructure and public health. Ruins of Indus cities, dating back to at least 3000 BCE, indicate urban drainage systems were integrated into the layout plan of the cities, with household wastewater guided along settling channels prior to entering stormwater drainage sewers in the streets (Webster, 1962; Burian & Edwards, 2002). Other ancient civilizations, including the Romans, Egyptians, and Mesopotamians, also constructed some of the earliest known drainage systems to prevent flooding and waterlogging in densely populated areas. The *Cloaca Maxima* of ancient Rome (Figure 1-1), built around 600 BCE, stands as one of the earliest examples of a combined sewer system, designed to convey both stormwater and wastewater away from the city and discharge it into the Tiber River (Gest, 1963; Hodge, 2002). In ancient Egypt and Mesopotamia, canals and surface drainage systems helped manage seasonal inundations and protect farmlands and settlements (Burian & Edwards, 2002; Echols & Pennypacker, 2015; Grant, 2016).

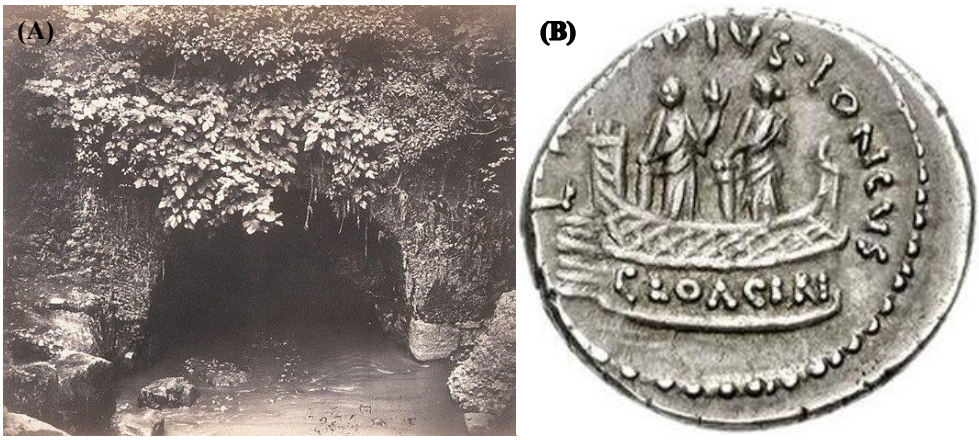


Figure 1-1. A) An opening of the *Cloaca Maxima* in Rome, where effluent flows into the Tiber River. Some parts of the system are still in use today. [Photo: Robert Macpherson, c. 1859. Licensed under Creative Commons Zero.]; B) A Roman coin depicting two statues of Cloacina, the Roman goddess who watched over the *Cloaca Maxima*. [Photo: Wikimedia Commons, licensed under Creative Commons].

During the medieval period in Europe, rudimentary gutter systems and open ditches were used to divert stormwater away from buildings and streets, often directly into nearby watercourses (Burian & Edwards, 2002). However, these systems frequently carried raw sewage alongside stormwater, contributing to deteriorating urban water quality and recurring outbreaks of waterborne disease (Grant, 2016). It was not until the 19th century, in response to the demands of rapid industrialization and widespread drainage maintenance issues, that stormwater infrastructure became more formalized, with designs incorporating engineering calculations of catchment sizes of various slopes (e.g., Roe's Table; McMath, 1887). The proliferation of impervious surfaces such as cobblestone streets and rooftops increased the frequency and severity of urban flooding. In response, cities across Europe and North America developed or reconstructed extensive underground conveyance systems - engineered storm sewers designed to rapidly remove surface water and reduce the risk of public exposure to sanitary waste (Novotny et al., 2010; Burian & Edwards, 2002). For example, the famed Paris sewer system (the backdrop of Hugo's *Les Misérables)* was reformed in the 1830s to replace open channels with gutters leading to the underground system (Reid, 1991). These early systems prioritized hydraulic efficiency and public sanitation, but they made no attempt to treat the stormwater or account for its ecological impact.

"The runoff and pollution from a contemporary city result not so much from the number of human beings, as from the lavish support given to their automobiles in land use and land development." - Bruce K. Ferguson

The advent of the automobile in the early 20th century dramatically reshaped the urban landscape, with profound implications for stormwater management. In 1904, nearly all roads in America were unpaved (Southworth & Ben-Joseph, 1995). As car ownership surged, cities expanded outward, giving rise to low-density suburban development characterized by wide roads, large parking lots, and cul-de-sac street networks (Ferguson, 1998). This auto-centric urban form increased impervious surface coverage dramatically, interrupting natural infiltration and exacerbating runoff volumes (Chocat, 1997; Booth & Jackson, 2007). The demand for high-speed mobility led to extensive highway construction and the paving-over of large swaths of land, further fragmenting hydrological connectivity (Ferguson, 1998). In many North American cities, auto-centered transportation infrastructure, including roads and parking lots, constituted a significant portion of urban land cover, often exceeding 60% in highly developed areas (Freund & Martin, 1993; Arnold & Gibbons, 1996). The automobile also influenced zoning policies and land use planning, encouraging the separation of residential, commercial, and industrial areas, each

serviced by its own network of impermeable surfaces (Duany et al., 2000) and the movement of industry from city centres to concentrated suburban industrial "parks" (Ferguson, 1998). This sprawling development pattern not only magnified stormwater runoff but also dispersed pollutant sources across the landscape, complicating both monitoring and management efforts (Arnold & Gibbons, 1996; Cappiella & Brown, 2001).

Well into the mid-20th century, the engineering paradigm of drainage efficiency using man-made technologies with conventional materials - often referred to as "grey infrastructure" - dominated stormwater management (Burns et al., 2012; Bell et al., 2019). The approach focused narrowly on drainage and flood prevention, with runoff treated as a nuisance to be quickly conveyed away from urban areas (Roesner & Matthews, 1990; Tourbier, 1994). However, as downstream ecosystems became increasingly degraded, largely due to rapid, post-World War II development, the limitations of this approach became evident (Marsalek, 2013; Bertrand-Krajewski, 2021). Urban streams receiving untreated stormwater exhibited altered hydrologic regimes, increased pollutant loads, and substantial declines in aquatic biodiversity (Porcella & Sorensen, 1980). The cumulative effects of impervious cover, flashy flows, and contaminant pulses led to widespread issues such as erosion, eutrophication, sedimentation, and the loss of sensitive taxa in receiving waterbodies (Leopold, 1968; Chocat, 1997; Marsalek et al., 2008).

This growing awareness, coupled with the emergence of environmental protection legislation in the 1970s - such as the Clean Water Act in the United States (Federal Water Pollution Control Act, 1972) and comparable water quality frameworks in Canada (e.g., the Canada Water Act in 1970) and Europe (e.g., the European Economic Community Directives on Water Quality in 1975, followed by the EU Water Framework Directive in 2000) - catalyzed a paradigm shift in stormwater management (Holman-Dodds, 2007; Bertrand-Krajewski, 2021). No longer viewed solely through the lens of drainage and flood control, stormwater began to be recognized as a significant vector for diffuse pollution and ecosystem degradation (Holman-Dodds, 2007; Echols & Pennypacker, 2015). The development of Best Management Practices (BMPs) in the late 20th century marked the first significant attempt to address water quality in stormwater planning (Schueler, 1992; Holman-Dodds, 2007). These practices included retention basins, vegetated swales, and sedimentation ponds designed to remove suspended solids, reduce peak flows, and protect downstream water quality (McFarland et al., 2019).

In recent decades, stormwater management has further evolved to embrace principles of ecological restoration and sustainability. Contemporary approaches, often framed under banners such as Low Impact Development (LID), Green Infrastructure (GI), or Sustainable Drainage Systems (SuDS), seek to restore aspects of the natural hydrologic cycle within the built environment (Burns et al., 2012; Fletcher et al., 2015). These systems aim to reduce runoff at its source, encouraging infiltration, evapotranspiration, and on-site retention (Burns et al., 2012; Bell et al., 2018). Bioretention cells, permeable pavements, rain gardens, green roofs, and stormwater wetlands now represent the leading edge of stormwater design, providing not only hydrologic control and pollutant removal, but also co-benefits such as urban heat mitigation, wildlife habitat, and aesthetic enhancement (Burns et al., 2012; Ashley et al., 2013; Dhakal & Chevalier, 2017; Bell et al., 2018).

Stormwater management ponds (Figure 1-2), initially developed primarily for flood control, have evolved alongside these broader shifts in urban environmental governance—from reactive, engineered solutions to adaptive, multifunctional landscapes (Chocat et al., 2001; Bertrand-Krajewski, 2021). As urbanization intensified and the hydrologic cycle became increasingly disrupted by impervious surfaces and altered drainage patterns, these engineered basins became ubiquitous components of suburban infrastructure, particularly in North America (Walsh et al., 2005; Roy et al., 2008). Most commonly constructed as wet ponds that permanently retain water, they now aim to serve a range of functions, including water retention, sedimentation (Clar et al., 2001), and nutrient and contaminant sequestration (Stanley, 1996). Their design reflects a strategic shift away from conveyance-based systems toward integrated infrastructure that incorporates features such as forebays, vegetated littoral zones, and other enhancements that improve pollutant removal and ecological value (MOE, 2003; USEPA, 2005; Hogan & Walbridge, 2007). In the face of climate change, aging infrastructure, and growing urban density, stormwater ponds are seen as critical to urban resilience (Marsalek & Schreier, 2009; van Duin et al., 2021; Barreiro et al., 2024).



Figure 1-2. A stormwater pond in Brampton, Ontario. [Photo by G. Izma, 2022].

Today, stormwater ponds are recognized not only for regulating water quantity and improving water quality, but also for offering aesthetic and recreational benefits (Baxter et al., 1985; Heller, 2020) and supporting urban biodiversity (Hassall, 2014; Oertli & Parris, 2019) by providing semi-natural habitat in otherwise urbanized landscapes (Ferzoco & McCauley, 2024; McKercher et al., 2024). However, these multifunctional systems are also recognized as pollutant sinks (Gallagher et al., 2011; McKercher et al., 2024), where metals (Karlsson et al., 2010), hydrocarbons (Flanagan et al., 2021), pesticides (Izma et al., 2024), and other emerging contaminants (Crane, 2019) can accumulate. This raises concerns about long-term maintenance, water quality variability, and the ecological suitability of these systems as aquatic habitat (Marsalek et al., 2005; Rooney et al., 2015; Monaghan et al., 2016; McKercher et al., 2024; Ferzoco & McCauley, 2024), highlighting the importance of evaluating their dual role as both biodiversity-supporting ecosystems and contaminant reservoirs. Given their ubiquity and ecological significance, understanding these trade-offs is key to informing and improving watershed management and urban biodiversity conservation.

1.4 Stormwater management ponds: Infrastructure and urban habitat

Stormwater management ponds are primarily flood-mitigation infrastructure. Design standards for stormwater ponds vary regionally, but common principles apply. Ponds are typically sized to accommodate the maximum incoming water volume (i.e., runoff from a peak rainfall event), with a permanent pool that facilitates sedimentation and pollutant removal (Shamaa & Zhu, 2001; OME, 2003; Clar et al., 2004). Many designs incorporate forebays to intercept coarse sediments and reduce resuspension (OME, 2003), as well as vegetated littoral zones that enhance nutrient uptake (Collins et al., 2010) and provide habitat heterogeneity (Sinclair et al., 2021). In Ontario, design guidance is provided by the Ministry of the Environment, Conservation and Parks (MECP), which encourages features that support both hydraulic performance and ecological function. The province's Stormwater Management Planning and Design Manual (2003) specifies that ponds be designed to remove 80% of total suspended solids from the water quality event, among other performance benchmarks (OME, 2003).

Beyond hydraulic and water quality functions, stormwater ponds have emerged as *de facto* urban aquatic habitats (Ferzoco & McCauley, 2024). Studies have shown that they can support communities of aquatic invertebrates (Hill et al., 2016), amphibians (Holtmann et al., 2017), waterfowl (Bishop et al., 2000), and fish (McIsaac, 2022), depending on their design, maintenance, and connectivity to other waterbodies (McKercher et al., 2023). However, these communities are shaped by the highly altered and often unstable conditions imposed by urban runoff (Perron & Pick, 2020) – such as thermal fluctuations, nutrient loading, periodic drying, and exposure to a cocktail of contaminants. Research has shown that stormwater ponds can harbour both functional and taxonomic biodiversity, but this is often constrained by frequent disturbance, simplified habitat structure, and chronic pollutant exposure (Marques & Mandrak, 2024; Ferzoco & McCauley, 2024).

The ecological role of stormwater ponds is thus complex and context-dependent. In some settings, they offer refuge and breeding habitat for aquatic organisms and support ecosystem services such as nutrient cycling and carbon sequestration (Zuniga-Palacios et al., 2021; McKercher et al., 2023). In others, they may act as ecological traps, attracting wildlife to suboptimal or toxic environments (Clevenot et al., 2018), or contribute to downstream pollution if not properly maintained (Taguchi et al., 2020a). The accumulation of contaminants in pond sediments, for instance, can pose long-term risks to benthic

organisms (Tixier et al., 2023) and higher trophic levels (Grung et al., 2016), particularly where dredging or resuspension events remobilize buried pollutants (Taguchi et al., 2020b).

Contaminant accumulation is one of the most pressing concerns associated with stormwater ponds. These systems often act as sinks for a suite of urban pollutants, including heavy metals (e.g., copper, lead, zinc; Karlsson et al., 2010), polycyclic aromatic hydrocarbons (PAHs; Flanagan et al., 2021), nutrients (e.g., nitrogen, phosphorus; Lusk et al., 2025), chloride from road salts (Marsalek, 2003), and emerging contaminants such as microplastics (Olesen et al., 2019) and pharmaceuticals (Liu et al., 2019). Over time, these pollutants can accumulate in sediments and biofilms, potentially entering the food web through benthic or periphytic exposure pathways. In some cases, stormwater ponds act as a source of contaminants for receiving water bodies (Casey et al., 2013; Yazdi et al., 2021). The long-term fate of these contaminants, particularly in the absence of regular dredging, raises questions about pond aging, maintenance costs, and risks to downstream waterbodies (Al-Rubaei et al., 2016; Ivanovsky et al., 2018; Lusk et al., 2025).

Despite these challenges, stormwater ponds are a critical part of the urban water landscape. In Ontario, their use is widespread in residential and commercial developments, governed by both provincial and municipal policies. The MECP requires stormwater management to be considered at multiple scales (i.e., site, subdivision, and watershed) and mandates that ponds meet specific design and maintenance criteria (OME, 2003). Municipalities such as Brampton and Mississauga, located within the jurisdiction of the Toronto and Region Conservation Authority (TRCA), have developed inventories and performance monitoring programs for existing ponds (TRCA, 2012; City of Brampton, n.d.; City of Mississauga, n.d.). These include assessments of hydrologic function, vegetation health, sediment accumulation, and water quality indicators, with the aim of ensuring long-term performance and compliance with TRCA's stormwater management criteria.

However, despite their widespread use and policy support, stormwater ponds are not a panacea. A growing body of research suggests that their efficacy may be overestimated (Booth et al., 2002; Bradford & Gharabaghi, 2004; Williams et al., 2013), particularly with regard to the removal of dissolved contaminants (e.g., Camponelli et al., 2010; Lusk & Toor, 2016) or the long-term sequestration of pollutants in sediment (Lusk et al., 2025). Maintenance lapses (Blecken et al., 2017), poor design (e.g., undersized basins, short residence times; Hancock et al., 2010), and high pollutant loads from upstream

land uses can reduce effectiveness (Crane, 2019). Maintenance remains a persistent issue: sediment forebays can fill more quickly than anticipated (Erickson et al., 2018), invasive vegetation can outcompete native plantings (Sinclair et al., 2020), and public concerns about aesthetics (Rooney et al., 2015) and mosquito breeding (Gingrich et al., 2006) may influence design and management priorities. Moreover, climate change introduces additional uncertainty, as increased storm intensity and frequency may exceed the design thresholds of existing infrastructure (Yu et al., 2022). The use of ponds as ecological surrogates for natural wetlands or streams must be approached with caution; while they can offer limited habitat value, they are fundamentally engineered environments with constrained biodiversity potential (Rooney et al., 2015).

Still, stormwater ponds remain a valuable lens through which to study urban aquatic ecosystems. Their dual role as infrastructure and habitat positions them uniquely within the urban landscape as sites where human and ecological processes intersect, often in unpredictable ways. Integrating contaminant exposure profiles from across the urban landscapes, stormwater ponds provide an underappreciated opportunity to monitor urban contaminants. Their physical accessibility and structural similarity also make them ideal "living laboratories" for examining contaminant dynamics, bioaccumulation, microbial processes, and ecological responses to urban stressors. As climate change intensifies storm events and urban expansion continues to replace pervious surfaces, the function and design of stormwater ponds will only grow in importance. Studying these systems can yield critical insights into the fate of urban contaminants, the resilience of aquatic communities, and the effectiveness of green-gray infrastructure in real-world conditions.

1.5 Knowledge gaps and research objectives

Despite decades of investment in stormwater infrastructure and water quality research, substantial knowledge gaps remain in the understanding of contaminant fate, ecological impacts, and the effectiveness of current monitoring and management strategies. Conventional stormwater management has primarily emphasized peak flow reduction and contaminant settling, yet growing attention is being paid to chemical complexity, contaminant mobility, and biological effects (Chocat et al., 2001; Gallagher et al., 2011; Tixier et al., 2011; Marques & Mandrak, 2024). Urban-use pesticides, for instance, are frequently detected in stormwater runoff (Masoner et al., 2019; Meftaul et al., 2020), but are not consistently monitored in stormwater ponds, and their partitioning between water, sediment, and biological compartments remains poorly understood (Chen et al., 2019). This gap is compounded for

hydrophilic and emerging contaminants, such as pharmaceuticals and personal care products, which are often present in the dissolved phase, making them difficult to capture with sedimentation-based BMPs (LeFevre et al., 2015; Masoner et al., 2019; Spahr et al., 2020). As Müller et al. (2020) note, systematic and long-term studies of these contaminants are rare, and environmental risk assessments remain incomplete due to limited concentration data, restrictive detection limits, and inconsistent sampling strategies.

There is a critical need to advance monitoring approaches that more effectively characterize contaminant dynamics in stormwater systems. Most existing data come from grab sampling or automated samplers (McCarthy et al., 2018; Spahr et al., 2020), which may fail to capture the episodic and pulse-driven nature of contaminant loads during storm events (Xing et al., 2013; Fairbairn et al., 2018; La Cecilia et al., 2021). Passive samplers, which offer time-integrated contaminant profiles, are increasingly proposed as complementary tools for stormwater monitoring (Page et al., 2014; Pinasseau et al., 2019), particularly for tracking trace organic compounds with low particle affinity (Spahr et al., 2020). However, further research is required to validate their performance under variable flow conditions, develop standardized protocols, and assess their ability to capture ecologically relevant exposure pathways (Birch et al., 2013; Mutzner et al., 2019; Spahr et al., 2020). In parallel, there remains a lack of consensus on which contaminants should be prioritized for monitoring. Criteria such as toxicity, persistence, and ubiquity are rarely harmonized across studies, limiting the comparability of results and the formulation of evidence-based regulatory frameworks (Zgheib et al., 2010; Masoner et al., 2010; Ma et al., 2019; Müller et al., 2020; Saifur & Gardner, 2021; Pamuru et al., 2022; Mutzner et al., 2023).

Equally underexplored are the biological consequences of contaminant accumulation in benthic compartments, particularly stormwater pond biofilms. Biofilms, as complex microbial assemblages at the base of aquatic food webs, are known to accumulate both heavy metals and organic pollutants (Ancion et al., 2014; Rooney et al., 2020; Mahler et al., 2020; Ijzerman et al., 2023), yet their role in mediating contaminant transfer to higher trophic levels remains poorly understood. While controlled microcosm experiments have demonstrated contaminant transfer from biofilms to invertebrate consumers (e.g., Kim et al., 2012; Golding et al., 2013), few studies have examined this process *in situ* or under realistic environmental conditions (Ruhí et al., 2016). Dietary exposure remains a largely neglected pathway in ecotoxicological assessments, despite growing recognition of its relevance for benthic grazers (Bonnineau

et al., 2020; Ijzerman et al., 2023). The kinetics of contaminant sorption, transformation, and trophic transfer within biofilms must be further elucidated to accurately assess ecological risks (Roessink et al., 2010; Chaumet et al., 2019; Fernandes et al., 2020), especially in systems like stormwater ponds that often function as contaminant sinks (Ancion et al., 2014).

In addition to being affected by chemical exposure, stormwater ponds play a complex and context-dependent role in supporting or undermining freshwater biodiversity. Although these engineered ecosystems provide aquatic habitat in urban landscapes, their ecological function varies widely depending on design, contaminant load, hydrology, and landscape connectivity (Ferzoco & McCauley, 2024; Oertli & Parris, 2019; McKercher et al., 2024). Key drivers of biological community composition in stormwater ponds, including vegetation structure, pond isolation, pollutant mixtures, and local land use, are still not well understood, especially for microbial assemblages such as diatoms or invertebrates (Gillett et al., 2017; Sun et al., 2019; Minelgaite et al., 2020; Meland et al., 2020; Sinclair et al., 2021; McKercher et al., 2024). As such, more research is needed to characterize the conditions under which these systems can contribute to urban biodiversity goals, rather than serving as ecological traps (Oertli & Parris, 2019; McKercher et al., 2024). Furthermore, studies that show the relationships among water chemistry, contaminant loads, and biological community structure across multiple scales are essential to support more integrated and ecologically informed stormwater management (Ancion et al., 2010; Pamuru et al., 2022; Lusk et al., 2025)

In response to these knowledge gaps, my thesis sets out five research objectives: (1) to evaluate pesticide monitoring tools, including water grab samples, passive samplers, and biofilm matrices, for their sensitivity and ecological relevance; (2) to quantify pesticide bioconcentration in stormwater pond biofilms and investigate the physicochemical mechanisms driving contaminant uptake; (3) to assess dietary exposure risks associated with biofilm consumption by freshwater macroinvertebrates; (4) to characterize the breadth and co-occurrence patterns of contaminants in stormwater ponds and assess their potential as indicator profiles for monitoring; and (5) to explore how local pond characteristics and surrounding landscape features influence diatom and macroinvertebrate community composition. Together, these objectives aim to advance our understanding of contaminant exposure pathways, improve ecological risk assessments, and support evidence-based management of stormwater ponds as multifunctional urban water infrastructure.

1.6 Overview of study context

My field research primarily took place at 21 SWPs in Brampton, Ontario. Brampton is a large city (population = 656,480; Statistics Canada, 2021) located in the northwestern part of the Greater Toronto Area in southwestern Ontario. The city is situated on the traditional territory of the Anishinaabe, Haudenosaunee, and Huron-Wendat peoples, where Indigenous communities, including most recently the Mississaugas of the Credit, have lived for thousands of years (Moreau, 2012). This area has undergone tremendous alterations to the natural hydrological cycles due to rapid urbanization and high rates of development since European settlement. Contemporaneously, the City of Brampton is among the fastest growing municipalities among Canada's large cities. With a 10% increase in population size between 2016 and 2021, the City of Brampton is predicted to reach a population of 800,000 by 2026 (Statistics Canada, 2022; City of Brampton, 2022; Canada Population, 2022). This expansion has resulted in large swaths of suburban and industrial land use; land now dominated by impervious surfaces covering 7,888 ha, or 45.5% of the City's area (TRCA, n.d.). Even at imperviousness levels as low as 10%, the ecological health and biodiversity of a watershed can be reduced due to the resulting degradation in water quality (CWP, 2003). In the 1980s, the City of Brampton began to build stormwater infrastructure to mitigate the consequences of this expanding development and its effect on water quality and flooding (TRCA, n.d.) and to date has over 190 SWPs in operation (City of Brampton, n.d.).

The 21 SWPs I surveyed for my research had previously been the focus of my lab mate and field partner Danny McIsaac's Master's thesis, in which he characterized the anuran and fish communities living in the ponds (McIsaac, 2022). McIsaac selected these ponds to cover a gradient in urbanization, which he achieved by selecting sites across two independent gradients. First, the sites sat in landscapes that ranged from a high proportion of impervious cover and low tree canopy cover to a low proportion of impervious cover and correspondingly higher tree canopy cover. Second, and independently, the landscapes surrounding the sites ranged from a low proportion of open water to a higher proportion of open water. McIsaac's research provides a valuable foundation and context for my research questions and his experience studying the same SWPs enabled productive and intensive field surveying to collect as much information about these sites as possible. To build on McIsaac's work to further understand the extent of contamination, water quality, and ecological state of these SWPs, I used a multidisciplinary approach that employed the use of chemical and biological tools in both the field and in the laboratory.

The research I present in this thesis centres around one core field season in 2022, where I collected water grab samples, harvested biofilms cultured on artificial substrates, and deployed passive samplers in the 21 SWPs. These samples were analyzed for pesticides and other urban-use contaminants. During this field season, I also conducted extensive water quality surveys and sampled the aquatic macroinvertebrate communities in the ponds. Throughout my thesis, I explore the data I collected from this field season to answer the five core objectives of my research, listed above. Using one set of samples collected from one field season enabled a more in-depth study of the conditions of my study sites, and allowed for integration across data chapters, where each chapter built on the proceeding one. This integration strengthened the inferences I could make about contaminant profiles of the SWPs and their ecological conditions, however this approach came at the expense of obtaining a greater temporal record. Due to the high costs of analyses, I was unable to repeat my sampling regimes in subsequent years. This limitation is further discussion in Chapter 7. The research contained within this thesis is the result of my effort to make the best use of the data I collected, despite this imperfection.

Throughout much of this thesis, my characterization of stormwater contamination within the SWPs focuses largely on pesticides. Pesticides are bioactive by design and have known negative direct and indirect effects on both target and non-target organisms (Wan et al., 2025; Cloyd, 2012; Ware, 1980), biological communities (Tooker & Pearsons, 2021, Carson, 1962), and ecosystem processes (deNoyelles et al., 2020). Monitoring for pesticides is largely focused on agriculturally affected surface waters; for example, the Ontario Ministry of Environment, Conservation, and Parks (OMECP) and the Ontario Ministry of Agriculture, Food, and Rural Affairs (OMAFRA) run a collaborative, province-wide pesticide monitoring program, with nearly all sampling sites located in agricultural areas (Raby et al., 2022). However, pesticide use is not restricted to rural areas, and in some cases application rates in urban centres even outpaces use on agricultural lands (Schuler, 2000). Pesticides have a multitude of applications in cities, from herbicides in turfgrass maintenance, to insecticides in urban forestry and domestic pest control, to fungicides in construction and building materials (Meftaul et al., 2020). With higher coverage of impervious surfaces in developed areas compared to agricultural lands, urban stormwater can accumulate pesticides from across the landscape at higher rates (Wittmer et al., 2011). Yet, monitoring for pesticides in SWP is seldom done; for example, the last known assessment of pesticides in water samples from SWPs was in 1994 (Struger et al., 1994). Although other urban contaminants, such as polycyclic aromatic hydrocarbons (PAHs) and polychlorinated buphenyls (PCBs), are also widespread and of ecological concern (e.g. Burant et al., 2018; Zhang et al., 2020), they are more well studied in SWPs (e.g.,

Stephansen et al., 2020; Flanagan et al., 2021). Thus, my research objectives focussed on characterizing pesticide contamination in SWPs to address this dearth in available information about exposure profiles faced by wildlife inhabiting these ponds.

1.7 References

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Chapter 2
Three complementary sampling approaches provide comprehensive characterization of pesticide contamination in urban stormwater

2.1 Overview

Urban areas are expanding rapidly and experience diverse and complex contamination of their surface waters. Addressing these issues requires different tools to describe exposures and predict toxicological risk to exposed biota. We surveyed 21 stormwater management ponds in Brampton, Ontario using three types of sampling methods deployed concurrently: time-integrated water sampling, biofilms cultured on artificial substrates, and organic-diffusive gradients in thin films (o-DGT) passive samplers. Our objective was to compare pesticide occurrences and concentrations to inform monitoring in stormwater ponds, which reflect pesticide pollution in urban areas. We detected 82 pesticides across the three sampling matrices, with most detections occurring in o-DGT samplers. The *in situ* accumulation of pesticides in o-DGTs during deployment and the high analytical sensitivity achieved establishes o-DGTs as excellent tools for capturing the mixtures of pesticides present. Water and biofilm sampling demonstrated that pesticide concentrations available for uptake are relatively low, with most below toxicological thresholds. Yet our results demonstrate that urban areas are subject to a wide range of pesticides, including herbicides, insecticides, and fungicides, and underscores the urgency of research to quantify the risks of chronic exposure to this chemical mixture.

2.2 Introduction

Globally, urbanization is accelerating rapidly, with urban land cover expanding at a rate of 1.16 km^2 per hour since 1990 (Behnisch et al., 2022). Urban pollution is intensified by the widespread coverage of land with impervious surfaces resulting from this rapid development and land clearing (Walsh et al., 2005). In areas with low rates of infiltration, contaminants are concentrated in runoff as water moves along surfaces and into constructed stormwater management systems (e.g., including stormwater management ponds (SWPs)) (Goonetilleke et al., 2005; Masoner et al., 2019), or directly into surface waters. This phenomenon drives the high diversity of substances found in stormwater, from organic contaminants like pesticides (Chen et al., 2019; Izma et al., 2024a [Chapter 3]), pharmaceuticals (Masoner et al., 2019), and tire compounds (Chalis et al., 2021), to metals (Hwang et al., 2016), oils (Stenstrom et al., 1984), and salt (Marsalek, 2003), which are subsequently released into receiving water bodies like creeks and streams.

Such discharges are known contributors to downstream habitat degradation (Walsh et al., 2005) and the impairment of benthic communities (Carpenter et al., 2016; Goldyn et al., 2018).

Urban-use pesticides are prevalent in stormwaters (Wolfand et al., 2019; Nowell et al., 2021; Izma et al., 2024a [Chapter 3]), including herbicides (Raina et al., 2011), insecticides (Weston et al., 2009; Weston et al., 2015), and fungicides (Bollmann et al., 2014), several of which are known toxicants to aquatic organisms (e.g., Schiff et al., 2002; Jiang et al., 2016). The large assortment of potential pesticide contaminants presents a challenge for characterizing contamination, because even within this singular contaminant type, there are a variety of physico-chemical properties represented, which affect the environmental fate of each compound. For example, some herbicides, such as 2,4-D and mecoprop, are hydrophilic, while fungicides, such as azoxystrobin, are relatively hydrophobic. The propensity to dissolve in water, among other physico-chemical properties, can determine which environmental compartments a pesticide will partition into once released (Seiber, 2002). Relying solely on sampling a single environmental compartment overlooks the other ways in which biota may be exposed (Ijzerman et al., 2024). To sufficiently predict the potential ecotoxicological risk of pesticide contamination in urban aquatic environments, it is crucial to accurately monitor the complete exposure profile. Below, we describe our study of the breadth of pesticides occurring in stormwater ponds in one of Canada's fastest growing municipalities and compare three methods of sampling, (1) time-integrated water sampling, (2) biofilms cultured on artificial substrates, and (3) passive sampling with organic-diffusive gradients in thin films (o-DGT) samplers, yielding recommendations for future pesticide monitoring and research.

2.2.1 Water, biofilm, and passive sampling for monitoring urban pesticide contamination

The Canada-Ontario Agreement (COA) on Great Lakes Water Quality and Ecosystem Health represents the commitment of the federal and provincial governments working together to meet their obligations under the Canada-United States Great Lakes Water Quality Agreement, which was first signed in 1972 by both nations to restore, protect, and conserve the health of the Great Lakes (MECP, 2021). Although much is understood about the contribution of agricultural lands to pesticide loading in the Great Lakes, research on the contribution of urban lands is relatively underrepresented. In 2021, a Wastewater and Stormwater Annex was added to COA under the priority area Protecting Waters, which reflects growing concern around the impacts urban developments can have on water quality in the Great Lakes and their watersheds (MECP, 2021).

Water grab sampling is the mainstay of monitoring pesticide contamination in surface waters. For example, the monitoring of agricultural pesticides in Ontario's streams, led by the Ontario Ministry of Agriculture, Food, and Rural Affairs (OMAFRA) and the Ontario Ministry of Environment, Conservation, and Parks (MECP), consists of monthly grab sampling during ice-free months (Raby et al., 2022). Instantaneous water sampling is also used by the U.S. Geological Survey for measuring organic contaminants in lentic waters in their National Water Quality Program (USGS, 2006). In 2024, Health Canada's Pest Management Regulatory Agency (PMRA) completed a nation-wide pesticide monitoring pilot program consisting of biweekly grab samples from over 100 sites across the country, as a model for a forthcoming Canadian Water Monitoring Program for Pesticides (Health Canada, 2024). Exposure thresholds to avoid adverse effects on freshwater biota are available for many common pesticides (e.g., CCME's Canadian Environmental Quality Guidelines; Health Canada Pest Management Regulatory Agency's Aquatic Life Reference Values; the U.S. EPA Office of Pesticide Program's Aquatic Life Benchmarks) and can be used for comparison with measured concentrations in water samples. However, water grab sampling represents a "snapshot" of contaminant concentrations and biotic exposure, which may fluctuate in urban stormwaters due to the flashy hydrology characteristic of urban runoff (Walsh et al., 2005). The lack of temporal integration when taking a single grab sample of water means that contaminant pulses can be missed (Xing et al., 2013); thus, the predicted exposure profile and toxic risks can be inaccurate over a longer period than when the sample was taken (la Cecilia et al., 2021).

Over the past two decades, the use of biofilm sampling has been developed as a means of monitoring pesticide concentrations that better represent average conditions through time than single grab samples of water (Sabater et al., 2007; Huerta et al., 2016; Fernandes et al., 2020; Rheinheimer dos Santos et al., 2020; Rooney et al., 2020; Ijzerman et al., 2024; Izma et al., 2024a [Chapter 3]). Biofilms are microbial assemblages held together by a matrix of extracellular polymeric substances (EPSs). The surfaces of biofilms are heterogenous, and this complexity offers a diverse set of potential sites for contaminant interaction (Flemming et al., 2007; Bonnineau et al., 2021). Biofilms are known to bioaccumulate both hydrophilic (e.g., MCPA; Rooney et al., 2020) and hydrophobic (e.g., azoxystrobin; Izma et al., 2024a [Chapter 3]) pesticides, although recent evidence suggests compounds with higher octanol–water partition coefficients are more likely to accumulate in biofilms (Ijzerman et al., 2024). Biofilm sampling was demonstrated to be more sensitive than traditional water grab sampling for detecting pesticides in agricultural streams (Rheinheimer dos Santos et al., 2023; Fernandes et al., 2023); however, this is not always the case (e.g., Ijzerman et al., 2024). As key components in aquatic food

webs, biofilms are consumed by other biota, representing an alternative route of exposure to contamination (Guasch et al., 2016). Thus, in addition to being valuable biological indicators for environmental contamination, biofilms are also important tools for assessing toxicological risks (e.g., Izma et al., 2024b [Chapter 4]), although published ecological thresholds for dietary exposure to pesticides do not currently exist, and research into pesticide exposure via dietary pathways is not nearly as well studied as exposure via immersion or contact pathways.

Passive sampling devices for monitoring low level environmental contamination of organic compounds have been used since the 1990s as another alternative technology to traditional water sampling (Huckins et al., 1990). The diffusive gradients in thin films for an organic contaminant (o-DGT) passive sampler have been used since 2012 (Chen et al., 2013). Differential chemical potential drives the sequestration of contaminants from the ambient water onto a binding gel via a diffusive hydrogel layer contained within the sampler. The concentration of analyte in the gel and the duration of deployment can be used to model time-weighted average (TWA) concentrations in the surrounding water (Challis et al., 2016). The o-DGT sampler differs from other similar passive sampling devices because of the inclusion of a semi-permeable membrane and diffusive gel to minimize the influence of environmental conditions on sampling performance; this adaptation of the o-DGT sampler has increased the accuracy of this method for detecting polar organic contaminants compared to other passive sampling devices (Challis et al., 2018). Not only can passive samplers integrate contamination through time, catching acute but periodic contaminant fluxes, but the accumulation of contaminants within the binding gel enhances the sensitivity to trace contaminants compared to traditional water grab sampling (Vrana et al., 2005). However, the use of TWA concentration data from o-DGTs poses challenges in assessing risk to exposed biota, as relatively higher pulses of contaminant concentrations that may exceed toxicity thresholds are averaged across the period of sampler deployment. Passive sampling for pesticides with o-DGTs was studied under laboratory conditions (e.g., Guibal et al., 2017; Li et al., 2019) and used successfully for monitoring pesticides in Canada (Challis et al., 2018) and New Zealand (Hageman et al., 2019).

2.2.2 Objectives and hypotheses

To tackle the complex challenge of assessing urban pesticide contamination in the Great Lakes Basin, we require many different tools and approaches. Yet, understanding how these tools and approaches compare to each other in effectiveness and biological relevance is critical. The overall objective of our study was to compare three sampling methods for extensively assessing pesticide occurrences and concentrations in

stormwater ponds, and to make recommendations for future pesticide monitoring programs in the Great Lakes watershed. These sampling methods were the following: a composite of weekly water grab samples, biofilm cultured on artificial substrates, and o-DGT samplers (summarized in Table 2-1). Considering the high cost of pesticide lab analysis, we recognize a trade-off in pesticide monitoring between a comprehensive assessment of multiple matrices, each of which has advantages and disadvantages (Table 2-1), and the economy of minimizing the number of sample types at the risk of failing to detect important pesticides present in the system but not well represented by the chosen sampling matrix. We hypothesized that (1) the three sampling methods would yield an integrated understanding of pesticide concentrations in the stormwater management ponds and consequently predicted that time-weighted average concentrations of pesticides estimated from o-DGTs would be well correlated with concentrations measured in weekly water sample composites. We also hypothesized that (2) compared to water and biofilm samples, the number and frequency of pesticide detections would be higher in o-DGT samplers due to the increased analytical sensitivity, but that including the water samples and biofilm samples would increase the range of pesticides types (with different physico-chemical properties) detected and provide important insight into potential exposure routes that could help contextualize exposure risk.

Table 2-1. Summary of three sampling methods for assessing pesticide contamination in surface waters.

	Grab water sampling	**Biofilm sampling**	**Passive sampling with o-DGTs**
Description of method	Instantaneous sampling of a water body by filling a sampling bottle with water directly from the study site	Harvesting biofilm grown in-situ at a study site from either artificial or natural substrates	Deployment of passive sampling device (e.g., o-DGT) in-situ at a study site
Benefits of this method	Concentrations in water are comparable with other studies and with ecotoxicological benchmarks or thresholds	Accumulation of pesticides in biofilms from the surrounding water can help detect pesticides at relatively low levels; time integration allows capture of fluctuations in concentrations; biologically relevant as many aquatic organisms consume biofilm directly	Accumulation of pesticides from the water can help detect pesticides at relatively low levels; time integration allows capture of fluctuations in concentrations; well-supported in the literature

Limitations of this method	Snapshot of concentrations in time may not represent all conditions or exposures; bias towards hydrophilic compounds	No dietary ecotoxicological thresholds or benchmarks exist for comparison	Artificial media is not as biologically relevant; assumptions in the calculation of TWA concentrations complicate informed risk assessments

2.3 Methods

2.3.1 Study context

We sampled surface water and biofilms in a heavily urbanized area within the Lake Ontario watershed to assess pesticide contamination in stormwater management ponds (see also Chapter 3; Izma et al., 2024a). Due to the high cost of pesticide analysis (the cost of analysis was approximately CAD 500 per sample per matrix submitted to the Agriculture and Food Lab in Guelph, Ontario), we were constrained to these two matrices. However, co-authors with the federal agency Agriculture and Agri-Food Canada (AAFC) enabled us to deploy o-DGT passive samplers, which AAFC's lab analyzed in kind. Differences in the analytical methodologies used by these two laboratories constrained our ability to compare the performance of the three sampling methods yet provided an excellent opportunity to compare the value of monitoring the three different matrices with different advantages and disadvantages (Table 2-1). Throughout this research, we consider the merits of working collaboratively and with different sampling and analytical methods, and we leverage these data sources to make recommendations for pesticide monitoring in urban areas.

2.3.2 Study area

Our sampling took place at 21 stormwater management ponds in the City of Brampton, located in Southwestern Ontario, Canada (Figure 2-1; Please see Appendix A for GPS coordinates). As part of the Greater Toronto Area, Brampton is one of the most rapidly expanding municipalities in Canada (Statistics Canada, 2022) and its highly urbanized watershed represents an ideal location for monitoring urban pesticides in stormwater ponds. The selected ponds were wet ponds, designed to hold water year-round, and were a minimum of 10 years old with no previous dredging within the past 10 years. The surface area of the ponds ranged from 1051 to 3686 m^2 (mean = 2272 ± 851 m^2). The sites represent a gradient of urbanization, with impervious cover within a buffer of 300 m surrounding each pond ranging from 10.9 to 55.2% (McIsaac, 2022).

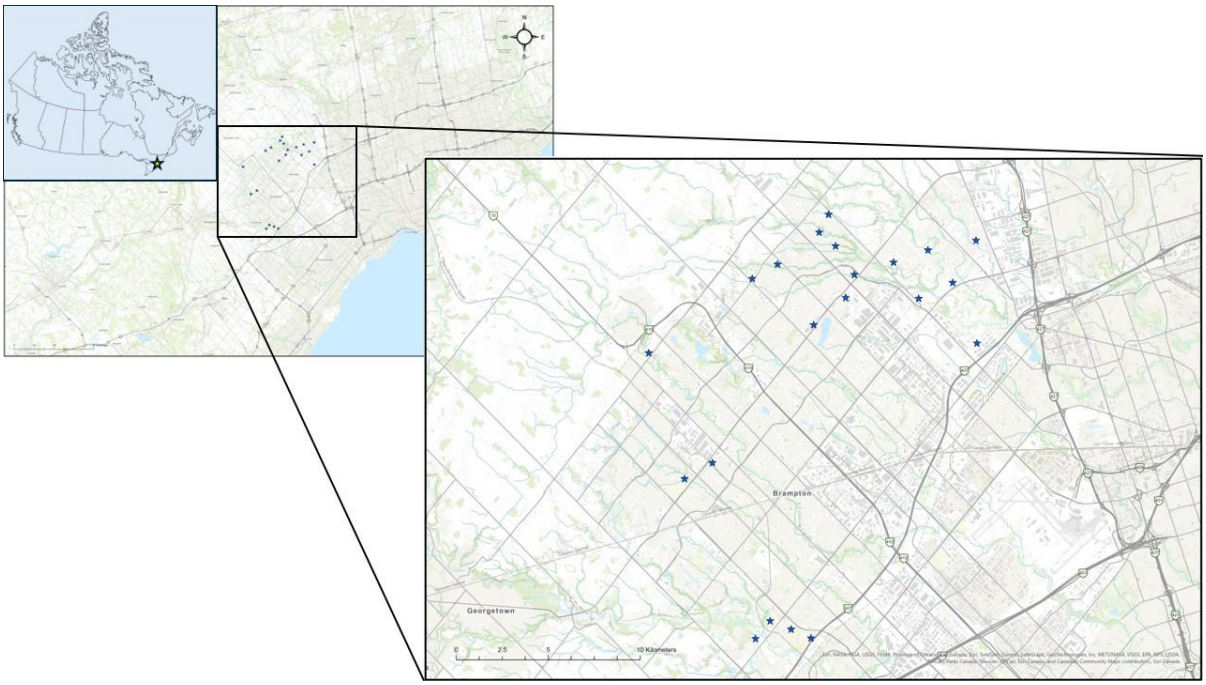


Figure 2-1. Map of stormwater pond sites surveyed in Brampton, Ontario, Canada. Blue stars represent stormwater pond locations.

2.3.3 Sampling

We used three environmental media types (water, biofilms, and o-DGTs) concurrently to assess pesticide contamination, described in the following sections. The survey period took place over the course of nine weeks, from 25 May to 25 July 2022.

2.3.3.1 Water

We followed the methods described in a previously published paper (Izma et al., 2024a); please refer to Chapter 3 for a more detailed description of these methods. Briefly, we created a 1.35 L time-integrative composite sample composed of nine 150 mL weekly water grab samples taken at 20 cm depth using an HDPE polyethylene bottle, which was kept frozen at −20 °C until submission for pesticide analysis. Constrained by the high costs of pesticide analysis, we used a temporal composite rather than keeping the instantaneous grab samples of water separate and analyzing each independently. The effect of the holding

period (i.e., time between sampling and extraction) on pesticide degradation in water samples is minimal when stored frozen, compared to storage at above-zero temperatures (Albaseer et al., 2011).

2.3.3.2 Biofilm

The sampling of biofilm also followed the methods previously described (Izma et al., 2024a); please refer to Chapter 3 for a more detailed description of these methods. To culture biofilms in situ, we deployed floating biofilm sampler racks, holding ten acrylic plates (20.2 cm by 44.4 cm), in each pond, secured with anchors (Figure 2-2). After submersion for 54 days, we harvested the biofilm by scraping both sides of each plate into a composite container for each site, which was subsequently stored frozen at −20 °C. We freeze-dried 300–500 g (wet weight) of each composite biofilm sampling using a FreeZone I Labconco benchtop freeze dryer (Labconco Corporation, Kansas City, MO, USA) prior to pesticide analysis. Insufficient biofilm mass from two of the SWP sites meant these were only analyzed for phenoxy herbicides and polar pesticides, as these screens contained the highest number of analytes. All other stormwater pond sites (n = 19) had adequate biofilm mass to analyze for all the intended pesticide screens.



Figure 2-2. Biofilm samplers deployed at stormwater pond sites for in situ culturing. Shown from above (top), at water surface (bottom left), and underwater (bottom right). [Photos by G. Izma, 2022].

2.3.3.3 o-DGT samplers

At each pond, we deployed two o-DGT (DGT Research®; LSND-AT with PTFE membranes; exposed area = 3.14 cm^2) duplicate samplers mounted on custom-built holders (Figure 2-3), which were attached to the floating biofilm sampler rack. Each set was replaced after 27 days of deployment, for a total of 54 days between the two sets, to match the duration of biofilm and water sampling. To detect any potential contamination from handling, we used a field travel blank for each day of deployment and collection (n = 7). Following collection, o-DGTs were removed from their holder, wrapped in clean aluminum foil, and stored frozen at −20 °C.



Figure 2-3. o-DGT samplers fixed to custom-built holder. [Photo by G. Izma, 2022].

Prior to analysis, we disassembled each o-DGT casing and transferred the gel membranes into 25 mL Falcon tubes. We then performed sequential methanol extractions into 15 mL polypropylene centrifuge tubes (Corning reference 430052; see Renaud et al., 2022 for detailed method). The supernatant extracts were evaporated to dryness using a Buchi Multivapor™ P-6. The dried residue was reconstituted with 400 µL of 80:20 methanol/water, and transferred to an amber glass high-performance liquid chromatography (HPLC) vial with glass insert for analysis.

2.3.4 Pesticide Analysis

Water and biofilm samples were submitted to the Agriculture and Food Lab (AFL) of the Laboratory Service Division at the University of Guelph, Ontario (ISO/IEC 17025 accredited). Water and biofilm samples were analyzed for 542 current-use and legacy pesticides using high-performance liquid chromatography paired with electrospray ionization and tandem mass spectrometry (HPLC-ESI-MS/MS) and gas chromatography paired with tandem mass spectrometry (GC–MS/MS). Details of this analysis are described in Appendix A, and a list of analytes and associated method detection limits (MDLs) and method quantification limits (MQLs) can be found on the FigShare repository, linked in the Appendix A.

Extracts from the o-DGT samplers were analyzed by the Agriculture and Agri-Food Canada (AAFC) chemical laboratory in London, ON, Canada. Samples were analyzed for 491 chemical contaminants by HPLC-ESI-MS/MS using a Vanquish-Duo HPLC coupled to a Thermo Altis triple quadrupole mass spectrometer. Details of this analysis are described in Appendix A. Target analytes, along with their limits of detection (LODs), limits of quantification (LOQs), multiple reason monitoring (MRM) transitions, and retention times are available in the FigShare repository, linked in Appendix A.

2.3.5 Calculation of detection frequencies on common set of analytes

To appropriately compare pesticide occurrences among the three matrices analyzed by two different chemical laboratories, we created a common set of analytes composed only of compounds targeted by both laboratories. Among the 542 compounds screened for by AFL and 491 screened for by AAFC, 238 were screened for by both labs.

We calculated pesticide detection frequencies (%) as the number of detections divided by the number of stormwater pond sites (i.e., 21). For o-DGT samplers, we define a detection as the occurrence of a compound above the detection limit in at least one sampler out of the two duplicates deployed in each pond for each deployment period. As the detection frequency represents the entire sampling period (two deployment periods), if a compound is detected in one deployment period, but not the other, we still define the occurrence as one detection. If a compound is detected in more than one sampler in a pond or across both deployment periods, the overall occurrence of that compound is still defined as one detection.

2.3.6 Calculation of Time-Weighted Average concentrations in o-DGT samplers

2.3.6.1 Modeling of diffusion coefficients

We used the Hayduk–Laudie diffusion model (Equation (1)) to estimate the diffusion coefficients of pesticides (Hayduk & Laudie, 1974).

(1)

$$D_w = \frac{1.326 \times 10^{-4}}{\eta^{1.14}\ V^{0.589}}$$

where D_w is the diffusion coefficient ($cm^2\ s^{-1}$); η is the viscosity of water (centipoise) at the average temperature of pond water (24.28 °C); and V is the molar volume ($cm^3\ mol^{-1}$) calculated from the structure of each compound.

2.3.6.2 Sampling rates

Sampling rates for each pesticide analyte in the o-DGT samplers were calculated using Equation (2).

$$R_s = \frac{D_w\ A}{\Delta g} \quad \textbf{(2)}$$

where R_s is the sampling rate ($cm^3\ s^{-1}$); D_w is the diffusion coefficient ($cm^2\ s^{-1}$) of the analyte; A is the exposed surface area of the o-DGT (3.14 cm^2); and Δg is the thickness of the o-DGT diffusive gel (0.094 cm).

2.3.6.3 Time-Weighted Average concentrations

Any quantity of analyte detected in a field blank was first subtracted from its paired sample prior to calculating concentrations. Equation (3) was used to determine the time-weighted average (TWA) water concentrations (C_w).

$$C_w = \frac{M}{R_s\ t} \quad \textbf{(3)}$$

where C_w is the estimated concentration in water (ng L^{-1}); M is the mass of analyte on the o-DGT sampler (ng); Rs is the sampling rate (L d^{-1}); and t is the deployment time (27 d).

2.3.6.4 Method Detection Limits

We derived method detection limits (MDLs) from the limits of detection (LODs) for pesticide analytes in o-DGT samplers using Equation (3), with M representing the LOD (pg per sampler). We note that these are not true MDLs, as they are not calculated based on repeated measurements, thus we refer to these values as *derived-MDLs* (d-MDLs). We used these d-MDL values (in µg L^{-1}) for comparison with the MDL values for the water samples (Table A-1.1).

2.3.7 Statistical analysis

Statistical analyses were completed with R Statistical Software (version 2.2.2) in RStudio (version 2023.06.01; Posit Team RStudio, 2023). To compare the concentrations of pesticides detected in the water samples and o-DGTs, we used the lm function in base R to perform linear regressions between the concentrations in water and the TWA concentrations calculated from o-DGT samplers. We were not able to investigate relationships between concentrations in biofilms and o-DGTs due to a lack of data > MQL

in the biofilm samples. We only investigated pesticides which were present above the MQL at least twice in the water samples; thus, we investigated 5 of the 11 compounds detected in both water samples and o-DGTs: flupyradifurone, imidacloprid, mecoprop, 2,4-D, and MCPA. We log transformed the water concentrations for flupyradifurone and imidacloprid because they appeared to have a saturation effect in the o-DGTs. To obtain a singular data point representative of the four o-DGTs deployed in each pond, we averaged the concentrations measured across both deployments in each pond to represent average conditions over the same period as the biofilm sampler deployment and the composite water sampling for each pond. We only included data points where the compound was detected in at least one of the two matrices being assessed; sites with no detections in both matrices were excluded. If a compound was detected at a site in one matrix but not in the other, we replaced the non-detect with ½ the MDL value to rule out type II errors. For detections below the MQL, we used the MDL value of that compound to avoid overestimating the concentration. We evaluated the model fits of these regressions by assessing the adjusted r^2 values and the statistical significance ($p < 0.05$). We created plots (Figure 2-4) where each concentration is represented as a point, with the linear regression represented by a solid (significant) or dashed (not significant) line (R packages *ggplot2*, *patchwork*).

2.4 Results and discussion

2.4.1 Pesticide detections

We detected 82 pesticides across the 3 sampling matrices (Table 2-2): 14 pesticides were detected in water samples, 9 in biofilm samples, and 78 in o-DGT samplers (see also Table A-1.2). The total number of pesticides detected at each site ranged from 22 to 52 pesticides, with an average of 36 pesticides detected per site. There were 17 pesticides detected at all sites and 31 pesticides were detected in at least half of the sites (Table 2-3).

Table 2-2. Summary of the number of pesticides out of the 82 we detected in total that were detected by each sampling matrix: either in water, biofilm, o-DGT samplers, or in all three sample types combined. The values on the diagonal represent the number of pesticides detected exclusively by that sampling matrix, whereas the values in the triangular matrix represent the number of pesticides detected in common by the pair of sampler types indicated by the column and row headings.

	Water	Biofilm	o-DGT	All 3 matrices

Water	3			5
Biofilm	0	1		
o-DGT	6	3	64	

Many of the most commonly detected pesticides in our stormwater ponds were also widely detected elsewhere in urban surface water studies. Seventeen pesticides were detected in all of our stormwater pond sites: 2,4-D, atrazine, azoxystrobin, carbendazim, chlorantraniliprole, clomazone, fluopyram, MCPA, mecoprop, metalaxyl, metolachlor, propazine, propiconazole, simazine, tebuconazole, tebufenozide, and thiabendazole. Of these 17 pesticides, all but six were previously detected in urban stormwaters by other studies in Canada or the United States (Raina et al., 2011; Metcalfe et al., 2016; Burant et al., 2018; Fairbairn et al., 2018; Nowell et al., 2021). These six exceptions were chlorantraniliprole, clomazone, fluopyram, metalaxyl, tebufenozide, and thiabendazole. Chlorantriliprole and fluopyram are both relatively new pesticides, and clomazone, metalaxyl, tebufenozide, and thiabendazole are more commonly associated with agricultural runoff (Struger et al., 2016; Santos et al., 2017; Barizon et al., 2022; Raby et al., 2022; Wang et al., 2023). Ubiquitous and other commonly detected pesticides will be discussed in greater detail in a forthcoming publication. Identifying commonly occurring pesticides in stormwaters is important to gain a better understanding of common sources, landscape drivers, and the potential toxicity of pesticide contamination in urban areas (Nowell et al., 2021).

Table 2-3. Detection frequencies (%) of pesticides in water, biofilm, and o-DGT samples. n.d. = not detected or concentration was below MDL. Detection frequency is the number of detections divided by number of sites (n = 21) multiplied by 100%. Pesticides are listed in order from highest to lowest detection frequency. Note that detection limits vary by matrix and the analytical laboratory where analysis was completed.

Pesticide	Detection Frequency (%)		
	Water	**Biofilm**	**o-DGT**
2,4-D	100	14	90
atrazine	n.d.	n.d.	100
azoxystrobin	n.d.	43	100
carbendazim	5	10	100
chlorantraniliprole	n.d.	n.d.	100
clomazone	n.d.	n.d.	100

Pesticide	Detection Frequency (%)		
	Water	Biofilm	o-DGT
fluopyram	n.d.	n.d.	100
MCPA	100	5	43
mecoprop	100	n.d.	95
metalaxyl	n.d.	n.d.	100
metolachlor	n.d.	n.d.	100
propazine	n.d.	n.d.	100
propiconazole	n.d.	n.d.	100
simazine	n.d.	n.d.	100
tebuconazole	n.d.	19	100
tebufenozide	n.d.	n.d.	100
thiabendazole	n.d.	5	100
diuron	5	19	95
imazethapyr	n.d.	n.d.	95
prometon	n.d.	n.d.	95
triclopyr	95	n.d.	n.d.
clothianidin	33	n.d.	90
flupyradifurone	38	n.d.	90
paclobutrazol	n.d.	n.d.	90
imidacloprid	62	n.d.	86
dimethenamid	n.d.	n.d.	81
ametryn	n.d.	n.d.	71
tebuthiuron	n.d.	n.d.	71
difenoconazole	n.d.	n.d.	67
carbaryl	n.d.	n.d.	52
epoxiconazole	n.d.	n.d.	52
hexazinone	n.d.	n.d.	48
myclobutanil	n.d.	n.d.	48
cyantraniliprole	n.d.	n.d.	38
sulfentrazone	n.d.	n.d.	38
acetamiprid	n.d.	n.d.	33
diazinon	n.d.	n.d.	33
ethiofencarb	n.d.	n.d.	33
benalaxyl	n.d.	n.d.	29
metribuzin	n.d.	n.d.	29
pyrimethanil	n.d.	n.d.	29

Pesticide	Detection Frequency (%)		
	Water	Biofilm	o-DGT
bentazon	24	n.d.	14
bromoxynil	n.d.	n.d.	24
indaziflam	n.d.	n.d.	24
dichlorprop	19	n.d.	5
dithiopyr	n.d.	n.d.	19
flonicamid	19	n.d.	n.d.
flufenoxuron	n.d.	n.d.	19
flutriafol	n.d.	n.d.	19
prometryn	n.d.	n.d.	19
pyridate	n.d.	n.d.	19
spiroxamine	n.d.	n.d.	19
dimethomorph	n.d.	n.d.	14
fenpropimorph	n.d.	n.d.	14
pyridaben	n.d.	n.d.	14
terbuthylazine	n.d.	n.d.	14
terbutryn	n.d.	n.d.	14
trifloxystrobin	n.d.	n.d.	14
picolinafen	n.d.	n.d.	10
pyraclostrobin	n.d.	n.d.	10
pyriproxyfen	n.d.	n.d.	10
spiromesifen	n.d.	n.d.	10
alanycarb	n.d.	n.d.	5
benfuracarb	n.d.	n.d.	5
benzoximate	n.d.	n.d.	5
bifenazate	n.d.	n.d.	5
bromacil	n.d.	n.d.	5
bromuconazole	n.d.	n.d.	5
bupirimate	n.d.	n.d.	5
butafenacil	n.d.	n.d.	5
chlorpropham	5	5	5
etoxazole	n.d.	n.d.	5
fenazaquin	n.d.	n.d.	5
fenobucarb	n.d.	n.d.	5
fluazifop-p-butyl	n.d.	n.d.	5
fludioxonil	n.d.	n.d.	5

Pesticide	Detection Frequency (%)		
	Water	Biofilm	o-DGT
hexaconazole	n.d.	n.d.	5
imazapyr	5	n.d.	n.d.
indoxacarb	n.d.	n.d.	5
methiocarb	n.d.	n.d.	5
ofurace	n.d.	n.d.	5
picoxystrobin	n.d.	n.d.	5
propoxur	n.d.	n.d.	5
quizalofop-ethyl	n.d.	n.d.	5
simetryn	n.d.	n.d.	5
tetraconazole	n.d.	n.d.	5
thiacloprid	n.d.	n.d.	5
thiophanate-methyl	n.d.	5	n.d.

2.4.1.1 The influence of analytical sensitivity on pesticide detections

Overall, the o-DGT samplers detected substantially more pesticides and had more overlapping detections with the other matrices than the water or biofilm samples. Notably, pesticides that were widespread in o-DGTs were not frequently detected in the other matrices. Of the fourteen pesticides with a detection frequency of 100% in o-DGTs, only four were also detected in one or both other matrices. Moreover, 64 pesticides were detected only in o-DGT samplers (Table 2-2), and would have been missed without the use of this passive sampling device. Yet we must question whether these results are due to the sensitivity of o-DGT devices themselves, or due to differences in analytical sensitivities.

Pesticide analyses took place at two different analytical laboratories utilizing different analysis techniques and equipment. Prior to considering the drivers of differences in detections among the three matrices, we first inquired whether the analytical differences may have contributed to the results. To do so, we compared the MDLs of pesticides in water with the d-MDLs of pesticides in o-DGT samplers. Unfortunately, we were not able to include biofilm MDLs in this investigation on account of the discrepancy in units, as these are measured on a per mass basis ($\mu g\ kg^{-1}$) whereas the MDLs for water samples and d-MDLs for o-DGTs are measured on a per volume basis ($\mu g\ L^{-1}$). The MDLs for analysis in water samples were 1 to 6 orders of magnitude higher than the d-MDLs for analysis in o-DGTs (Table A-1.1). For example, atrazine, metolachlor, propazine, and tebuconazole all had detection frequencies of

100% in o-DGTs and 0% in the water samples (Table 2-3). The MDLs for these four pesticides were five orders of magnitude higher in water samples than the d-MDLs of o-DGTs (Table A-1.1). It is therefore plausible that the differences in detection limits between the water and o-DGT samples are highly influential in the resulting differences in pesticide detections.

To explore the potential magnitude of impact that the analytical sensitivity had on pesticide detection, we standardized by removing detections in the o-DGT samplers that were below the MDL used for the analysis of water samples. As shown in Table A-1.3, only 12 of the 78 pesticides detected in o-DGTs would have been detected if the o-DGT analytical detection limits were as high as those used for water analysis. When differences in analytical sensitivities are factored out, we can see that more compounds were detected in water samples (n = 14) than in o-DGTs (n = 12) and at higher detection frequencies. This begs the question, if the detection limits for analyzing water samples were as low as those in the o-DGTs (down to the ng L^{-1} or pg L^{-1} level), what pesticides would have been detected, and at what frequency? Although o-DGTs have an inherent sensitivity because they accumulate contaminants over time, therefore sampling a greater volume of water than the grab water sampling, the analytical conditions are evidently influential on pesticide detections. Without the relatively low detection limits used for the analysis of o-DGTs, our sampling would have missed the majority of pesticides present in the stormwater ponds. We can resolve that monitoring programs aiming to characterize the presence of pesticides could benefit substantially from allocating money and resources into lowering the detection limits of their analytical methods as we infer that the majority of pesticides in urban aquatic systems are present at trace levels. However, programs concerned with assessing hazards arising from elevated contaminant concentrations would not need to quantify concentrations at such low levels and would alternatively benefit from allocating resources toward increasing the temporal resolution of water grab sampling to more accurately describe contaminant exposure.

In addition to these important analytical differences, other factors may have contributed, albeit less significantly, to the differing detections in water and o-DGT samples. Pesticides may be undetected in water samples because of their physico-chemical properties (e.g., hydrophobicity) or because at the time of grab sampling, they were not present or occurred at trace levels at which the method (i.e., the volume of water and the approach of analysis) was not sensitive enough to detect them. The highly dynamic nature of water and contaminant influxes from the stormwater system means that instantaneous water grab sampling may miss sporadic pulses, which may have short yet potentially serious effects on exposed biota (Norman et al., 2020; la Cecilia et al., 2021). The apparent absence in water samples of

compounds detected in o-DGTs could imply that pulses in pesticide occurrence took place out of sync with our weekly sampling regime. It is important to note that our water sampling approach is atypical: traditional sampling involves the submission of one grab sample, whereas our submissions were composed of a time-integrative composite sample of nine weekly grab samples. In either case, passive sampling may be preferred for detecting pesticides whose occurrences are too episodic to detect with instantaneous sampling, such as those entering stormwater ponds (discussed further in Section 2.4.3).

Biofilm sampling is also a form of passive sampling, yet there are a few key differences with o-DGTs that may explain their differing detections, in addition to an assumed difference in instrumental detection limits between the analyses of the two matrices, which is likely the key driver of these results. Even with standardized biofilm samplers, biofilms are as variable as any living being: their growth and composition are influenced by the conditions of their surroundings (Ponsati et al., 2016), which are also variable across sites (Izma et al., 2024a [Chapter 3]). Biofilms are subject to grazing, and new growth throughout the growing season may result in a dilution effect. Biofilms may also support the microbial degradation of pesticides (Tien et al., 2013). Although some biofouling can occur on the o-DGTs, it was noted that such fouling does not have significant effects on measuring organic contaminants in water (Wang et al., 2020; Wang et al., 2022). Although both biofilm samplers and o-DGT samplers were deployed at the same time, the commencement of the accumulation of contaminants within biofilms is delayed because biofilm colonies must first establish, whereas o-DGTs have a defined mass of binding material that can start collecting contaminants upon immersion. Conversely, the large surface area of the biofilm samplers (837 cm^2 per side per slide) can increase the opportunities for interactions with compounds, compared to the relatively small surface area of o-DGTs (3.14 cm^2 each), although our results suggest this factor does not necessarily increase sensitivity. Finally, binding and transport processes of chemicals differ between the two matrices, with binding and transport within the EPSs of biofilms likely more variable than within the standardized, well-characterized binding materials of the o-DGTs.

2.4.1.2 Sampling pesticides with three different environmental matrices

Optimizing contaminant monitoring techniques requires the assessment and comparison of currently used methods. Yet, research comparing the performances of biofilms, passive sampling devices, and water grab samples is uncommon, and we do not know of any existing literature comparing DGT-type passive samplers to biofilm sampling. Rheinheimer dos Santos et al. (2023) compared biofilm and water sampling

with polar organic compound sampling devices (POCIS; a passive sampling device that does not contain a semi-permeable membrane or diffusive gel layer; Alvarez et al., 2004) in agriculturally affected rivers in Brazil, finding that the combined use of biofilms and POCIS allowed for a more holistic characterization of pesticide contamination compared to water grab sampling. Biofilms detected several contaminants entirely absent in POCIS samplers and were better able to describe contamination on a smaller scale. The authors concluded that because pesticides encompass a range of differing molecular properties (e.g., some are highly soluble in water while others are insoluble), integrating different types of matrices helps to address the variety of compound–matrix interactions (e.g., adsorption, accumulation, mineralization) that are specific to each compound (Rheinheimer dos Santos et al., 2023).

Our results differ from the aforementioned study as the contribution of detections in biofilm samples to the overall contamination characterization was not as pronounced. This could be explained by the substantial differences in detection limits among the sampling approaches. The o-DGTs in our study, aided by relatively low MDLs, far outperformed the other matrices in terms of sensitivity, and all but four of the compounds found in water and biofilm samples were also detected by the o-DGTs. We discuss these four exceptions below.

Flonicamid, imazapyr, and triclopyr were only detected in water samples. These pesticides have a relatively high solubility in water and a low log octanol–water partition coefficients (Log K_{ow}) and effective log octanol–water distribution coefficients (Log D_{ow}), the latter of which accounts for the ionizing behavior of a pesticide in water (Table A-1.4; see Li et al., 2011). Compounds with low Log D_{ow} values are less likely to be sorbed by the o-DGT samplers (Li et al., 2011). However, a study by Morin et al. (2013) found that the uptake behavior of organic micropollutants into passive samplers can be variable, often specific to the compound, and not consistently predicted by physico-chemical properties. Flonicamid is a selective systemic pyridine insecticide used to control greenhouse pests, such as aphids (University of Hertfordshire, 2020), and would have been missed at 19% of pond sites without time-composited weekly water sampling. Imazapyr is a nonselective imidazolinone herbicide used to control broad-leaf weeds, perennial grasses, and woody plants in turfgrass, urban forestry, ponds and wetlands, and industrial sites. It is the only pesticide approved for use in Canada over standing water to control invasive wetland plants, such as *Phragmites australis* (PMRA, 2021). Imazapyr was only detected at one site. Triclopyr is a selective pyridine herbicide used to control broad-leaf and woody weeds in uncultivated areas, such as on industrial sites, turfgrass, or rights-of-way (University of Hertfordshire, 2020). Triclopyr was widespread in water samples (Table 2-3), all of which would be missed without

water sampling. The apparent absence of these three pesticides in o-DGTs, despite the enhanced detection limits in the analysis of o-DGTs, suggests there may benefits to including multiple matrices in pesticide monitoring depending on the objective and scope of the study.

Thiophanate-methyl was detected only in biofilm samples. This compound has low solubility, which may explain its apparent absence in water samples, and its low Log D_{ow} may explain its absence in o-DGTs (Table A-1.4). It is a parent compound of carbendazim, which was detected in o-DGTs at all sites, suggesting it is only present momentarily prior to its rapid breakdown in water via hydrolysis (Selling et al., 1970). Thiophanate-methyl is a carbamate fungicide used to control fungal diseases in turfgrass and greenhouses and is also used in veterinary medicines (University of Hertfordshire, 2020). However, this fungicide was only detected at one site. In our study, biofilms did not contribute significantly to the overall detection of contaminants, and did not detect notable contaminants that were missed by other matrices, unlike the findings of Rheinheimer et al. (2023). However, biofilms provide a unique and biologically relevant insight into contaminant bioconcentration and fate, as well as an alternative route of entry for contaminants into the food web. This is discussed further in Section 2.4.3.

2.4.2 Pesticide concentrations

Of the 82 total pesticide detections, 10 of 14 detections were quantified in water, 4 of 9 detections were quantified in biofilm, and 76 of 78 detections were quantified in o-DGTs (Table 2-4). We compared concentrations in water samples and estimated TWA concentrations in o-DGT samplers to the US EPA's Aquatic Life Benchmarks (ALB; USEPA, 2024) and found two instances of exceedances. The maximum concentration in water samples of imidacloprid, 0.012 µg L^{-1}, surpassed the ALB of 0.01 µg L^{-1} (EC_{50}) for chronic exposure to freshwater invertebrates. The maximum TWA concentration of diuron estimated from o-DGTs (0.42 µg L^{-1}) surpassed the ALB for acute exposure to vascular aquatic plants (0.13 µg L^{-1}; IC_{50}) and was within the same order of magnitude as the ALB for chronic exposure to freshwater invertebrates (0.83 µg L^{-1}; EC_{50}). In several other instances, maximum concentrations either measured in water samples or estimated in o-DGT samplers approached but did not surpass benchmarks or guidelines. For example, maximum concentrations of carbaryl (0.025 µg L^{-1}), clothianidin (0.0011 µg L^{-1}), difenoconazole (0.043 µg L^{-1}), pyridaben (0.007 µg L^{-1}), and tebuconazole (1.7 µg L^{-1}) were within one order of magnitude from their respective ALBs for chronic exposures to freshwater invertebrates (0.5, 0.05, 0.86, 0.044, and 11 µg L^{-1}, respectively). We were unable to systematically compare concentrations in the biofilm samples to appropriate thresholds for dietary exposure due to the lack of available

information for this type of exposure. Given the diversity and large number of pesticides detected at each site, an evaluation of mixture toxicity would be necessary to assess the risk of toxicity to aquatic organisms living in stormwater ponds or in aquatic habitats receiving stormwater runoff.

Table 2-4. Minimum and maximum pesticide concentrations in water, biofilm, and o-DGT samples. Concentrations for pesticides in water and o-DGTs are reported in µg L^{-1} to ease comparisons with ecotoxicological thresholds, which are commonly reported in the same units. Notation of "<MQL" refers to a pesticide that was detected (i.e., > MDL) but the concentration was lower than the MQL.

	Water (µg L^{-1})		Biofilm (µg kg^{-1})		o-DGTs (µg L^{-1})	
Pesticide	**Min**	**Max**	**Min**	**Max**	**Min**	**Max**
2,4-D	0.0036	0.87	<MQL	<MQL	0.0002	0.019
acetamiprid					<MQL	0.00016
ametryn					<MQL	0.0011
atrazine					0.0056	0.087
azoxystrobin			< MQL	35	0.000016	0.022
benalaxyl					<MQL	0.000019
bentazon	<MQL	<MQL			0.000022	0.000031
bromacil					<MQL	0.0021
bromoxynil					0.00031	0.00089
bromuconazole					0.00024	0.00024
bupirimate					0.000075	0.000075
carbaryl					0.00029	0.025
carbendazim	0.52	0.52	<MQL	<MQL	0.000026	0.019
chlorantraniliprole					0.000036	0.01
chlorpropham	< MQL	< MQL	33	33	0.44	1.36
clomazone					0.00011	0.00074
clothianidin	< MQL	< MQL			0.00024	0.0011
cyantraniliprole					0.0004	0.0011
diazinon					0.00042	0.00044
dichlorprop	0.0022	0.028			0.00097	0.00098
difenoconazole					0.000039	0.043
dimethenamid					0.00017	0.00038
dimethomorph					<MQL	0.00023
dithiopyr					<MQL	0.00035
diuron	< MQL	< MQL	<MQL	23	0.00014	0.423
epoxiconazole					<MQL	0.00063
ethiofencarb					0.00017	0.0009

	Water (µg L^{-1})		Biofilm (µg kg^{-1})		o-DGTs (µg L^{-1})	
Pesticide	**Min**	**Max**	**Min**	**Max**	**Min**	**Max**
etoxazole					0.000056	0.000056
fenazaquin					0.00029	0.00029
fenobucarb					<MQL	0.000072
fenpropimorph					0.00031	0.00046
flonicamid	< MQL	0.014				
fluazifop-p-butyl					<MQL	<MQL
fludioxonil					0.0013	0.004
flufenoxuron					0.0021	0.0035
fluopyram					0.00095	0.0043
flupyradifurone	< MQL	0.014			0.00026	0.01
flutriafol					0.00067	0.0017
hexaconazole					0.00016	0.00016
hexazinone					0.000032	0.00016
imazapyr	0.0089	0.0089				
imazethapyr					<MQL	0.000054
imidacloprid	<MQL	0.012			0.00031	0.0084
indaziflam					0.00018	0.00025
indoxacarb					<MQL	<MQL
MCPA	<MQL	1.2	<MQL	<MQL	<MQL	0.0077
mecoprop	< MQL	0.47			0.000054	0.010
metalaxyl					0.000038	0.0023
methiocarb					0.000086	0.000086
metolachlor					0.00073	0.032
metribuzin					0.00062	0.0024
myclobutanil					0.00038	0.0011
ofurace					0.00070	0.00070
paclobutrazol					0.000068	0.0026
picolinafen					0.00065	0.0011
picoxystrobin					0.000043	0.000043
prometon					<MQL	0.00026
prometryn					0.000033	0.00009
propazine					0.000035	0.00044
propiconazole					0.00042	0.049
propoxur					0.00037	0.00062
pyraclostrobin					0.000053	0.00014
pyridaben					0.00050	0.0070

	Water (µg L^{-1})		Biofilm (µg kg^{-1})		o-DGTs (µg L^{-1})	
Pesticide	**Min**	**Max**	**Min**	**Max**	**Min**	**Max**
pyridate					0.00094	0.010
pyrimethanil					0.00015	0.0028
pyriproxyfen					0.000023	0.00025
quizalofop-ethyl					0.0012	0.0012
simazine					0.00014	0.0015
simetryn					<MQL	0.000042
spiromesifen					0.00074	0.0031
spiroxamine					0.000016	0.000039
sulfentrazone					0.00014	0.0096
tebuconazole			<MQL	29	0.0051	1.72
tebufenozide					0.00064	0.0039
tebuthiuron					<MQL	0.00059
terbuthylazine					0.00039	0.00074
terbutryn					<MQL	0.00020
tetraconazole					0.00064	0.00064
thiabendazole			<MQL	<MQL	0.000032	0.020
thiacloprid					0.000055	0.00013
thiophanate-methyl			< MQL	<MQL		
triclopyr	< MQL	0.065				
trifloxystrobin					<MQL	0.000082

The extrapolation of concentration data from o-DGT samplers to inform risk assessment is challenging because it provides a TWA of the chemical concentration in the water during the exposure period. It is well-known that stormwater systems experience dynamic fluctuations in water quality and contaminant loads (Hatt et al., 2004; Walsh et al., 2005), where relatively short pulses of relatively high concentrations of contaminants can enter into the water body where the o-DGTs were deployed. The calculation of a TWA concentration of exposure over the course of the deployment period from the quantities of contaminants present in the o-DGTs does not provide insight into the frequency, duration, or magnitude of any spikes in concentration. This creates a scenario where the TWA concentrations may not exceed a threshold of toxicity, but pulses could have exceeded that threshold, resulting in adverse effects to biota. When conducting a risk assessment, using TWA concentration data from o-DGTs could result in type II errors (i.e., false negatives, where a risk is predicted to be absent when in fact it may exist).

However, this same limitation exists for grab samples of water and biofilm sampling as a measure of exposure to biota in surface water.

2.4.2.1 Pesticide concentrations in water samples vs. o-DGTs

We predicted that if a compound is detected only in o-DGTs, the calculated TWA concentration in water should be lower than the method detection limit (MDL) for water samples. Of the 67 compounds that were detected in o-DGTs but not in water (Table 2-3), 63 had maximum concentrations in o-DGTs that were lower than the MDLs for water samples. This suggests that the o-DGTs are able to detect pesticides at lower concentrations than water grab samples due to their lower detection limits and the time-integrated accumulation of pesticide compounds from the surrounding water. The exceptions were atrazine, difenoconazole, metolachlor, and tebuconazole. Both the maximum TWA concentration in o-DGTs and the MDL values for water samples were in the same order of magnitude for atrazine, difenoconazole, and metolachlor; however, the maximum concentration of tebuconazole (1.723 $\mu g\ L^{-1}$) is much higher in the o-DGTs than the MDL for water (0.1 $\mu g\ L^{-1}$). This suggests that a pulse of tebuconazole was missed by water sampling but captured by the o-DGTs.

We also predicted that we would observe a positive relationship between the concentration of contaminants measured in the water, and their measured quantities in o-DGTs. We expected that pesticides that are not detected in water samples appear to be absent because they are either present at very low concentrations (below the detection limits) or not present at all. In this case, the more sensitive o-DGT samplers (with relatively lower detection limits) should have correlated quantifications with the water samples. Of the five pesticides we investigated (flupyradifurone, imidacloprid, mecoprop, 2,4-D, and MCPA; Figure 2-4), all had a significant positive relationship ($p < 0.05$) with a relatively high coefficient of determination (adjusted $r^2 > 0.60$; Table A-1.5). We can reasonably conclude here that the detection of these five pesticides in o-DGTs was likely due to the increased sensitivity from lower detection limits or from time-integration. The significant fit of the linear regressions on the log-transformed concentrations of flupyradifurone and imidacloprid could indicate a saturation effect in o-DGTs at higher concentrations; however, previous work has shown that effective binding capacities are rarely reached during deployment periods of up to 43 days, even for high contaminant concentrations (Guibal et al., 2017). The uptake of chemicals from water into passive samplers is known to be variable; compounds can accumulate linearly, with an inflection point, randomly, or not at all (Morin et al., 2013), and we require more data points to properly test these relationships as we are constrained by the majority

of concentrations being near the detection limits. It must be stressed that clarification of these relationships is also difficult due to the differences in analytical sensitivity, small sample size, and because differences in pesticide behavior in passive samplers may be compound specific; all are areas requiring further investigation.

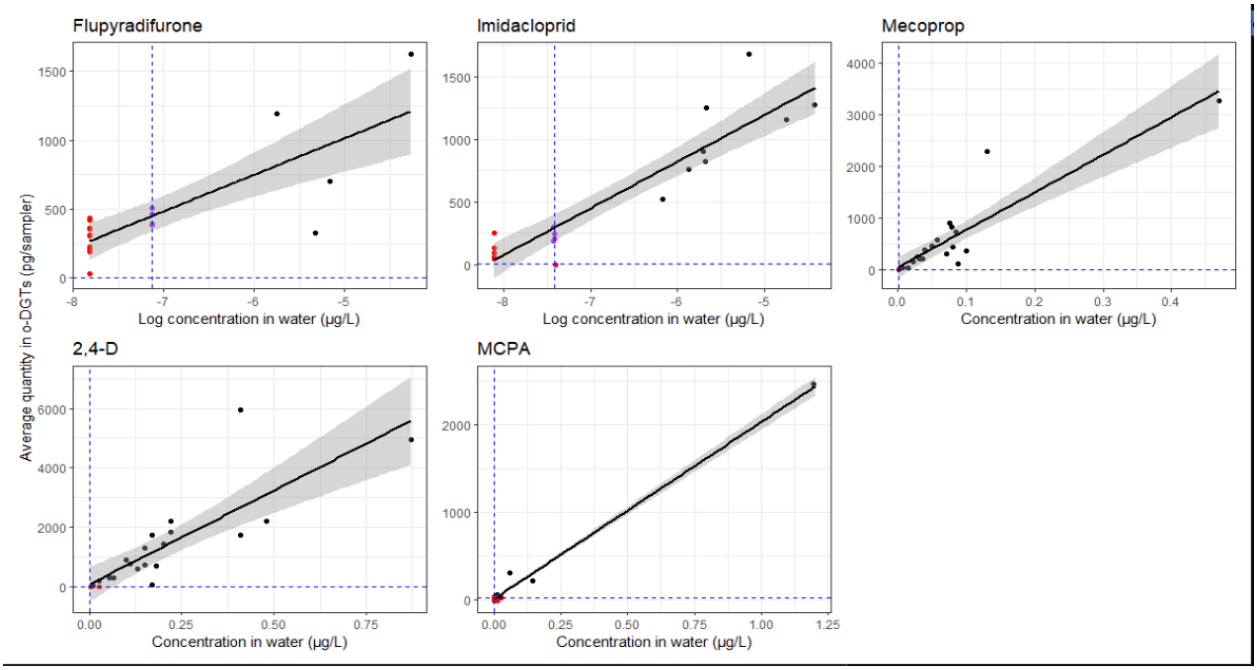


Figure 2-4. Linear regressions describing the relationship between concentrations in the water and in the o-DGT samplers for 5 pesticides. The horizontal dashed lines represent the limit of detection for o-DGTs, while the vertical dashed lines represent the limit of detection for the water samples. The solid black lines indicate a significant linear relationship ($p < 0.05$). In cases where a compound was not detected at a site in one of the matrices, half of the MDL value of that compound was used (indicated by a red dot). In cases where a compound was detected but not quantified (<MQL) at a site in the water samples, the MDL value of that compound was used (indicated by the purple dots). The black dots represent instances where the compound was quantified in both matrices at that site. The dots are jittered. The results are shown in Table A-1.5.

2.4.3 Practicality and reproducibility

2.4.3.1 Variability in o-DGT duplicates

An advantage of sampling with o-DGTs is the ability to easily incorporate duplication to increase the reliability of detections (biofilms, on the other hand, require substantially more surface area to collect adequate biomass for chemical analysis). We predicted that due to their identical deployment and exposure conditions, duplicates should have a high level of agreement in both the detection and quantification of compounds. Here, we summarize our findings on the variance between duplicates deployed at the same instance at the same sites. Importantly, this is not the variance between two deployment periods at the same site.

We found relatively high levels of agreement in the detection of pesticides across the duplicate o-DGTs (average detection agreement ranged from 65 to 100% between duplicates, with an average agreement of 81 ± 8.7%); these results are encouraging and may suggest high reproducibility for presence–absence analysis (Table A-1.6).

We expected that time-integration would also result in good correlations between concentrations in the duplicates, especially with their close spatial proximity and matching deployment orientation; however, the large range in concentration agreement (mean concentration differences ranged 3.16–24,290 pg/sampler, averaging 676 ± 3115 pg/sampler) may signify uncertainty in the reliability of concentration measurements (Table A-1.7). The coefficient of variance (COV), representing the between-duplicate variability in target analyte recovery, averaged 86 ± 74%, and for pesticides detected in both duplicates, COV values ranged from 7 to 295%. Also, variation between duplicates did not decrease with an increase in the mass of analyte present (Table A-1.8), although at such trace levels, uncertainty around concentrations makes sense as even the tiniest difference in dilution volume or exposure history can have a relatively large impact on concentration. As suggested by Challis et al. (2018), uptake variability may be caused by physical interference of particles settling from the water column, and it is apparent that stormwater ponds are subject to relatively high concentrations of suspended sediments (Marsalek et al., 2002; also see Figure 2-2). The relatively small surface area of the exposed face of o-DGTs may also be a contributing factor (Renaud et al., 2022). In either case, deploying replicate samplers at a site is clearly important to characterize the variation in resultant concentrations measured across o-DGTs.

2.4.3.2 Limitations and recommendations for pesticide monitoring in urban areas

This study aimed to improve the understanding of urban pesticide contamination by assessing the performances of three sampling approaches and to inform how pesticide monitoring could be implemented in urban areas of the Great Lakes watershed. To our knowledge this is the first study to report on the performance of o-DGT samplers in stormwater ponds for the detection of pesticide contamination. Although the o-DGT samplers clearly detected more pesticide compounds and had lower limits of detection than our composite water samples or biofilm samples, we cannot dismiss the value of water and biofilm sampling. Each matrix has benefits and limitations, and each sampling approach has different purposes for which they are best suited.

Instantaneous water sampling is likely insufficient for describing such temporally complex pollutant fluxes as seen in urban stormwater systems (Paul & Meyer, 2001; Harmel et al., 2010). Yet, the sample volume required for analysis (approximately 1 L per site for all three pesticide screens) is easily achieved, and low equipment and labor costs can allow for more samples to be taken across a wider spatial or temporal scale (e.g., the use of automatic high-frequency water sampling (la Cecilia et al., 2020). Importantly, direct comparisons in concentrations can be made with other studies and with ecotoxicological risk thresholds and guidelines, of which most report exposure in terms of the concentration in the surrounding water (e.g., Tang et al., 2013; Pamuru et al., 2022).

Biofilms are important integrators of pollutants, and the biofilm sampling approach is ecologically relevant, giving insight into potential exposures through the food chain (Bonnineau et al., 2021). Compared to o-DGT samplers (which are not consumed), biofilms represent a direct dietary exposure pathway and a more realistic route of entry for contaminants (see Chapter 4; Izma et al., 2024b). However, we currently have very little understanding of the impacts of pesticides through dietary exposure routes in aquatic ecosystems, which limits our ability to draw comparisons to other research or to ecotoxicological risk guidelines. Additionally, the biofilm community can be variable - with different compositions even under the same environmental conditions (Roeselers et al., 2006) - thus, its standardization can be difficult to achieve from a monitoring perspective, although this may be irrelevant if the focus is on dietary exposure characterization. The surface area required to meet the mass requirements for chemical analysis can make the duplication of biofilm samplers much more difficult. For example, despite the total surface area of 1.67 m^2 for each biofilm sampler, adequate biomass for analysis was often difficult to obtain. The total amount of biofilm harvested at each site ranged widely from as little as 0.0018 g cm^{-2} to 0.098 g cm^{-2}, and due to the high water content of most biofilms, between 300

and 500 g of wet biofilm was needed to meet the required 4–6 g of freeze-dried biofilm to conduct all three pesticide screens. The construction and deployment of biofilm samplers was time-consuming in our case because each sampler was made by hand and assembled on site (we are not currently aware of adequately-sized commercialized biofilm samplers for deployment in lentic systems). The harvesting of biofilm was also more labor intensive than water sampling or o-DGT collection; for example, at each site, we spent 45–60 min thoroughly scraping the 10 acrylic plates on both sides to obtain our composite sample, whereas the collection of water samples or o-DGTs took less than 5 min. However, both passive sampling techniques (biofilm and o-DGT) reduce the number of required site visits, enabling time-integration without added field labor costs.

What o-DGTs lack in biological relevance, they make up for in sensitivity. Based on our findings, these devices are capable of concentrating compounds present at trace levels across a variety of molecular properties to allow for their detection and quantification otherwise missed by other sampling approaches. This is supported by other similar work with o-DGTs for monitoring pesticides (Challis et al., 2018; Hageman et al., 2019). o-DGTs are highly applicable in urban settings due to the wide variety of contaminant types they can detect (Guibal et al., 2019) and allow for a more holistic picture of the mixture of chemicals present. o-DGTs allow for field blanks and duplication, both of which are either not possible or more effort to conduct in other methods. However, given the high degree of variability in concentration between duplicate samplers, there remains uncertainty in pesticide compound quantification extrapolated from o-DGTs. Inferences about pesticide occurrence (i.e., presence/absence) are much more reliable than inferences about pesticide concentrations. An additional trade-off with time-integrated sampling is that because pulses are averaged over the exposed period, extremes are not captured in an interpretable way, and this may lead to inaccurate perceptions of ecological risk.

Analytical differences drove an increase in sensitivity to pesticide detection in the o-DGT samplers compared to water and biofilm samples. Analytical methods are critically important for determining the profile of pesticide contamination because so many pesticides occurred at trace levels. For the effective characterization of pesticide contamination, programs clearly need research-grade methods with lower detection limits (i.e., in the ng L^{-1} range) to detect what pesticides are present. Because most pesticides detected in our stormwater ponds were detected by o-DGTs, the costs of analyzing more than one matrix seem to outweigh the benefits of the additional insight provided by these.

A limitation of our study was that sampling occurred during a single field season, which limits the interpretability of the data collected. This lack of temporal representation means that factors potentially influencing the presence and levels of contaminants, such as pesticide use patterns or climatic conditions, which may be variable across seasons and years are not assessed. Thus, the effect of these factors on the utility of each sampling approach is unknown. For example, a shift in pesticide use towards higher applications of fungicides, perhaps due to the onset of favourable weather conditions for fungal growth affecting the integrity of building materials or turfgrass, may cause a subsequent shift in the number of hydrophobic pesticides entering stormwaters (as fungicides are typically more hydrophobic compared to other pesticide classes). This shift could necessitate the use of a sampling matrix, such as biofilms, capable of capturing these types of contaminants. Assessing sampling approaches across seasons and years is an important avenue for future research.

We recommend careful consideration of the monitoring goals prior to selecting any sampling method. When the objective is to cast a wide net to survey the range of pesticides present in the environment and to track trends in their relative usage/release, o-DGTs should be considered because of their high sensitivity to a large range of pesticides and capacity for time-integration. Allocating funds and resources toward lowering detection limits, rather than sampling more than matrix, would better equip these types of programs. When the goal is to characterize contaminant exposure for the purpose of comparison with water quality guidelines and ecotoxicological risk thresholds or to contrast with other research studies (given how commonly environmental monitoring relies on water grab samples), then high-frequency water sampling is best selected. To support the assessment of contaminant exposure and ecological risk from data obtained from o-DGTs, we suggest targeted, high-frequency water sampling, particularly for specific areas where there is concern about contaminant pulses surpassing ecological thresholds. When the objective is to understand the environmental fate of pesticides or potential risk to the aquatic food chain, biofilm sampling should be included in the program. Biofilms are also useful as a substrate for use in bioassays to assess the dietary exposure of pesticides (e.g., see Chapter 4; Izma et al., 2024b). When resources are available and objectives are manifold, we recommend measuring multiple matrices. The collaborative monitoring of stormwater management ponds undertaken in this research provided a more comprehensive understanding of the diversity of pesticides present in the urban environment.

2.5 Conclusions

In our study of urban stormwater management ponds, we found widespread pollution with pesticides, including an assortment of 82 different herbicides, insecticides, and fungicides in water, biofilm, and using o-DGT samplers. These findings raise important questions about the ecological impact of chronic exposure to these chemical mixtures. Such extensive contamination of the urban environment concerns the well-being of both residents and wildlife in cities and must be addressed to ensure the sustainability of urban water systems and the health of urban biota.

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Chapter 3: Urban-use pesticides in stormwater ponds and their accumulation in biofilms

3.1 Overview

Stormwater ponds frequently receive urban runoff, increasing the likelihood of pesticide contamination. Biofilms growing in surface waters of these ponds are known to accumulate a range of aquatic contaminants, paradoxically providing both water purification services and potentially posing a threat to urban wildlife. Thus, sampling biofilms in stormwater ponds may be a critical and biologically relevant tool for characterizing pesticide contamination and toxicity in urban environments. Here, we aimed to investigate pesticide occurrences at 21 stormwater ponds in Brampton, ON, one of Canada's fastest growing municipalities, and to quantify their accumulation in biofilm. Over nine weeks, we collected time-integrated composite water and biofilm samples for analysis of ~500 current-use and legacy pesticides. Thirty-two pesticide compounds were detected across both matrices, with 2,4-D, MCPA, MCPP, azoxystrobin, bentazon, triclopyr, and diuron having near-ubiquitous occurrences. Several compounds not typically monitored in pesticide suites (e.g., melamine and nicotine) were also detected, but only in biofilms. Overall, 56% of analytes detected in biofilms were not found in water samples, indicating traditional pesticide monitoring practices fail to capture all exposure routes, as even when pesticides are below detection levels in water, organisms may still be exposed via dietary pathways. Calculated bioconcentration factors ranged from 4.2 to 1275 and were not predicted by standard pesticide physicochemical properties. Monitoring biofilms provides a sensitive and comprehensive supplement to water sampling for pesticide quantification in urban areas, and identifying pesticide occurrences in stormwater could improve source-tracking efforts in the future. Further research is needed to understand the mechanisms driving pesticide accumulation, to investigate toxicity risks associated with pesticide-contaminated biofilm, and to evaluate whether pesticide accumulation in stormwater pond biofilms represents a route through which contaminants are mobilized into the surrounding terrestrial and downstream aquatic environments.

3.2 Introduction

In urban areas, stormwater management ponds are employed to accommodate hydrological alterations imposed by urbanization and sprawl. Runoff accumulates in these retention ponds via stormwater drainage systems and can be loaded with sediment and contaminants (Göbel et al., 2007), including road

salt, heavy metals (Pitt et al., 1995; Zgheib et al., 2012), pesticides (Burant et al., 2018), oil (Brown et al., 1985), and fecal bacteria (Valenca et al., 2022). Discharge from stormwater ponds is an important source of chemical and thermal pollution into freshwater systems, with downstream consequences, such as habitat degradation and biodiversity loss, being well documented (e.g., Hatt et al., 2004; Burns et al., 2012; Park and Roesner, 2012; Zgheib et al., 2012). Frequently overlooked, however, are the ecological implications of these stressors within the ponds, which have become urban habitat for aquatic wildlife (Le Viol et al., 2009; Perron and Pick, 2020).

Although stormwater ponds primarily serve flood mitigation objectives, they are also frequently designed to improve water quality, in recognition of their role in transforming and accumulating contaminants to protect downstream waters (e.g., MOE, 2003; EPA, 2009). However, stormwater ponds are not routinely monitored for contaminants. In particular, levels of current-use and legacy pesticides in stormwater ponds are not well documented; for example, the only other evaluation of pesticide levels in stormwater ponds in Ontario was published in 1994 by Struger et al. A stormwater pond study was conducted in 2000 by Bishop et al. (2000), but they only looked at pesticides present in sediment, not water. This knowledge gap is emphasized in a critical review by Chen et al. (2019) that covered pesticides in stormwater and identified an urgent need for research quantifying a wider scope of pesticide mixtures and investigating the biological and physicochemical drivers of pesticide occurrence variability in urban runoff. Urban-use pesticides, such as methylchlorophenoxypropionic acid (MCPP) and 2,4-dichlorophenoxyacetic acid (2,4-D), accumulate in stormwater ponds (Struger et al., 1994; Raina et al., 2011; Chen et al., 2019; Flanagan et al., 2021) and could present toxicological risks to urban wildlife residing in stormwater ponds (Allinson et al., 2015; Flanagan et al., 2021). Moreover, contaminant retention within the ponds can be compromised, particularly during flood events when pond sediments are disturbed (Vulava et al., 2019; Spahr et al., 2020). Sediment resuspension can cause remobilization of contaminants and threaten downstream ecosystems. The lack of routine monitoring in stormwater ponds complicates efforts to predict both within-pond risks to biota, and the potential for pesticide remobilization into surrounding aquatic ecosystems.

Pesticides can enter stormwater in urban environments through a variety of uses, including construction activities, personal and pet care (e.g., bed bug or flea treatments), household cleaning, and municipal landscaping (Meftaul et al., 2020; Spahr et al., 2020; OMAFRA, 2021). Pesticides may also be applied directly to stormwater ponds, for example, methoprene is a larvicide used to kill mosquitos and mitigate the public health risk of West Nile Virus in some stormwater ponds in Ontario (Peel Public

Health, personal communication, October 28, 2022). The persistence and accumulation of pesticides in aquatic environments is highly dependent on their physiochemical properties, such as their solubility in water, which influences the partitioning of pesticides into water, sediment, or organic material (Gramatica and Di Guardo, 2002). Thus, traditional assessment using water sampling techniques may fail to capture pesticides in biologically relevant material, such as biofilms (Rooney et al., 2020; Mahler et al., 2020; Bonnineau et al., 2021). Recent evidence suggests that biofilms can accumulate a range of contaminants, including pesticides (Mahler et al., 2020; Fernandes et al., 2020; Beecraft and Rooney, 2021; Ijzerman et al., 2023). Beecraft and Rooney (2021) found glyphosate concentrations to be two to four times higher in biofilms compared with surrounding water, while Lawrence et al. (2001) proposed that biofilms are sinks for herbicides after discovering that diclofop methyl and atrazine were detected in river biofilm samples despite not being detected in water samples. Most recently, Rheinheimer Dos Santos et al. (2023) found pesticide occurrences were underrepresented in grab water samples compared to epilithic biofilm collected from natural substrates in both agricultural and urban stream systems. Primary production and decomposition in aquatic ecosystems are driven by microbial activity (Hall Jr and Meyer, 1998; Zou et al., 2016), thus biofilms are key energy sources for many invertebrate and fish species but may represent trophic links through which contaminants can be passed (Bonnineau et al., 2021). Not only do biofilms containing pesticides potentially pose a toxic threat to biota through dietary transfer, but connections between aquatic and terrestrial environments (e.g., during emergence of adult stage invertebrates or amphibians) could facilitate mobilization of pesticides from stormwater systems into neighboring terrestrial habitats. Thus, it is critical to understand contamination in both stormwater pond water and biofilm matrices and to measure the magnitude of accumulation within biofilms.

With a high surface area to volume ratio, biofilms can be exposed to contaminants from both the water and the surfaces to which they attach. Pesticide accumulation is thought to occur via sorption and passive diffusion (Flemming, 1995; Chaumet et al., 2019b), where the hydrophobicity of a pesticide has been previously hypothesized as the key determinant of uptake and accumulation within biofilms. For example, Nikkilä et al. (2001) monitored atrazine uptake and depuration by a river biofilm and found that atrazine was rapidly taken up by periphyton but was minimally bioconcentrated due to low hydrophobicity. Capacity for pesticide accumulation in biofilms may also be influenced by surrounding water quality parameters, such as salinization, which is known to lower diatom diversity (Ziemann et al., 2001; Coring and Bäthe, 2011; Van Meter et al., 2011), microbial respiration in heterotrophic biofilms (Entrekin et al., 2019; Martínez et al., 2020), and photosynthetic rates in autotrophic biofilms (Cook and

Francoeur, 2013). Although environmental drivers of pesticide accumulation remain understudied, changes resulting in altered biofilm community structures, and thus their lipid content, may influence biofilms' capacity to accumulate organic chemicals (Wang et al., 1999).

In this study, our objectives were to: 1) determine the maximum concentration and occurrence of herbicides, insecticides, and fungicides in stormwater pond water and in biofilms, and compare those concentrations to exposure thresholds to assess potential risk to aquatic biota; 2) evaluate the relationship between pesticide occurrences in each matrix and the physicochemical properties of the pesticides; 3) compare the concentrations of pesticides in water to those in cultivated biofilm to quantify accumulation using bioconcentration factors; and 4) investigate the relationship between bioconcentration factors and the physicochemical properties of the pesticides we detected alongside the general quality of the water in the stormwater ponds and the chlorophyll-*a* content and ash-free dry weight of the biofilms. We hypothesized that the accumulation of pesticides into biofilm would be related to the physicochemical properties of the pesticides, and that pesticides that are more soluble in water would accumulate in biofilm the least and be detected more frequently in the water matrix. We also hypothesized that pesticides with higher octanol-water (Log K_{ow}) or soil adsorption (Log K_{oc}) coefficients would accumulate in biofilm more and be more frequently detected in biofilm than in water.

3.3 Materials and methods

3.3.1 Location of study

The City of Brampton, Ontario, is one of Canada's fastest growing municipalities and experiences high rates of development and urban expansion (Statistics Canada, 2021; City of Brampton, 2022). The resulting impervious cover totals 7,888 ha, or 45.4%, of the City's area (TRCA, n.d.). Located within two major watersheds, the West Humber River watershed and the Credit River watershed, Brampton is home to > 180 stormwater ponds that ultimately feed into Lake Ontario (City of Brampton, n.d.). Twenty-one of these stormwater ponds were selected for this study (Figure 3-1). The impervious cover within a 300 m buffer surrounding each stormwater pond ranged from 10.9 to 55.2% (range = 69,630–247,521 m^2, mean = 159,060 m^2, standard deviation = 45,224 m^2) to yield a gradient in urbanization intensity (McIsaac, 2022). The 21 stormwater ponds drain into either Etobicoke Creek, Mimico Creek, the Credit River, or the Humber River. As wet ponds, these locations are designed and managed to retain water all year long. The ponds ranged in size from 1051 to 3686 m^2 (mean = 2272 m^2, standard deviation = 851 m^2), were a minimum of 10 years old, and had no history of dredging within the past 10 years.

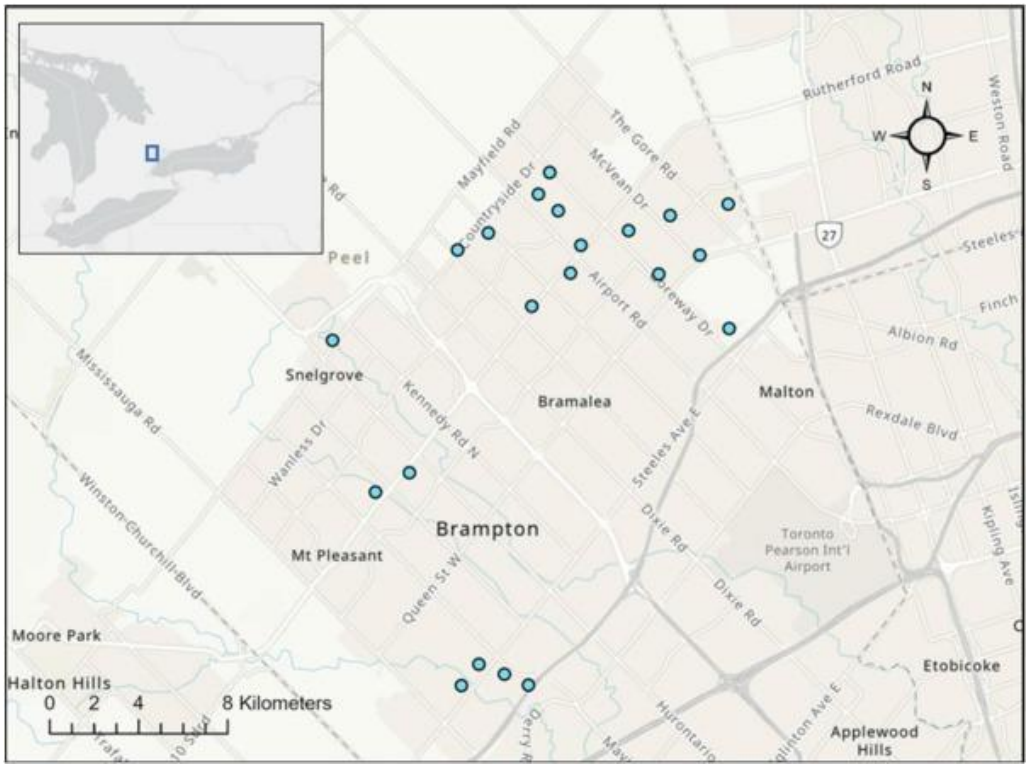


Figure 3-1. Map of stormwater pond sites (blue circles) in Brampton, Ontario. Map created in ARCGIS on July 5, 2023.

3.3.2 Collection and analysis of water samples

The water samples used in this chapter are the same samples presented in Chapter 2. Briefly, we collected a time-integrated stormwater pond surface sample to characterize pesticide concentrations in each pond over a nine-week study period between May 25 and July 25, 2022. This was achieved by adding a 150 mL subsample, taken at ~20 cm depth, each week to create a 1.35 L composite that we kept at −20 °C until submission to the Agriculture and Food Laboratory (AFL) (ISO/IEC 17025 accredited) in Guelph, Ontario, for a comprehensive analysis of 542 current-use and legacy pesticides using multi-residue liquid chromatography/electrospray ionization-tandem mass spectrometry (LC/ESI-MS/MS) and gas chromatography-tandem mass spectrometry (GC–MS/MS). Details of this analysis, including lists of analytes and their detection and quantification limits are provided in Appendix B. Unlike in Chapter 2, where our exploration of these results was restricted to 238 analytes screened for in three matrices, as this chapter focuses specifically on pesticide concentrations in water and biofilm samples only, we were able to assess the full results of the analysis of 542 pesticide analytes.

We measured water quality regularly over the same nine-week period (Table 3-1). At each stormwater pond, at three points in the open water and ~20 cm deep, we measured water temperature (°C) and conductivity (mS cm^{-1}), using a Hach HQ1140 Portable Conductivity/TDS Meter (Hach Sales and Services L.P., London, Canada) and we measured dissolved oxygen (DO; mg L^{-1}), using a YSI ProSolo ODO Optical Dissolved Oxygen Meter (Xylem Inc., Miami, FL).

Table 3-1. Water chemistry parameters measured at each stormwater pond site (n = 21) in Brampton, Ontario. WWL = Waterloo Wetland Lab; ALS = ALS Laboratories; AFL = University of Guelph Agriculture and Food Laboratory.

Parameter	Location of analysis	Method	Frequency of analysis
Surface water temperature	In-situ	Hach multi-probe meter	Weekly
Conductivity	In-situ	Hach multi-probe meter	Weekly
DO	In-situ	Hach multi-probe meter	Weekly
pH	WWL	Thermo-Orion pH probe and benchtop meter	Weekly
Chloride	WWL	Colorimetric	Weekly
Chlorophyll-*a*	WWL	Fluorometric	June and July
TSS	WWL	Gravimetric	Weekly
Ammonia	ALS	Fluorometric	Once
Nitrate	ALS	Ion chromatography	Once
Nitrite	ALS	Ion chromatography	Once
Orthophosphate	ALS	Colorimetric	Once
E. coli and coliforms	AFL	Plate counts	Once
Pesticides	AFL	Multi-residue LC-MS	Once[a]

[a]analysis completed on time-integrated composite of weekly water samples.

Each week, we combined 50 mL surface water grab samples from ~20 cm depth within a high-density polyethylene Nalgene bottle to make an additional time-integrated composite for the purpose of *ex situ* measurements. We kept the weekly water sample on ice during transport to the Waterloo Wetland Lab (WWL) in Waterloo, Ontario, where we determined total suspended solids (TSS; mg L^{-1}) gravimetrically, and measured pH using an Orion 9156BNWP Combination pH Electrode and Orion Star A211 Benchtop pH Meter (Thermo Fisher Scientific, Waltham, MA). We measured chloride ion concentration (Cl^-; mg L^{-1}) colorimetrically, using a Chloride Checker® HC (Hannah Instruments, Woonsocket, RI). Once in June (week of June 21, 2022) and once in July (week of July 11, 2022), we collected additional composite water grab samples for the fluorometric determination of chlorophyll-*a* (ug

L^{-1}) using a Turner Designs TD 10 AU fluorometer (Turner Designs Inc., Sunnyvale, California) at the WWL. This machine is calibrated annually using a calibration curve which was created from a dilution series of chlorophyll-*a* standard and validated with a Biochrom Ultrospec 3100 spectrophotometer (Biochrom Ltd., Cambridge, UK).

Between June 21 and 24, 2022, we collected a water grab sample from each site and submitted them for nutrient analysis at ALS laboratories in Waterloo, Ontario, which included fluorometric determination of ammonia (NH_3), ion chromatographic determination of nitrate (NO_3) and nitrite (NO_2), and calorimetric analysis of orthophosphate (PO_4). Finally, following a precipitation event on June 7, 2022, we collected and submitted surface grab water samples from each stormwater pond to the Agriculture and Food Laboratory (AFL) in Guelph, Ontario, for plate-count analysis of *E. coli* and total coliform bacteria. The rationale behind collecting these samples following a precipitation event was to capture potential maximum loadings of bacterial contaminants into the stormwater.

We used the Canadian Council of Ministers of the Environment (CCME) Water Quality Index (WQI) Calculator version 1.2 to calculate WQI scores for each stormwater pond site. The following 13 parameters were included in this calculation: pH, conductivity (mS cm^{-1}), DO (mg L^{-1}), surface water temperature (°C), chloride concentration (mg L^{-1}), chlorophyll-*a* concentration (µg L^{-1}), TSS (mg L^{-1}), ammonia (mg L^{-1}), nitrate (mg L^{-1}), nitrite (mg L^{-1}), phosphorus (mg L^{-1}), coliforms (cfu per 100 mL), and *E. coli* (cfu per 100 mL). The Canadian Environmental Quality Guidelines for the protection of aquatic life reported by CCME (accessed at: https://ccme.ca/en/resources#) were prioritized for these calculations, and where none existed, we used guidelines from the Ontario Provincial Water Quality Objectives (accessed at: https://www.ontario.ca/page/water-management-policies-guidelines-provincial-water-quality-objectives#section-3), the USEPA Water Quality Criteria for Aquatic Life (accessed at: https://www.epa.gov/wqc/national-recommended-water-quality-criteria-aquatic-life-criteria-table), and the USEPA Aquatic Life Benchmarks (https://www.epa.gov/pesticide-science-and-assessing-pesticide-risks/aquatic-life-benchmarks-and-ecological-risk#aquatic-benchmarks). Table B-1 shows the WQI scores for each stormwater pond site.

3.3.3 Collection and analysis of biofilm samples

The biofilm samples used in this chapter are the same samples presented in Chapter 2. To culture biofilm in-situ, we deployed ten standard substrates (acrylic plates 20.2 by 44.4 cm in area) in each of the 21 stormwater ponds, following the deployment protocol described by Rooney et al. (2020). In brief, we

acid-bathed the plates, rinsed them three times with deionized water, and gently scuffed them with fine sandpaper prior to deploying them on buoyant racks (Figure B-1. A) that suspended them ~10 cm beneath the water's surface (Figure B-1. B). We anchored the racks at depths between 60 and 100 cm in areas clear of vegetation and shading. After 54 days of deployment, we retrieved the plates and harvested the biofilm by scraping each side of each plate into a composite sample for each sampler rack, and quantitatively transferring the resulting biofilm slurry using a measured amount of distilled water to rinse.

We removed an aliquot of 300–500 g (wet weight) from each composite biofilm sample and freeze-dried it using a FreeZone I Labconco benchtop freeze dryer (Labconco Corporation, Kansas City, Missouri). We then submitted the freeze-dried biofilm to AFL for analysis of the same suite of pesticides as described for water samples. Of note, two sites did not produce adequate biofilm mass for all pesticide analysis screens and were only analyzed for phenoxy herbicides and polar pesticides (see Appendix B for a list of analytes). However, the other sites (n = 19) had sufficient biofilm mass to complete all pesticide screens. As noted in Section 3.3.2, although the samples discussed in this chapter are the same as in Chapter 2, because we focus here specifically on pesticide concentrations in water and biofilm samples only, we were able to assess the full results of the analysis of 542 pesticide analytes.

For each biofilm sample, we measured dry mass and ash-free dry weight (AFDW) gravimetrically using a Thermo Scientific F6010 Thermolyne furnace (Thermo Fisher Scientific Inc., Asheville, North Carolina). To measure chlorophyll-*a* levels in the biofilm fluorometrically, we sonicated the samples at 90% amplitude for three 30-s intervals and centrifuged at 3500 rpm for 6 min prior to fluorometric assessment using the same method applied to the water samples.

3.3.4 Characterization of pesticides

We used databases such as the Pesticide Properties Database (PPDB; accessed at: https://sitem.herts.ac.uk/aeru/ppdb/en/index.htm) and the Pesticide Action Network Pesticide Database (http://www.pesticideinfo.org/) to extract information regarding the chemical structures and properties of the pesticides detected in our samples. We supplemented these sources with chemical profiles reported by PubChem online database (accessed at: https://pubchem.ncbi.nlm.nih.gov/) and ChemSpider online database (accessed at: https://www.chemspider.com/Default.aspx). Specifically, we collected each chemical's CAS number, chemical class, mode of action, octanol-water partition coefficient (Log K_{ow}), solubility in water, soil adsorption coefficient (Log K_{oc}), persistence in aquatic environments, and potential sources. We also used Health Canada's Pesticide Product Information Database (PPID; accessed

at: https://pest-control.canada.ca/pesticide-registry/en/product-search.html) to determine the status of pesticide permits.

3.3.5 Data analysis

3.3.5.1 Pesticide occurrences and concentrations and comparison to toxicity thresholds

We visualized the relative occurrence of pesticide types and their detections in water versus biofilm (objective 1) using Microsoft Excel. To support comparison to previous studies (e.g., Struger et al., 1994; Rooney et al., 2020), we grouped detected pesticides into four categories based on type: herbicides, insecticides, fungicides, and other biocides. Some pesticides bridged more than one type (e.g., nicotine is historically associated with insecticidal uses, but present-day detections are more likely with the result of improper cigarette disposal), and in these cases we selected the most probable type given the urban setting.

To facilitate comparisons between water and biofilm samples, we report pesticide concentrations as parts per billion (ppb), rather than as $\mu g\ L^{-1}$ in water samples and $\mu g\ kg^{-1}$ in biofilm samples. To estimate potential risks posed to stormwater organisms, the levels of pesticides detected in the stormwater were compared to known levels of concern for aquatic exposures provided by the Canadian Council of Ministers of the Environment (CCME) and the Pesticide Properties Database (PPDB).

3.3.5.2 Pesticide detections and their physicochemical properties

We tested whether the number of detections of pesticides in water and biofilm matrices depended on the pesticide properties (Log K_{ow}, Log K_{oc}, water solubility; objective 2) using generalized linear models with a Poisson distribution and log link function, because of its suitability for count data. These properties were selected because of their known influence on chemical fate in the environment (Gramatica & Di Guardo, 2002). As active uptake of pesticides by biofilms is unlikely, we hypothesized accumulation would be dependent on each chemical's inclination for sorption and absorption on organic surfaces, in addition to its concentration in the water.

3.3.5.3 Bioconcentration factor calculations

The accumulation of heavy metals, pharmaceuticals, and pesticides from the surrounding water and sediment into biofilm has been characterized as bioconcentration by other researchers (Ancion et al., 2014; Bonnineau et al., 2021; Rooney et al., 2020). This characterization is pragmatic: microbial

organisms in biofilms are not analyzed individually to measure bioconcentration by each microbe, but biofilm is consumed whole by many aquatic organisms. Thus, the increase in contaminant concentration from water and sediment to biofilm creates an exposure magnification that is analogous to bioconcentration from an ecotoxicology perspective. However, because it is not certain whether pesticides adsorb onto the extracellular polymeric substance (EPS) matrix or to the surface of microbial cells, or whether pesticides enter microbial organisms through active or passive transport across cell membranes, the general term "accumulation" is more appropriately used over "bioconcentration" or "bioaccumulation.". In this study we use the calculation of bioconcentration factors (BCFs) as useful indices of pesticide accumulation, but we do not imply that pesticides enter the cells of microorganisms in the biofilms.

Dry weight bioconcentration factors (BCF_{DW}) for each pesticide at each site were calculated using the pesticide concentrations reported in both the stormwater and in the freeze-dried biofilm by AFL (objective 3). We used the calculation:

$$BCF_{DW} = \frac{Pesticide\ concentration\ in\ biofilm\ (ppb)}{Pesticide\ concentration\ in\ stormwater\ (ppb)}$$

as described by Beecraft and Rooney (2021) and Rooney et al. (2020). We divided our reported BCF_{DW}s into two groups: calculated and estimated BCF_{DW}s, where calculated BCF_{DW}s represent a more confident assessment in which pesticide concentrations were measured above the method quantification limit (MQL) in both the biofilm and the water from the stormwater ponds. If a pesticide was quantified in a biofilm sample, but not in the surrounding stormwater, we used the method detection limit (MDL) value for the water to estimate a BCF_{DW} that was as conservative as possible. Estimated BCF_{DW}s represent instances where concentrations in the biofilm were below the MQL but above the MDL, in which case the MDL limit value was used. Both estimated and calculated BCF_{DW}s were reported, however only calculated BCF_{DW}s were averaged and used in statistical analyses.

3.3.5.4 Bioconcentration factors and pesticide physicochemical properties

We used generalized linear models to investigate the relationship between calculated bioconcentration factors (BCF_{DW}s) and Log K_{ow}, Log K_{oc}, and water solubility of the pesticides, water quality in each stormwater pond, and chlorophyll-*a* content and AFDW of biofilm (objective 4). These generalized linear models used a gamma distribution with a log link function, chosen for its suitability in accommodating

the positively skewed BCF_{DW} data. An average calculated BCF_{DW} was used for pesticides with more than one calculated BCF_{DW}.

We used R Statistical Software (version 4.2.2.) in RStudio (version 2023.06.1; Rstudio Team 2021) for all analyses. To implement generalized linear models, we used the glm function in base R. We evaluated model fit for all the generalized linear models by comparing the null deviance to the residual deviance and assessing statistical significance of each model term.

3.4 Results and discussion

3.4.1 Concentration and occurrence of pesticides in stormwater and biofilm

We found 32 pesticides across our 21 sampling sites and matrices. The minimum, maximum, and mean concentrations of each pesticide observed are reported in Table 3-2. Pesticide contamination is clearly widespread in urban areas. For example, the herbicides 2,4-D, MCPA, MCPP, triclopyr, and bentazon were nearly ubiquitous in water samples. Neonicotinoids were also frequently found in water samples, with detectable levels of clothianidin and imidacloprid at 67% and 62% of sites, respectively. Despite provincial legislation introduced in 2015 to reduce their use by 80% (OMECP, 2015), neonicotinoids still have both urban and agricultural applications (e.g., in lawn care, pet care, and domestic pest management; PMRA, 2016; OMAFRA, 2017; Batikian et al., 2019) and are evidently as common in urban stormwater as in Ontario's agricultural streams (Raby et al., 2022). Fungicides were less commonly detected in stormwater, but by sampling biofilms we were able to determine that they are also widespread in urban areas. For example, biofilm samples had detectable levels of azoxystrobin and thiabendazole at 95% and 81% of sampling sites, respectively. Biofilm sampling also revealed the widespread presence of nicotine in 52% of the sites sampled.

Table 3-2. Detection and quantification of 32 pesticides found in water or biofilm samples collected from 21 stormwater ponds in Brampton, Ontario. A full list of the 542 pesticides tested for, with their respective limits of detection and quantification, is shown in Appendix B. MDL = Method Detection Limit, MQL = Method Quantification Limit.

Type	Pesticide	Detection in stormwater (ppb)				Detection in biofilm (ppb)			
		# of detections out of 21	Min	Max	Mean (± standard deviation)	# of detections out of 21	Min	Max	Mean (± standard deviation)
Herbicide	2,4-D	21	0.0036	0.87	0.1980 (±0.2025)	9	5 (MDL)	30 (MQL)	
Herbicide	2,4-DP	4	0.0022	0.028	0.0099 (±0.0122)	0			
Herbicide	2,4,5-T	5	0.0007 (MDL)	0.0052		0			
Herbicide	Atrazine	10	0.07 (MDL)	0.07 (MDL)		0			
Herbicide	Bentazon	20	0.0008 (MDL)	0.002 (MQL)		0			
Herbicide	Chlorpropham	1	0.2 (MQL)	0.2 (MQL)		1	33	33	33
Herbicide	Dicamba	9	0.038	0.44	0.1251 (±0.1313)	0			
Herbicide	Diuron	4	0.2 (MDL)	0.6 (MQL)		18	5 (MDL)	23	
Herbicide	Glufosinate	2	1 (MDL)	1 (MDL)		0			
Herbicide	Imazapyr	1	0.0089	0.0089	0.0089	0			
Herbicide	MCPA	21	0.002 (MQL)	1.2		1	30 (MQL)	30 (MQL)	
Herbicide	MCPP	21	0.002 (MQL)	0.47		7	5 (MDL)	5 (MDL)	
Herbicide	Pendimethalin	0				1	51	51	51
Herbicide	Triclopyr	20	0.004 (MQL)	0.065		0			
Insecticide	Acetamiprid	4	0.0006 (MDL)	0.0006 (MDL)		0			
Insecticide	Clothianidin	14	0.002 (MDL)	0.006 (MQL)		0			
Insecticide	Flonicamid	4	0.005 (MQL)	0.014		0			
Insecticide	Flupyradifurone	8	0.2 (MQL)	0.014		0			
Insecticide	Imidacloprid	13	0.002 (MQL)	0.012		0			
Fungicide	2-phenylphenol	0				3	5 (MDL)	20 (MQL)	
Fungicide	Azoxystrobin	0				20	5 (MDL)	35	

Type	Pesticide	Detection in stormwater (ppb)				Detection in biofilm (ppb)			
		# of detections out of 21	Min	Max	Mean (± standard deviation)	# of detections out of 21	Min	Max	Mean (± standard deviation)
Fungicide	Carbendazim	7	0.1 (MDL)	0.52		5	10 (MDL)	30 (MQL)	
Fungicide	Fenbuconazole	2	0.1 (MDL)	0.1 (MDL)		0			
Fungicide	Imazalil	0				2	5 (MDL)	5 (MDL)	
Fungicide	Tebuconazole	1	0.1 (MDL)	0.1 (MDL)		4	20 (MQL)	29	
Fungicide	Thiabendazole	0				17	4 (MDL)	10 (MQL)	
Fungicide	Thiophanate-methyl	0				1	20 (MQL)	20 (MQL)	
Other	Chlorate	8	1 (MDL)	150		0			
Other	2-Hydroxyethylphosphonic acid (HEPA)	0				3	130	710	483 (±310)
Other	Perchlorate	5	0.8 (MDL)	2 (MQL)		0			
Other	Melamine	0				1	63	63	63
Other	Nicotine	0				11	5 (MDL)	67	

Fewer pesticides were detected in biofilm (n = 16) than in stormwater samples (n = 23). This contradicts the findings of Rooney et al. (2020) who detected more pesticides in their biofilm samples (n = 20) than in water samples (n = 9). This contradiction could be explained by the contrasting catchment types, as Rooney et al. (2020) examined an area impacted by agricultural runoff where water moving from fields, through tile drainage, and into ditches, likely accumulates organic matter and transports hydrophobic chemicals that can then adsorb onto biofilms. In the catchments of the Brampton stormwater ponds, stormwater flows rapidly over impervious surfaces like roads, and may be moving primarily water-soluble chemicals that are less likely to adsorb onto biofilms. Application patterns could also play a role: if fewer pesticides with low solubility, for example, are applied in urban areas than in agricultural lands, fewer would be available to be transported into stormwater ponds. Additionally, contrasting limits of detections for the analysis of pesticides in water samples, compared with biofilm samples, may obscure the actual proportion of pesticide occurrences in each matrix type.

Figures 3-2A and B illustrate the distribution of pesticide detections in each matrix by pesticide type. A greater number of herbicides were detected in water (n = 13) than in biofilm (n = 6), while the opposite was true for fungicides (n = 7 in biofilm versus n = 3 in water; Table B-2). Five insecticides were detected in water samples, while none were detected in biofilm samples. We identified five other biocides in total, 2 in water samples and 3 in biofilm samples.

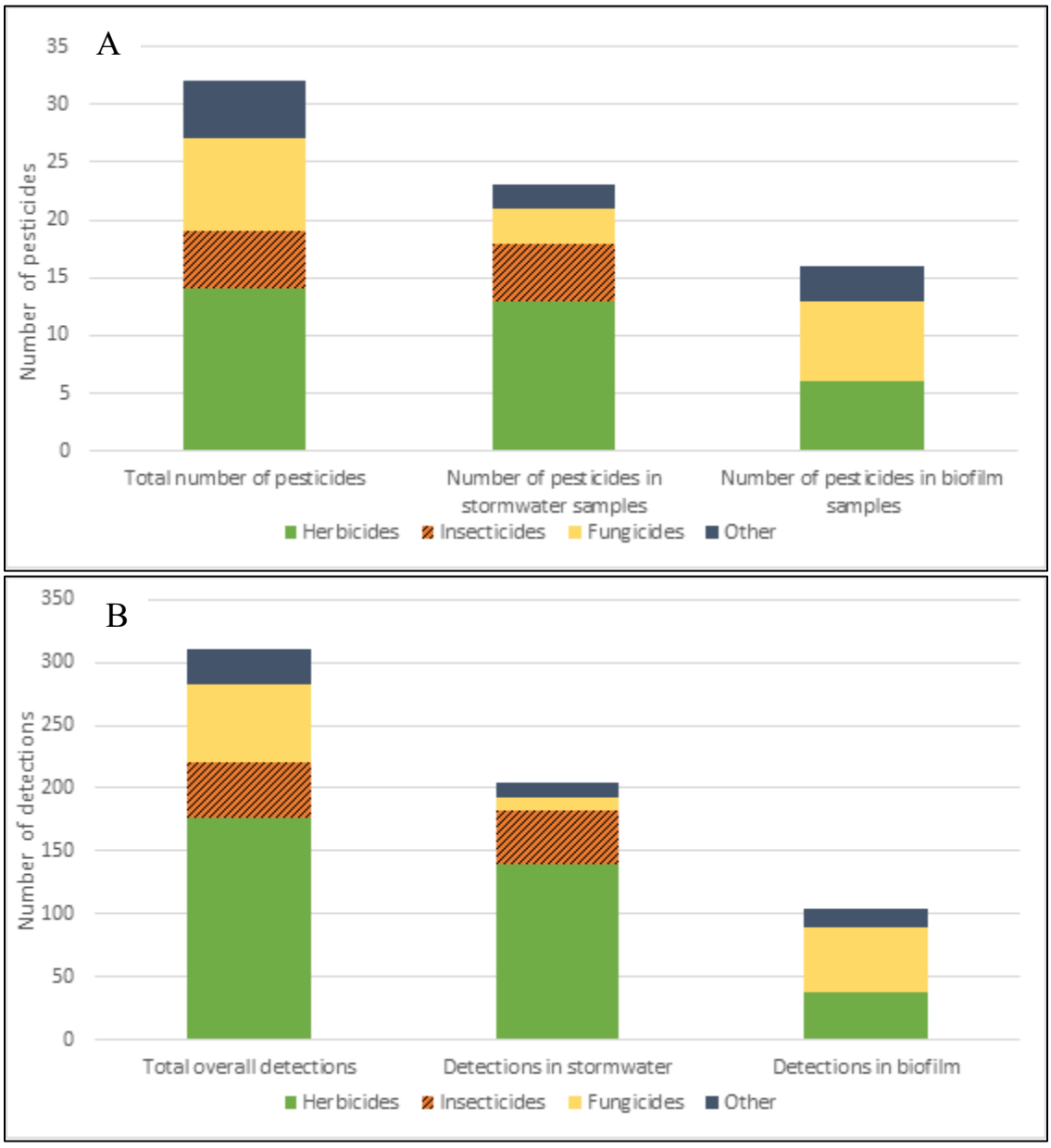


Figure 3-2. (A) Number of different pesticides detected in stormwater samples (n = 21) and biofilm samples (n = 21). (B) Number of pesticide detections in stormwater and biofilm samples across 21 stormwater pond sites.

Despite differing sampling approaches and suites of pesticide analytes, our herbicide detections were comparable to the only other known publication to report pesticide presence in water samples in Ontario's urban stormwater ponds. Of the 35 herbicides and insecticides analyzed, Struger et al. (1994)

similarly reported a prevalence of phenoxy acid herbicides, but also a collection of pesticides analyzed but not found in our samples (e.g., metolachlor, diazinon, and trifluralin). Pesticide-use profiles have certainly changed since 1994 because of since-introduced legislation (e.g., the use of pesticides for cosmetic landscaping purposes was banned in 2009 in Ontario under the Pesticides Act, R.S.O., 1990, c. P.11) as well as an increase in public awareness of environmental concerns from pesticide contamination (Arya, 2005; Cole et al., 2011). However, despite Ontario's current cosmetic pesticide-use ban, pesticide application is widespread in urban areas, including in turf management, in construction materials, in pest controls, municipal lands (e.g., right-of-ways), and in other non-cosmetic applications (Pesticides Act, R.S.O., 1990, c. P.11). Additionally, pesticide products used in urban applications may have different formulations but contain the same active ingredients as products restricted to agricultural uses. This could explain the commonalities between the pesticides we detected and those commonly reported in studies based in agriculturally dominated landscapes, even if the urban-use products contain lower concentrations of the active ingredient or different adjuvants than their agricultural counterparts. For example, in an agriculturally impacted coastal marsh, Rooney et al. (2020) found a similar dominance of herbicides in their water samples, compared to our urban samples. However, these two land use types were distinguished by clear differences in the ratios of insecticides and fungicides between water and biofilm samples. This pattern illustrates two contrasting pesticide use and fate profiles that are important for characterizing pesticide contamination across landscapes.

We also grouped pesticides based on their registration status in Canada, shown in Table 3-3, with most of the pesticides we detected (26 out of 32, or 81%) falling into the current-use category, one historic-use pesticide (2,4,5-T), and the remaining five with either unlisted (imazalil, HEPA, and perchlorate) or non-pesticidal uses (melamine and nicotine). Historic-use pesticides, such as DDT, are no longer permitted for use in the province. Although historic-use pesticides have been reported in contemporary samples of water (Raby et al., 2022) and biofilms (Rooney et al., 2020) collected from agriculturally impacted environments, we did not expect to find many historic-use pesticides in our stormwater ponds. Although Brampton is surrounded to its north and west sides by agricultural lands, the majority of stormwater contributed to stormwater ponds is delivered via the underground stormwater system in its sewer shed and so stormwater entering the ponds has not typically passed over agricultural lands where it could pick up sediments contaminated with historic-use pesticides. The category containing compounds with unlisted or non-pesticidal uses could be associated with chemicals unique to or more prevalent in urban areas; for example, melamine is found as a component of many common household

products (e.g., kitchenware, furniture, and cleaning products), as a nitrogen-based flame retardant, and in the manufacturing of plastics (PubChem). Importantly, pesticides in the unlisted or non-pesticidal use categories were detected more frequently in biofilm than in stormwater, necessitating the inclusion of biofilm sampling in urban monitoring to provide comprehensive assessment of contaminants.

Table 3-3. Chemical properties and other notable characteristics of pesticides detected in water and biofilm samples collected from urban stormwater ponds. CAS number, chemical class, chemical properties, and mode of action for each pesticide were obtained from the Pesticide Properties Database, PubChem online database, and ChemSpider online database. Current registration derived from Health Canada's Pesticide Product Information Database.

Type	Compound	Registration status	CAS	Class	Log K_{ow}	Log K_{oc}	Water solubility (mg L^{-1})	Mode of action
Herbicide	2,4-D	Current	94-75-7	Chlorophenoxy	2.81	1.59	24300	Systemic. Synthetic auxin.
Herbicide	2,4-DP	Current	120-36-5	Chlorophenoxy	3.43	1.87	350	Systemic. Synthetic auxin.
Herbicide	2,4,5-T	Historic	93-76-5	Chlorophenoxy	4	1.00	268	Systemic. Synthetic auxin.
Herbicide	Atrazine	Current	1912-24-9	Triazine	2.61	2.00	33	Systemic. Inhibition of photosystem II.
Herbicide	Bentazon	Current	25057-89-0	Thiadiazine	2.34	1.62	570	Systemic. Inhibition of photosystem II.
Herbicide	Chlorpropham	Current	101-21-3	Carbamate	3.51	2.67	89	Inhibition of mitosis.
Herbicide	Dicamba	Current	1918-00-9	Chlorophenoxy	2.21	1.13	23180	Systemic. Synthetic auxin.
Herbicide	Diuron	Current	330-54-1	Phenylurea	2.68	2.83	42	Systemic. Inhibition of photosynthesis.
Herbicide	Glufosinate	Current	51276-47-2	Organophosphate	0.1	2.63	1370000	Contact and systemic. Inhibition of glutamine synthetase and photosynthesis.
Herbicide	Imazapyr	Current	81334-34-1	Imidazolinone	0.22	0.94	11300	Systemic. Inhibition of plant amino acid synthesis.
Herbicide	MCPA	Current	94-74-6	Chlorophenoxy	3.25	1.75	250000	Systemic. Synthetic auxin.
Herbicide	MCPP	Current	7085-19-0	Chlorophenoxy	3.13	1.67	880	Systemic. Synthetic auxin.
Herbicide	Pendimethalin	Current	40487-42-1	Dinitroaniline	5.20	4.24	0.33	Inhibition of cell division and mitosis.
Herbicide	Triclopyr	Current	55335-06-3	Pyridine	-0.45	1.43	440	Systemic. Synthetic auxin.
Insecticide	Acetamiprid	Current	135410-20-7	Neonicotinoid	0.80	2.30	4250	Systemic. Modulation of nicotinic acetylcholine receptor (nAChR).
Insecticide	Clothianidin	Current	210880-92-5	Neonicotinoid	0.7	1.78	327	Systemic. Modulation of nAChR.
Insecticide	Flonicamid	Current	158062-67-0	Pyridine	0.30	1.40	5200	Systemic. Modulation of chordotonal organs.
Insecticide	Flupyradifurone	Current	951659-40-8	Pyridine	1.2	1.99	3200	Systemic. Modulation of nAChR.
Insecticide	Imidacloprid	Current	138261-41-3	Neonicotinoid.	0.57	2.68	610	Systemic. Modulation of nAChR.

Type	Compound	Registration status	CAS	Class	Log K_{ow}	Log K_{oc}	Water solubility (mg L^{-1})	Mode of action
Fungicide	2-phenylphenol	Current	90-43-7	Phenolic	3.09	3.83	700	Contact, broad spectrum.
Fungicide	Azoxystrobin	Current	131860-33-8	Strobilurin	2.50	2.77	6	Systemic. Inhibition of respiration.
Fungicide	Carbendazim	Current	10605-21-7	Carbamate	1.52	2.09	29	Systemic. Inhibition of cell division and mitosis.
Fungicide	Fenbuconazole	Current	114369-43-6	Triazole	3.79	3.37	2.47	Systemic. Inhibition of sterol biosynthesis.
Fungicide	Imazalil	Unlisted	35554-44-0	Imidazole	3.82	3.64	180	Systemic. Disruption of membrane function.
Fungicide	Tebuconazole	Current	107534-96-3	Triazole	3.70	3.01	36	Systemic. Disruption of membrane function. Inhibition of sterol biosynthesis inhibitor.
Fungicide	Thiabendazole	Current	148-79-8	Thiazole	2.47	3.60	50	Systemic. Inhibition of microtubule polymerization.
Fungicide	Thiophanate-methyl	Current	23564-05-8	Carbamate	1.40	2.52	26	Systemic. Inhibition of cell division and mitosis.
Other	Chlorate	Current	14866-68-3	Other	-4.63	1.54	1000000	Unknown
Other	2-Hydroxyethylphosphonic acid (HEPA)	Unlisted	22987-21-9	Organophosphate	-1.67	0.55	1000000	Unknown
Other	Perchlorate	Unlisted	14797-73-0	Other	-4.63	1.69	1000000	Unknown
Other	Melamine	Current (as biocide)	108-78-1	Triazine	-1.37	0.70	3.23	Hepatotoxin, nephrotoxin, possibly carcinogenic.
Other	Nicotine	Current (as non-prescription pharmaceutical)	54-11-5	Other	1.17	2.00	1000000	Neurotoxin.

3.4.1.1 Potential risks to biota

Although substantial concern surrounding contaminated stormwater centers on effects to downstream habitats (e.g., Carpenter et al., 2016), stormwater ponds are important hosts for urban biodiversity and should also be subjected to contaminant risk assessments (Rooney et al., 2015; McIsaac, 2022; Bradley et al., 2023). While pesticides were ubiquitous in our assessment of stormwater ponds, concentrations were generally low with most water and biofilm samples being below the limits of quantification. However, some pesticides can be toxic at very low levels (e.g., EC_{50} = 1.6 μg L^{-1} for *Neocloeon triangulifer* exposure to acetamiprid; Raby et al., 2018), thus we assessed whether the pesticides we observed in stormwater ponds could present a threat to aquatic biota.

Of the 16 compounds detected in biofilm samples, nine (56%) were not detected in stormwater samples, eight of which belong to either the fungicide or biocide category. Failure to capture these pesticides through water sampling methods suggests an underestimation of the toxicological burden experienced by aquatic biota and represents a critical gap in our understanding of pesticide contamination in ecosystems. Unfortunately, there remains a lack of available and appropriate dietary toxicity thresholds relevant to aquatic species. As such, it is difficult to estimate the proximity of quantified pesticide levels in biofilm to concentrations causing harm to the growth or survival of its consumers. Testing of dietary consumption of pesticide-contaminated biofilm is a novel ecotoxicological approach (Ijzerman et al., 2023), and thus we are unable to find suitable thresholds to which we can compare our pesticide concentrations and provide an estimation of risk. In Chapter 4, I further investigate the dietary consumption of contaminated biofilm by two common aquatic organisms. Hereafter, we discuss the potential risk to biota solely in the context of water exposure.

Many of the detected herbicides are synthetic auxins (see Table 3-3) meaning they regulate plant growth hormones. For example, 2,4-D causes dysregulation of ethylene production resulting in damaged vascular tissue in broad-leafed weeds such as dandelions (PPDB). These effects are not seen in narrow-leaf plants like grass, supporting its use in lawncare and turf management, and perhaps explaining its prevalence in stormwater ponds. This specificity also means that 2,4-D, along with most other synthetic auxins, have comparatively lower toxicity to algae: the 96-h NOEC for the algae *Chlorella vulgaris* is reported as 1×10^5 ppb, whereas the 7-day EC_{50} for the broadleaf vascular plant *Lemna gibba* is reported as 2700 ppb (PPDB). The maximum concentration of 2,4-D found in our samples was 0.87 ppb, and other synthetic auxins similarly fell substantially below reported toxicity thresholds. On the other hand, photosynthesis inhibitors were also observed in our stormwater pond samples. These affect both plants and algae as this mode of action hinders photosynthetic activity in most primary producers. The

remaining herbicides we detected included those with modes of action targeting mitosis and amino acid synthesis. Many of these herbicides fell below the MDL or MQL, and all observations were below reported thresholds for the protection of aquatic biota.

As a key part of an insects' central nervous system, the nicotinic acetylcholine receptor (nAChR) is targeted by many synthetic insecticides, including the majority of those detected in our samples (PPDB). Modulation of this neurotransmitter receptor is fairly specific to insects but may pose a risk to other aquatic organisms. The presence of neonicotinoids in stormwater is not surprising, given previous detections in Ontario urban and rural surface waters (Metcalfe et al., 2019; Raby et al., 2022), however measured concentrations were well below known toxicity thresholds. For example, the highest concentration of flonicamid was 0.014 ppb, which was considerably lower than the USEPA Aquatic Life Benchmark (ALB) for chronic exposure to freshwater invertebrates, 3000 ppb (USEPA). Imidacloprid, measured in our stormwater samples at 0.012 ppb, was below reported toxicity thresholds: the chronic NOEC for *Chironomus riparius* is 2.1 ppb (PPDB), and the CCME guideline for the protection of aquatic life is 0.23 ppb (CCME). However, this concentration was slightly higher than the USEPA ALB for chronic exposure to freshwater invertebrates, at 0.01 ppb. This was the only measured contaminant in stormwater samples to surpass a benchmark.

Carbendazim was the only fungicide that we detected above its quantification limit in stormwater samples. The maximum concentration we observed was 0.52 ppb. This is higher than reported by Metcalfe et al. (2016) in their surveys of urban surface waters (0.18 ppb) and yet it remains below the most sensitive threshold for aquatic biota, reported as 1.5 ppb for *Daphnia magna* (NOEC; PPDB). Other biocides detected in stormwater also fell below USEPA benchmarks and PPDB reported thresholds.

Concentrations of pesticides in the stormwater samples were low, never exceeding thresholds or guidelines for ecotoxicological risk to aquatic biota reported by the PPDB and CCME, with only one exceedance of a USEPA benchmark. However, these contaminants were always present as mixtures: a minimum of four pesticides were detected at each pond, and 57% of ponds had ten or more pesticides detected (See Appendix B for pesticide detections and concentrations in water samples). The mixtures included multiple pesticide types (herbicides, fungicides, insecticides) and multiple modes of action within a single type (e.g., synthetic auxins and photosynthesis inhibitors). Such complex mixtures may have greater toxicity to biota than predicted from any single pesticide as their toxicities may combine additively or synergistically (Hernández et al., 2013; Weisner et al., 2021). Consequently, despite the low

concentrations detected in our samples, the ubiquity of pesticides and the diversity of mixtures detected in our study raises concern for aquatic biota in stormwater ponds and emphasizes the need for more research on pesticide mixtures (Hernández et al., 2017). I further explore the characterization and potential consequences of urban stormwater contaminant mixtures in Chapter 5.

3.4.2 Relationship between occurrences of pesticides in each matrix and their physicochemical properties

We evaluated whether physicochemical properties of pesticides that are commonly invoked to understand their fate in aquatic environments (Table 3-3) were predictive of the number of detections of pesticides in stormwater and in biofilm from stormwater ponds using generalized linear models (Table 3-4). We preclude the presentation of these models by disclaiming the limited predictive ability of such an approach when using a relatively small sample size. We present these findings as preliminary and as a useful approach that future research with larger sample sizes can build upon to further clarify these relationships.

We found that water solubility did predict the number of pesticides detected in water samples (Figiure B-2B), but not in biofilm samples (Figure B-2A). The lack of relationship between water solubility and detection in biofilms could be due to the heterogeneous surface of biofilm, which provides diverse chemical sorption sites that contribute to its ability to accumulate a wide assortment of contaminants (Bonnineau et al., 2021). For example, although pesticides with lower water solubilities were indeed represented in biofilm samples, HEPA and nicotine, the two most soluble compounds detected across all sites with water solubilities over 1×10^6 mg L^{-1}, appeared exclusively in biofilm samples.

Table 3-4. Results of generalized linear models with gamma distributions for pesticide physicochemical properties (water solubility, Log K_{ow}, and Log K_{oc}) with the number of pesticide detections in water and in biofilm samples.

Predictor	Matrix of response variable	Dispersion parameter	Residual deviance & d.f.	Null deviance & d.f.	AIC value	Coefficient	p-value
Water solubility	Water	1	256.00 on 30 d.f.	237.74 on 31 d.f.	344.41	-5.822×10^{-7}	0.0102
Water solubility	Biofilm	1	236.65 on 30 d.f.	237.74 on 31 d.f.	292.37	-2.808×10^{-7}	0.315
Log K_{ow}	Water	1	263.85 on 30 d.f.	263.85 on 31 d.f.	352.26	-0.0007	0.982
Log K_{ow}	Biofilm	1	221.83 on 30 d.f.	237.74 on 31 d.f.	277.55	0.205	0.0002
Log K_{oc}	Water	1	227.78 on 30 d.f.	263.85 on 31 d.f.	316.18	-0.494	9.27×10^{-9}
Log K_{oc}	Biofilm	1	214.12 on 30 d.f.	237.74 on 31 d.f.	269.83	0.498	9.3×10^{-7}
Combined	Water	1	210.54 on 28 d.f.	263.85 on 31 d.f.	302.94	-6.813×10^{-7} (water solubility); 0.05019 (Log K_{ow}); -0.5998 (Log K_{oc})	0.0133 (water solubility); 0.2617 (Log K_{ow}); 5.12×10^{-10} (Log K_{oc})
Combined	Biofilm	1	210.90 on 28 d.f.	237.74 on 31 d.f.	270.61	3.592×10^{-7} (water solubility); 0.1335 (Log K_{ow}); 0.3621 (Log K_{oc})	0.279 (water solubility); 0.0778 (Log K_{ow}); 0.00409 (Log K_{oc})

The octanol-water partition coefficient (K_{ow}) indicates the ratio of a chemical's concentration in the non-polar octanol phase to its concentration in the polar aqueous phase of an octanol-water two phase system. Log K_{ow} was predictive of the number of pesticides detected in biofilm (Figure B-2C), but not in water (Figure B-2D). The Log K_{ow} is a metric of hydrophobicity and is often used to calculate the accumulation of a chemical into living biological material as a bioconcentration factor (e.g., Mackay and Fraser, 2000). Thus, we expected that pesticides with higher Log K_{ow} would more readily be accumulated out of the water phase and onto or into the living biofilms.

The soil adsorption coefficient (K_{oc}) is the ratio of a chemical's concentration in a soil or sediment phase to its concentration in a water phase. In our context, this coefficient represents the adsorption of pesticides onto the organic matter contained within biofilms, both intra- and extracellularly. We found that Log K_{oc} predicted the number of detections in both biofilm (Figure 3-3B) and water (Figure B-2E). We expected that pesticides with higher Log K_{oc} values may bind to organic-rich materials more strongly and thus have elevated occurrences in biofilms, while pesticides with lower Log K_{oc} values and weaker bonds with organics should be more dissolved and mobile in the water.

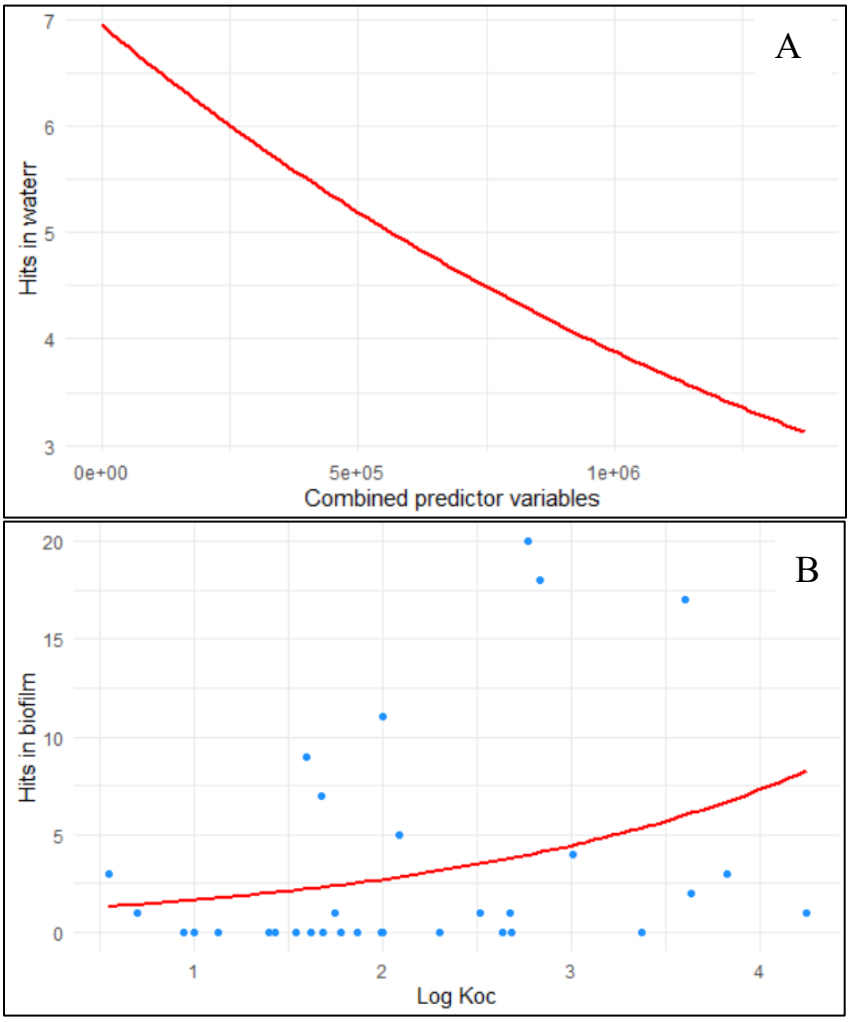


Figure 3-3. Generalized linear models that best predict the number of pesticides detected in water and biofilm samples. A combined model using water solubility, Log K_{ow} and Log K_{oc} best predicted the number of pesticides detected in water (A), while of the number of pesticide detections in biofilm was best predicted by Log K_{oc} (B).

Additionally, we tested combined models using all three variables as predictors of pesticide occurrences in stormwater (Figure 3-3A) and in biofilm (Figure B-2F). The model for detections in water and the model for detections in biofilm samples had delta-AIC values of 13.24 and 0.78, respectively (raw AIC values shown in Table 3-4). This indicates that the number of pesticide detections in water is better predicted by a combined model than by individual pesticide properties, but that the number of pesticides detected in biofilm is not better predicted by the combination of three pesticide properties than it was by Log K_{oc} alone.

3.4.3 Bioconcentration factors

3.4.3.1 Calculated and estimated bioconcentration factors

All BCF_{DW} values, both calculated and estimated, as well as averages for calculated BCF_{DW}s and residuals for the pesticides with multiple calculated BCF_{DW}s, are reported in Table 3-5. Calculated BCF_{DW}s spanned a wide range (4.2–1275). Of the 42 calculated and estimated BCF_{DW}s, 10 were attributed to herbicides, 18 to fungicides, and 14 to other biocides. No insecticides were found to have bioconcentrated in the biofilm, though this seems unrelated to physicochemical characteristics (Section 3.4.2). Bioconcentration factors (BCF_{DW}s) are valuable indices for comparing relative concentrations of pesticides in two matrices and for quantifying accumulation from ambient water. Recent studies have quantified the magnitudes of pesticide accumulation in biofilm (e.g., Mahler et al., 2020; Rheinheimer dos Santos et al., 2020), however only two other studies explored a similarly large set of pesticides and calculated bioconcentration factors (BCF_{DW}s; Rooney et al., 2020; Ijzerman et al., 2023). Our study is the first to document pesticide BCF_{DW}s in biofilm in a non-agricultural context.

Table 3-5. Calculated and estimated bioconcentration factors (BCF_{DW}s) of pesticides quantified in biofilms cultivated in stormwater ponds.

Pesticide	Type	BCF_{DW}s	BCF_{DW} type	Average calculated BCF_{DW}	Residual BCF_{DW}
2,4-D	Herbicide	12.2	Calculated	9.5 (±3.33)	-2.7
		10.4	Calculated		-1.0
		5.8	Calculated		3.7
MCPA	Herbicide	4.2	Calculated	4.2	
Chlorpropham	Herbicide	165.0	Calculated	165.0	
Pendimethalin	Herbicide	1275.0	Calculated	1275.0	
Diuron	Herbicide	27.5	Estimated	38.3	
		60.0	Estimated		
		75.0	Estimated		
		38.3	Calculated		
Azoxystrobin	Fungicide	166.7	Estimated	329.6 (±75.64)	
		155.6	Estimated		
		177.8	Estimated		
		144.4	Estimated		-59.3
		388.9	Calculated		
		133.3	Estimated		85.2
		244.4	Calculated		-25.9

Pesticide	Type	BCF_{DW}s	BCF_{DW} type	Average calculated BCF_{DW}	Residual BCF_{DW}
		355.6	Calculated		
		177.8	Estimated		
Tebuconazole	Fungicide	160.0	Estimated	290.0	
		290.0	Calculated		
		130.0	Estimated		
		120.0	Estimated		
Carbendazim	Fungicide	180.0	Estimated	19.2	
		19.2	Calculated		
Thiabendazole	Fungicide	257.1	Estimated	N/A	
Thiophanate-methyl	Fungicide	280.0	Estimated	N/A	
2-phenylphenol	Fungicide	80.0	Estimated	N/A	
HEPA	Other	130.0	Calculated	483.3 (±310.05)	353.3
		710.0	Calculated		-226.7
		610.0	Calculated		-126.7
Melamine	Other	70.0	Calculated	70.0	
Nicotine	Other	50.0	Estimated	513.3 (±140.12)	
		400.0	Calculated		113.3
		670.0	Calculated		-156.7
		150.0	Estimated		
		160.0	Estimated		
		470.0	Calculated		43.3
		83.0	Estimated		
		200.0	Estimated		
		110.0	Estimated		
		50.0	Estimated		

The BCF values reported in pesticide databases (such as the PPDB) for pesticides in aquatic ecosystems are typically based on fish. Although pesticide physicochemical properties are thought to dictate pesticide accumulation in aquatic organisms (Mackay and Fraser, 2000; Katagi, 2010), the diversity in characteristics of the biofilm surface and EPS matrix that orchestrate sorption and binding of pesticides (Lundqvist et al., 2012) could result in differences from fish BCFs. Also, whether reported BCF values were calculated from wet or dry biological material is not always indicated. However, these indices are still useful for contextualizing our reported BCF_{DW} values. While a few differences exist, we observed surprising agreement in the order of magnitudes of our reported BCF_{DW} values and those reported by pesticide databases and by other scientific studies (e.g., Rooney et al., 2020). Given the

variability in exposure levels, water quality, and the composition of biological tissues being considered by these information sources, we propose that only differences in the order of magnitude of BCF values are meaningful.

Our results generally agreed with those of Rooney et al. (2020), who also found that biofilm accumulates a wide range of pesticides from the water of coastal marshes, however our reported BCF_{DW} values were more consistent with those recorded for pesticides in the PPDB. For example, for 2,4-D, the PPDB lists a BCF in fish of 10, consistent with our average BCF_{DW} of 9.5 (± 3.33), yet lower than reported by Rooney et al. (2020), who calculated a BCF_{DW} of 42 in freshwater periphyton. Similarly, our lowest recorded BCF_{DW}, 4.2 for MCPA, was comparable to the PPDB reporting of 1 in fish, but again differs substantially from the BCF_{DW} of 2459.2 reported by Rooney et al. (2020). Atrazine was detected in 48% of stormwater samples but did not accumulate in our biofilm samples, also contradicting accumulation in periphyton found by Rooney et al. (2020); BCF_{DW} = 112 - 350 and Nikkilä et al. (2001; BCF = 215–350). Our samples were not filtered prior to analysis, and because stormwater systems are known to have relatively higher suspended sediment loads, it is possible that some of the pesticides measured in our stormwater samples were associated with particulates, which may have contributed to the differences seen among studies in different environments. Additionally,these differences could be driven by other environmental factors, such as catchment type or water quality, however more investigation is needed to understand the mechanisms of pesticide accumulation before we can determine the influence of external conditions.

No literature was found to measure the BCF of chlorpropham, but the PPDB listing for fish (144) was comparable to our calculation (165). A geometric mean BCF of 1878 for pendimethalin in aquatic organisms determined by Vighi et al. (2017) is likely a more appropriate comparison point than the BCF reported for fish in the PPDB, 5100, although both are within the same order of magnitude as our calculation of 1275. Diuron was measured to be bioconcentrated at a factor of 2 in freshwater fish by Call et al. (1987) and is reported by the PPDB as 9.45 in fish, both lower than our calculated BCF_{DW} of 38.3.

Similarly, BCFs for fungicides reported by the PPDB and most accessible literature (e.g., Andreu-Sánchez et al., 2012; Ju et al., 2019) measured accumulation in either fish or crop plants, the exception being those reported in periphyton by Rooney et al. (2020). Calculated BCFs for azoxystrobin and tebuconazole (688.0 and 712.9, respectively) by these researchers were higher yet within the same order of magnitude as our BCF_{DW} calculations (329.6 and 290.0, respectively). This discrepancy (as with

herbicidal BCFs) is possibly due to higher loads of agricultural pesticides delivered to their sampling environment. This explanation is substantiated by Ding et al. (2019), who described a wide range of cellular accumulation of carbendazim (BCFs = 12.1–1336.2) in dried algal samples when exposed to a range of concentrations over the span of 144 h. The relationship between cellular accumulation of carbendazim and initial exposure concentrations in Ding et al. (2019) may explain variations in periphyton or biofilm accumulation in environments with different exposures to pesticide loads. Both our calculated carbendazim BCF_{DW} (19.2), and that reported by the PPDB (25), fell into the range given by Ding et al. (2019). Thiabendazole and thiophanate-methyl had estimated BCF_{DW}s (257.1 and 280.0, respectively) higher than those reported by the PPDB for fish (96.5 and 75, respectively). No relevant BCFs for the remaining biocides were found in literature, although the PPDB reports a BCF of 21.7 (for an unknown organism) for 2-phenylphenol, which we estimated at 80.0 in our biofilm samples.

3.4.4 Chemical and environmental drivers of bioconcentration factors

3.4.4.1 Bioconcentration factors and pesticide physicochemical properties

We used generalized linear models to investigate how pesticide physicochemical properties influenced the magnitude of pesticide bioconcentration, the results of which are presented in Table 3-6. We did not find calculated BCF_{DW} values to be significantly predicted by water solubility (Figure B-3A), Log K_{ow} (Figure B-3B), or Log K_{oc} (Figure B-3C) alone. A model combining these three properties yielded a better fit (delta AIC = 3.74; Figure 3-4) than any of the three properties modelled alone, but within this best fitting model, only water solubility had a p-value below 0.05 (Table 3-6). This reveals that the three physicochemical properties were not good predictors of pesticide BCF_{DW}s in stormwater pond biofilms.

Table 3-6. Results of generalized linear models for analysis of pesticide physicochemical properties as predictors of bioconcentration factors.

Predictor	Dispersion parameter	Residual deviance	Null deviance	AIC value	Coefficient	p-value
Water solubility	2.304	22.832 on 9 d.f.	23.444 on 10 d.f.	150.08	5.539×10^{-7}	0.656
Log K_{ow}	1.507	20.403 on 9 d.f.	23.444 on 10 d.f.	148.51	0.1921	0.267
Log K_{oc}	1.455	21.230 on 9 d.f.	23.444 on 10 d.f.	149.06	0.3048	0.355
Combined	1.093	11.376 on 7 d.f.	23.444 on 10 d.f.	144.77	2.404×10^{-6} (water solubility); 0.7505 (Log K_{ow});	0.0369 (water solubility); 0.0751 (Log K_{ow});

-0.5317 (Log K_{oc}) 0.482 (Log K_{oc})

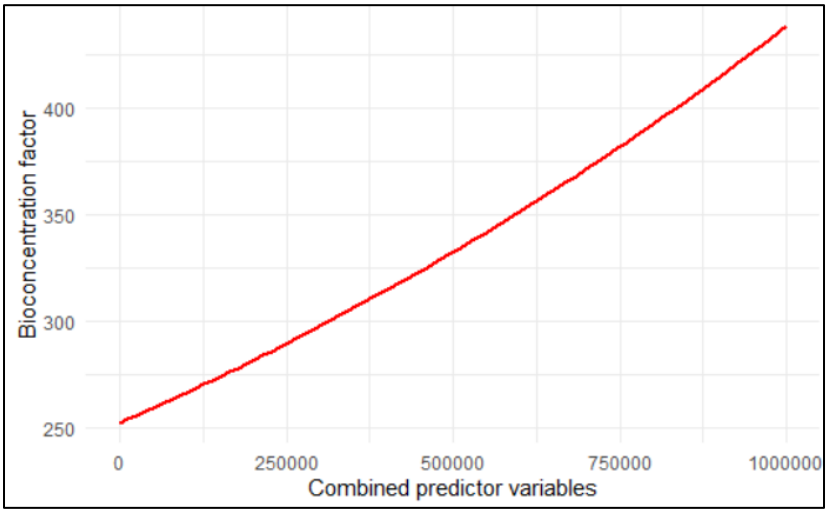


Figure 3-4. A combined generalized linear model using water solubility, Log K_{ow} and Log K_{oc} as predictors of the dry-weight bioconcentration factor of pesticides.

Although Lundqvist et al. (2012) and Nikkilä et al. (2001) found hydrophobicity was related to pesticide accumulation, Rooney et al. (2020) did not discern any strong physicochemical drivers of pesticide BCF_{DW}s in periphyton. Our generalized linear regressions (Figure B-3) agree with the findings of Rooney et al. (2020). From this, we conclude that BCF_{DW} values for pesticides accumulating in biofilms cannot reliably be estimated from physicochemical properties and that validation of pesticide BCF_{DW}s from field-based biofilm monitoring is required. Biofilm sampling is thus an important addition to contaminant monitoring.

3.4.4.2 Bioconcentration factors and other parameters

As pesticide properties were not the main drivers of pesticide accumulation in our biofilm samples, we explored whether characteristics of the environment might better predict BCF_{DW} variance across the stormwater ponds. The results of the generalized linear models are presented in Table 3-7.

Table 3-7. Results of generalized linear models with gamma distributions with water quality index (WQI), ash-free dry weight (AFDW) of biofilm, and chlorophyll-*a* content of biofilm as predictors of calculated bioconcentration factors (BCF_{DW}) of pesticides.

Predictor	Dispersion parameter	Residual deviance	Null deviance	AIC value	Coefficient	p-value
WQI	1.211	36.511 on 17 d.f.	36.587 on 18 d.f.	259.22	0.007	0.768
AFDW	1.357	36.281 on 17 d.f.	36.587 on 18 d.f.	259.07	0.195	0.586
Chlorophyll-*a* content	1.122	30.627 on 17 d.f.	36.587 on 18 d.f.	255.07	1.717	0.024

We did not find a significant relationship between calculated BCF_{DW} values, and the Water Quality Index (WQI) scores calculated for each pond site (Figure B-4). Some BCF_{DW}s varied widely, such as those calculated for HEPA, which ranged from 130 to 710. We had predicted that these observed variations could be features of the surrounding water quality and its impact on biofilm function and growth rate, resulting in more or less bioconcentration than expected. Deteriorated water quality could shift community composition (Tien et al., 2013) and pesticide tolerances (Larras et al., 2013), potentially leading to changes in accumulation capacities, and factors not included in the WQI score, such as artificial light pollution, could also alter community composition.

We also did not identify a significant relationship between BCF_{DW}s and ash-free dry weight (AFDW; Figure B-5), suggesting there was not a dilution effect (i.e., lower pesticide concentration with higher biofilm mass). We also used ash-free dry weight as a proxy for biofilm growth rate since all biofilm samplers had equivalent incubation periods at each site, and therefore biofilm samplers with higher ash-free dry weights must have grown at a faster rate. If there was a constant rate of pesticide uptake into biofilms, we would expect to see a negative linear relationship between BCF_{DW} values and ash-free dry weight. The lack of such a relationship implies that the rate of pesticide accumulation is variable but unrelated to the rate of biofilm growth.

We did find a statistically significant relationship between calculated BCF_{DW} values and the chlorophyll-*a* concentration measured in the biofilm, shown in Figure 3-5. A greater proportion of fungicides (n = 18) were accumulated relative to herbicides (n = 10), and at higher magnitudes (Table 3-5). This pattern could explain a tendency towards autotrophy and thus higher content of photosynthetic compounds in biofilms with higher BCF_{DW}s and could suggest that the community composition of a biofilm influences the capacity for pesticide accumulation. However, an in-depth assessment of microbial diversity is required to understand this relationship, and as such, is recommended here only as an avenue for future research.

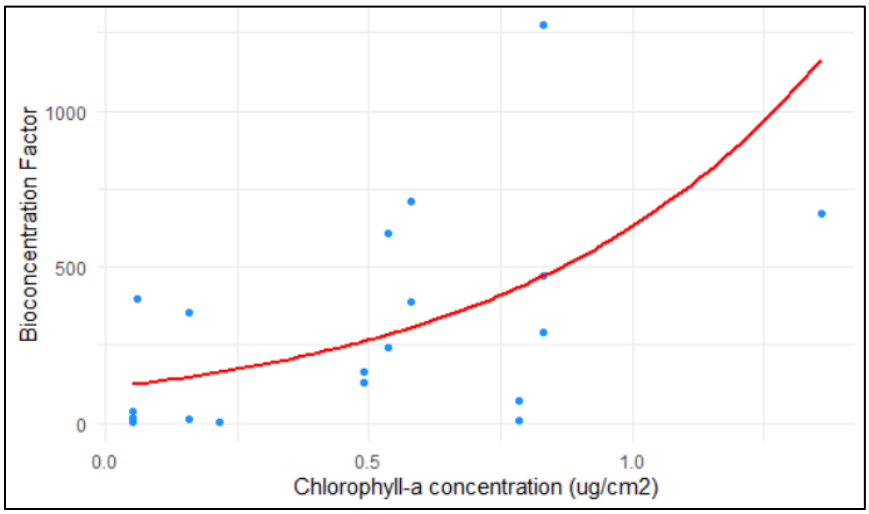


Figure 3-5. Chlorophyll-*a* concentration (µg cm^{-2}) in biofilm and calculated pesticide BCF_{DW} values (n = 17; p = 0.024) in a generalized linear model.

Our inability to find the expected drivers of accumulation as good predictors of pesticide BCF_{DW}s is likely due to an insufficient understanding of the mechanisms behind pesticide accumulation in biofilm and thus an incorrect selection of predictor variables. This is still an underrepresented area of research, frequently focusing on a small number of compounds in a controlled environment (e.g., Lundqvist et al., 2012; Chaumet et al., 2019a) and is often limited by technical constraints (e.g., in the quantification of contaminants within the EPS vs intracellularly; Bonnineau et al., 2021). The duration of pesticide exposure (Bonnineau et al., 2021), repartition dynamics between different environmental matrices (Headley et al., 1998), chemical transformation within the biofilms (Carles et al., 2019), and species composition (Lawrence et al., 2001; Berglund et al., 2005) are examples of plausible interacting drivers of pesticide accumulation as identified by other researchers. Yet, disentangling the vast assortment of possible mechanisms driving our calculated BCF_{DW}s was not feasible in our field-based experiment. Manipulating the conditions in which a biofilm grows and measuring the responding levels of accumulation of a single pesticide could provide valuable insight into the environmental influences of contaminant-biofilm interactions. For example, in the laboratory, Beecraft and Rooney (2021) found that the BCF_{DW} of glyphosate in biofilm was dependent on the concentration added, with higher BCF_{DW}s in lower concentrations, suggesting a saturation effect. This was not a relationship we were able to explore because only one pesticide (2,4-D) had both quantifiable concentrations in water samples and multiple calculated BCF_{DW} values. Another caveat of our study was our small sample size (n = 17) as we only

conducted analyses of possible relationships using our calculated BCF_{DW} values (not our estimated BCF_{DW} values). Future research conducting analyses on a much larger data set could help to better assess relationships between BCF_{DW}s and pesticide chemical properties, biofilm characteristics, and surrounding water quality in the real world. Finally, our study focussed on samples collected during a single field season, meaning that variations attributed to pesticide use and climatic patterns, which may change the contaminant profiles and environmental conditions year-by-year, were not captured and may have contributed to the unexplained results. This is also an important avenue for future research.

3.5 Conclusion and implications for future work

Our study is the first in over 25 years to identify and quantify pesticide contamination in stormwater ponds in Ontario, and the first since key pieces of legislation were put in place to reduce the use of neonicotinoids (OMECP, 2015) and the cosmetic use of pesticides (OMECP, 2014). Although known toxicity thresholds for pesticide exposure in water were not exceeded by levels found in the stormwater ponds, the risks to biota from exposure to complex mixtures and to contaminated biofilm are unknown. The implications of the observed diversity in modes of action for organisms living in these environments is an important area for future research. Our study is also one of the few to report pesticide bioconcentration in biofilm collected from the field. Pesticides, particularly current-use fungicides, were common in biofilms and several pesticides that were present in biofilms were not detected in the water samples, revealing that a comprehensive assessment of pesticide contamination in stormwater ponds really requires biofilm monitoring. The distribution of pesticides in water and biofilm was related to select physicochemical properties, with the Log K_{oc} being a strong predictor in both matrices. Our calculated BCF_{DW}s indicate pesticide accumulation can vary and may not be predicted by pesticide physicochemical properties or environmental factors, highlighting the need for more research on accumulation mechanisms. The use of biofilm sampling in contaminant monitoring can clearly provide more sensitivity than water sampling in pesticide monitoring programs, especially for chemicals shown to have high BCF_{DW} values. Water sampling may overlook pesticide risks, as even when pesticides are below detection limits in water samples, biota can still be exposed via consumption of biofilms. This represents an underexplored exposure pathway, further investigated in Chapter 4, and we likewise urge other researchers to consider the potential risks of chronic dietary consumption of pesticides when evaluating toxicological risks and setting ecological thresholds. Additionally, stormwater ponds could be mobilizing contaminants into other urban food webs, as many pond dwellers such as benthic macroinvertebrates and

birds act as bridges between aquatic and terrestrial ecosystems. Future work incorporating ecological assessments of these communities could help illustrate the breadth of stormwater contamination.

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Chapter 4: Dietary exposure of stormwater contaminants in biofilm to two freshwater macroinvertebrates

4.1 Overview

Aquatic habitats in urban environments are exposed to complex contaminant mixtures that may harm aquatic biota. The impact of contaminant transfer from contaminated biofilm through aquatic food webs is still understudied, as is the current state of knowledge on dietary exposure of urban contaminants to biota residing in stormwater ponds. Our overall objective was to characterize urban pesticide accumulation in a common aquatic food source (biofilm) in stormwater ponds and to investigate the potential toxicity of that food source by testing the responses of two freshwater macroinvertebrates to experimental exposure. We conducted two dietary bioassays using biofilm collected from 15 stormwater ponds in Brampton, Ontario: an acute exposure with the mayfly *Neocloeon triangulifer*, and a chronic exposure with the freshwater snail *Planorbella pilsbryi*. We screened for 542 current-use and legacy pesticides to measure pesticide burden (the number of pesticides detected) and the concentration of pesticides in the biofilm. We also quantified chlorophyll-*a*, pheophytin, and ash-free dry weight content which we used as indicators of biofilm quality. We found no correlations between pesticide burden and chlorophyll-*a*, pheophytin, or ash-free dry weight of the biofilm diets. Compared to control diets, biofilms collected from stormwater ponds caused a reduction in survival and growth endpoints for both test species, indicating that biofilm-consuming invertebrates living in stormwater ponds may be experiencing risks previously unaccounted for by traditional ecological risk assessments. Pesticide occurrences in biofilm diets did not relate to mayfly survival, growth, or biomass production, nor were they related to snail growth. This suggests that other contaminants in stormwater-cultivated biofilm are contributing to the observed effects. Snail survival and biomass production were negatively related to pesticide burden in the diets. This implies that duration of exposure may influence the degree and manifestation of pesticide toxicity via dietary exposure.

4.2 Introduction

As shown in previous chapters of this thesis, urban stormwaters are persistently laden with complex cocktails of contaminants (see also Masoner et al., 2019; Pamuru et al., 2022). Yet, stormwater management ponds, the end-of-pipe reservoirs for stormwater, are infrequently monitored for contaminants (Waara and Johansson, 2022; Tixier et al., 2023) and are generally managed as infrastructure (Stephansen et al., 2016; Hale et al., 2019). Often, their role as aquatic habitat for urban

wildlife is overlooked (Hassall and Anderson, 2015; Hill et al., 2017; McIsaac, 2022; McIsaac et al., 2024). With the swift and extensive replacement of natural wetland habitats by stormwater ponds (Birch et al., 2022), evaluating the toxicological risks faced by pond biota is critical for conserving urban biodiversity (Oertli and Parris, 2019).

Among the urban contaminants known to pollute stormwater, pesticides present a serious threat to wildlife due to their targeted and non-targeted effects on microbial, plant, invertebrate, and vertebrate species and their ubiquity in surface waters (Anderson et al., 2021; Raby et al., 2022). Previous pesticide toxicity experiments suggest that benthic macroinvertebrates may be particularly sensitive to pesticide exposure (e.g., Siegfried, 1993; Weston and Lydy, 2014). Due to the flashy hydrology typical of stormwater runoff and variations in pesticide application patterns, pesticide concentrations in urban areas are highly dynamic (Phillips and Bode, 2004; Rippy et al., 2017) and are often present as complex mixtures (Meftaul et al., 2020; see also Chapter 2 and 3) and in conjunction with other contaminants (e.g., metals; Zgheib et al., 2012). Laboratory-based toxicity testing often involves only single-compound exposures, and even mixture experiments rarely consider more than three pesticides at a time (e.g., Hasenbein et al., 2015; Wang et al., 2017). There are few tests of the toxicological risk from exposure to environmentally relevant mixtures (Rodney et al., 2013) and estimating the risk of single chemical exposures individually is often insufficient due to the potential for cumulative effects and additive or synergistic interactions (Côté et al., 2016).

Immersion in a solution containing pesticides is the dominant route of contaminant exposure in toxicity testing and bioassays involving aquatic biota (Ashauer et al., 2012), and there is limited knowledge about the sensitivity of invertebrates to dietary exposure pathways and the potential trophic transfer of pesticides within aquatic food webs. Because biofilm is an important food source for many aquatic organisms (Hall Jr and Meyer, 1998) and recent research has shown that biofilm can accumulate a wide array of contaminants (e.g., Bonnineau et al., 2021), including pesticides (e.g., Rooney et al., 2020; Ijzerman et al., 2024; Izma et al., 2024 [Chapter 3]), it matters that we do not know the toxicity risk of dietary exposure routes. This is particularly crucial in hydrologically dynamic environments, such as stormwater ponds, where biofilms may lengthen the exposure duration, and thus the persistence, of pesticides beyond what would be expected from immersion-based exposures.

We are particularly interested in the potential effects of contaminant accumulation in biofilms on the biofilm-invertebrate grazer relationship because of the importance of macroinvertebrates in food

webs, e.g., as fish food (Curtis et al., 2018), their connections between aquatic and terrestrial systems through emergence and contaminant transfer (Blais et al., 2007), and because they are sensitive indicators commonly used to assess ecological condition (Rosenberg and Resh, 1992). Exposure of aquatic invertebrates to mixtures of urban contaminants in stormwater may affect these organisms directly, through toxic effects on organism function or fitness. However, exposure may also affect aquatic biota indirectly, mediated by negative effects on their food. For example, herbicide exposure may not directly affect aquatic invertebrate grazers, yet a reduction in the standing crop of biofilm available to consumers (i.e., food quantity) or a compositional change in the biofilm community (i.e., food quality) may cause a trophic cascade. For example, Rybicki et al. (2012) reported a reduction in biofilm growth from exposure to the herbicide terbutryn, which resulted in signs of starvation of the mayfly *Rhithrogena semicolorata*. Konschak et al. (2021) observed that both the physiology and feeding activity of the freshwater snail *Physella acuta* were influenced by changes in the periphyton community composition following exposure to the herbicide diuron.

Such indirect effects may occur in conjunction with direct toxicity effects, particularly if pesticide mixtures are bioconcentrated in biofilms and consumers are exposed to elevated levels through dietary exposure. Ijzerman et al. (2023) assessed the toxicity of dietary exposure of agricultural pesticides in biofilms to *Neocloeon triangulifer* (McDunnough, 1931) mayfly nymphs. Using a newly developed 7-day acute toxicity dietary test, the authors found that the response of nymphs to environmentally-relevant pesticide mixtures in field-collected biofilms was related to catchment land use. Their study represents a pivotal, novel approach to characterizing pesticide exposure and risk, which we herein expand to a new environment (stormwater ponds) with a unique contaminant profile. Moreover, we extend this biofilm-grazer dietary exposure bioassay method to a novel and complementary test species: the freshwater pulmonate snail *Planorbella pilsbryi* (F.C. Baker, 1926).

Our objective was to assess the degree to which aquatic macroinvertebrates living in stormwater ponds are affected by consuming biofilm contaminated with environmentally relevant mixtures of urban contaminants such as pesticides. Specifically, we aimed to (1) estimate the effects of pesticide accumulation on biofilm chlorophyll-*a*, pheophytin, and ash-free dry weight content; and (2) measure the effects of consuming wild-cultured biofilm with environmentally-relevant contaminant mixtures on the growth and survival of two biofilm-consuming invertebrate grazers during their most sensitive developmental life stages. We used dietary bioassays to test our hypothesis that snail and mayfly test

organisms would exhibit reductions in growth and survival when exposed to contaminants typical of stormwater ponds through their biofilm diets.

4.3 Methods

4.3.1 Sample collection

We collected biofilm samples from 15 stormwater pond sites located in Brampton, Ontario (Figure 4-1). We refer to these sites by their identification numbers assigned by the City of Brampton; site numbers are thus discontinuous and are not correlated with variables of interest in this study. Water is delivered to these ponds from a highly urbanized landscape: impervious cover in a 300 m buffer surrounding each pond ranges from 69,630 to 220,322 m^2 (average = 152,487 ± 41,268 m^2; McIsaac, 2022). These biofilm samples are the same as those described in Chapters 2 and 3, in which we characterized pesticide contamination in the biofilms from 21 stormwater ponds. From these initial 21 biofilm samples, 15 had sufficient mass left over to conduct the dietary feeding bioassays..

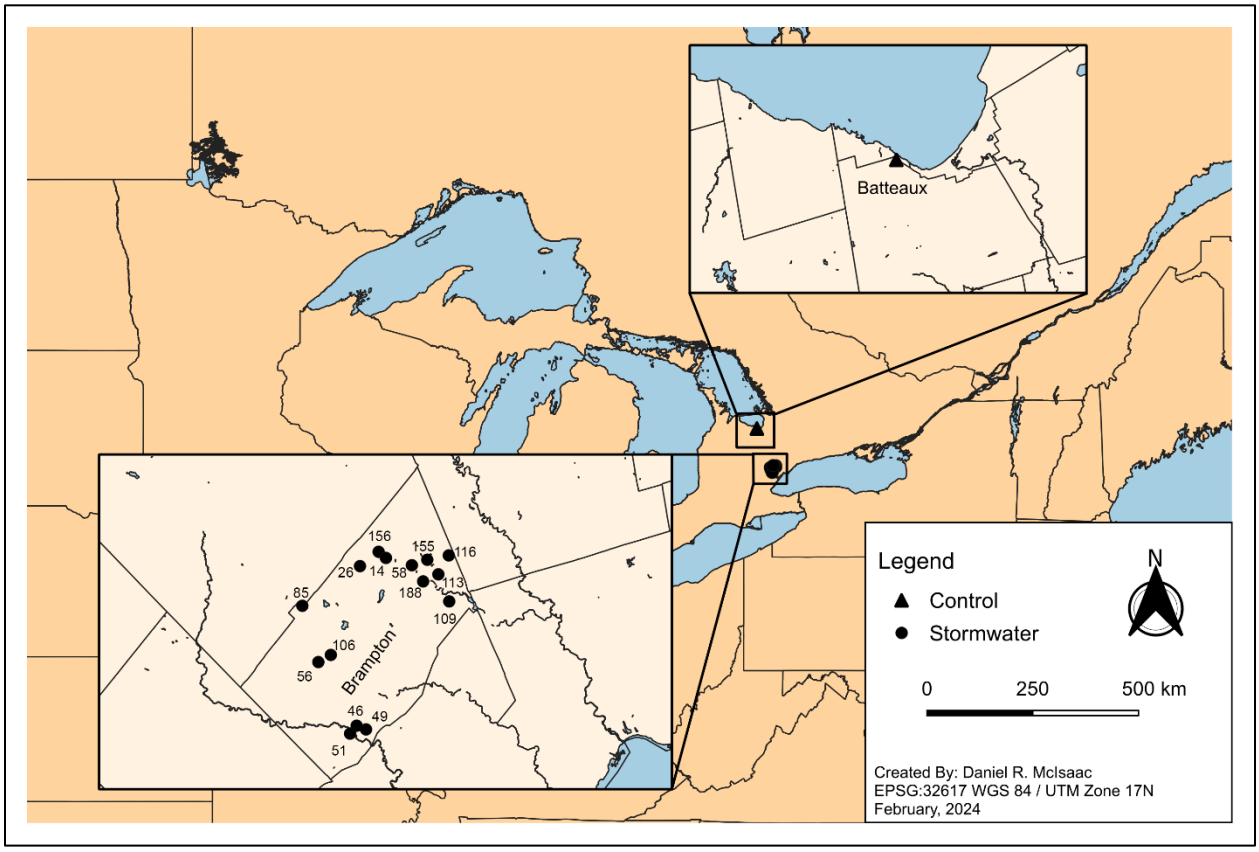


Figure 4-1. Map of field sites where biofilm was grown and harvested in situ for use in bioassay treatment diets. Biofilm samples were collected from 15 stormwater ponds (circles with site numbers) in Brampton, Ontario. A control biofilm (CB) sample was collected by Ijzerman in the relatively pristine Batteaux Creek (triangle) near Collingwood, Ontario (see Ijzerman et al., 2023 for more details).

Detailed biofilm sampling methodology is described in Chapters 2 and 3. We followed the methods described by Beecraft and Rooney (2021) and Izma et al. (2024) for the cultivation of biofilm on artificial substrates. Briefly, we used 10 acrylic plates (area = 20.2 × 44.4 cm) suspended from buoyant racks to cultivate biofilm in each of the 15 stormwater ponds. Following 54 days of incubation, we removed the substrates from the ponds and collected the biofilm from each plate into one composite sample per site. We transported biofilm samples on ice in coolers back to the laboratory, where we homogenized them by gently shaking for 1 min before taking aliquots for test treatments and chemical and nutritional analyses.

4.3.2 Biofilm analysis

The analysis of biofilm samples was previously described in Chapter 3; we provide a summary here. We measured chlorophyll-*a* and pheophytin content fluorometrically using a Turner Designs TD 10 AU fluorometer (Turner Designs Inc., Sunnyvale, California), following active extraction by sonication at 90% amplitude for three 30-s intervals (Sonic Dismembrator, Ultrasonic Liquid Processor by Fisher Scientific) and centrifugation at 3500 rpm for 6 min. We measured ash-free dry weight (AFDW) gravimetrically using a Thermo ScientificF6010 Thermolyne furnace (Thermo Fisher Scientific Inc., Asheville, North Carolina). We calculated the ratio of chlorophyll-*a* to pheophytin content, as discussed by Marshall (1978), as a useful measurement of the proportion of living to dead phototrophic microbial biomass. We freeze-dried 300–500 g (wet weight) of biofilm from each composite sample prior to submission to the Agriculture and Food Lab (AFL) in Guelph, Ontario, for analysis of 542 current and historical-use pesticides following three analytical methods. Although biofilms in urban areas are likely to accumulate substances other than pesticides, we chose this pollutant group as the focus of our study because of known negative impacts on macroinvertebrates and to address concerns expressed by the Ontario Ministry of Environment, Conservation and Parks who monitor pesticides in agricultural streams but have no urban monitoring program for pesticides. Details of chemical analysis procedures, including a complete list of pesticide analytes and their method detection limits (MDL) and method quantification

limits (MQL) in biological material is provided in Appendix B. Some samples contained compounds that were near yet below the MDL and were reported as DET (detection) and < MDL by the analytical laboratory. To ensure we took the most precautionary approach in assessing exposure to contaminants, we included these detections reported by AFL that were < MDL. This allowed us to prioritize controlling for type II errors (to limit false negatives) and characterize maximum exposure risk. The remaining fresh biofilm was stored, frozen at −20 °C, until the commencement of the bioassay tests (maximum storage duration was six months).

To gather information about pesticide analytes including their CAS number and mode of action, we used the Pesticide Properties Database (PPDB; https://sitem.herts.ac.uk/aeru/ppdb/en/index.htm) and PubChem (https://pubchem.ncbi.nlm.nih.gov/). We used the Government of Canada's Pesticide Product Information Database (PPID; accessed at https://pest-control.canada.ca/pesticide-registry/en/index.html) to determine the application status of pesticides detected in the biofilm diets. We searched for ecotoxicological thresholds provided by the Canadian Council of Ministers of the Environment (CCME) and the USEPA ECOTOX database (https://cfpub.epa.gov/ecotox/) to contextualize the concentrations of contaminants measured in our bioassay diets. These thresholds all reflect contact exposure through water because concentrations of concern for dietary exposure to aquatic organisms do not exist for any of these pesticides, highlighting the need for dietary-route ecotoxicology testing.

4.3.3 Dietary bioassay with *Neocloeon triangulifer*

Mayflies (order Ephemeroptera) are important bioindicators in aquatic invertebrate-based environmental assessment tools (e.g., Ontario Benthos Biomonitoring Network, Canadian Aquatic Biomonitoring Network) and are widely used for measuring stream ecological integrity (Bauernfeind and Moog, 2000; Jones et al., 2005; Pond, 2010). Mayflies have a hemimetabolous life history, whereby they spend the bulk of their lifespan as nymphs in the aquatic environment and moult to winged imagos to breed out of the water. Consequently, they are important in the transfer of nutrients and energy from aquatic to terrestrial ecosystems (Brittain, 1982; Vander Zanden and Gratton, 2011). The Triangle Small Minnow Mayfly, *Neocloeon triangulifer*, is considered an ideal test subject for ecotoxicological studies due to its relative sensitivity to contaminant exposure (Rowsey, 2015; Johnson et al., 2015; Weaver et al., 2015); for example, Raby et al. (2018) found this species was the most sensitive of seven mayfly species, two of which are commonly used in pesticide toxicity testing, to neonicotinoid exposure. *Neocloen triangulifer* has previously been used in dietary toxicity tests with metal-contaminated biofilm (Conley et al., 2009;

Conley et al., 2011; Kim et al., 2012; Lopez et al., 2016) and pesticide-contaminated biofilm (Ijzerman et al., 2023). These established methodologies can therefore be easily transposed into our study. Additionally, genetic variation as a confounding factor is limited in this species, due to their parthenogenetic reproduction (Funk et al., 2006). This is important for our study as differing organismal responses can be more confidently attributed to test conditions, rather than to variability in genetics.

We performed an acute dietary bioassay with the mayfly nymph *Neocloeon triangulifer* which was developed by Ijzerman et al. (2023). We chose to conduct a 7-day exposure test because of the sensitivity of the early life stages of this species (Soucek and Dickinson, 2015), and based on recent evidence of comparable sensitivities in endpoints among acute and longer tests (i.e., 7-day, 14-day, and complete life-cycle exposures; Soucek et al., 2022). Table C-1 summarizes the test conditions. We hatched *N. triangulifer* eggs (clone WCC-2) originating from Nautilus Environmental (Calgary, Alberta), who maintain a continuous culture.

We contrasted *N. triangulifer* endpoints in bioassays fed a treatment diet, which comprised biofilm collected from one of the 15 stormwater ponds, with end points for *N. triangulifer* administered control diets, of which there were three types. We used a control fed (CF) diet, consisting of cultured diatoms (*Navicula sp*.), the preferred culturing food for *N. triangulifer*, as a standard laboratory control. We cultured this food following the methods described by Ijzerman et al. (2023). We used a control biofilm (CB) diet of wild-grown biofilm collected and preserved from a stream site in southern Ontario (Batteaux River), in which no pesticides had been detected (see Ijzerman et al., 2023). This second control diet was included as an environmentally-relevant fed control, and to rule out the impact of consuming frozen and thawed biofilm on test endpoints. Biofilm from this site was stored in the same conditions and for the same duration as the biofilm collected from the stormwater ponds. Finally, we used a control starved (CS) treatment, in which no food was given during the test period, to distinguish between observed effects due to pesticide toxicity and effects attributable to organisms' aversion to consuming the provided biofilm. Each treatment (test diets and controls) was replicated five times for a total of 90 bioassays, each in separate test vessels. Each treatment, except for CS, was provided *ad libitum*. We administered the diet to each test vessel on Days 0, 3, and 5, and performed a complete water change on Days 3 and 5. We measured water quality (dissolved oxygen, conductivity, and pH) on Days 1 and 7, presented in Tables C-2.

We visually assessed survival using magnifying glasses on Days 0, 3, 5, and 7. We also checked for survival in the three control treatments after 24 h, which were all 100%. We determined mortality based on a lack of movement following gentle probing with a pipette, as recommended in Ijzerman et al. (2023).

At the end of the test period, we euthanized all surviving individuals with 70% ethanol and mounted them on microscope slides to measure body length at 12–18 X magnification using a stereomicroscope (Nikon SMZ-18; NIS Elements software). Mayfly growth was determined using the following equation:

$$\mathbf{G_{nymph} = BL_{nymph} - ABL_{nymph}}$$

Where G_{nymph} = indiviual nymph growth (µm); BL_{nymph} = body length of a nymph at the end of the trial (µm); and ABL_{nymph} = the average initial body length of the 15 individuals measured on Day 0 of the trial (µm).

Nymph body length was measured on surviving individuals following the test, and the initial average body length was calculated by averaging the body lengths of 15 individuals measured on Day 0 of the test. We used G_{nymph} to calculate average growth and biomass production. The difference between these two measurements is that average growth is calculated as the sum of the G_{nymph} from the surviving nymphs in a replicate divided by the number of survivors, whereas biomass production is the sum of the G_{nymph} in a replicate divided by the initial number of nymphs in each replicate (i.e., 3).

A graphical summary of bioassay methods for both test species is shown in Figure 4-2.

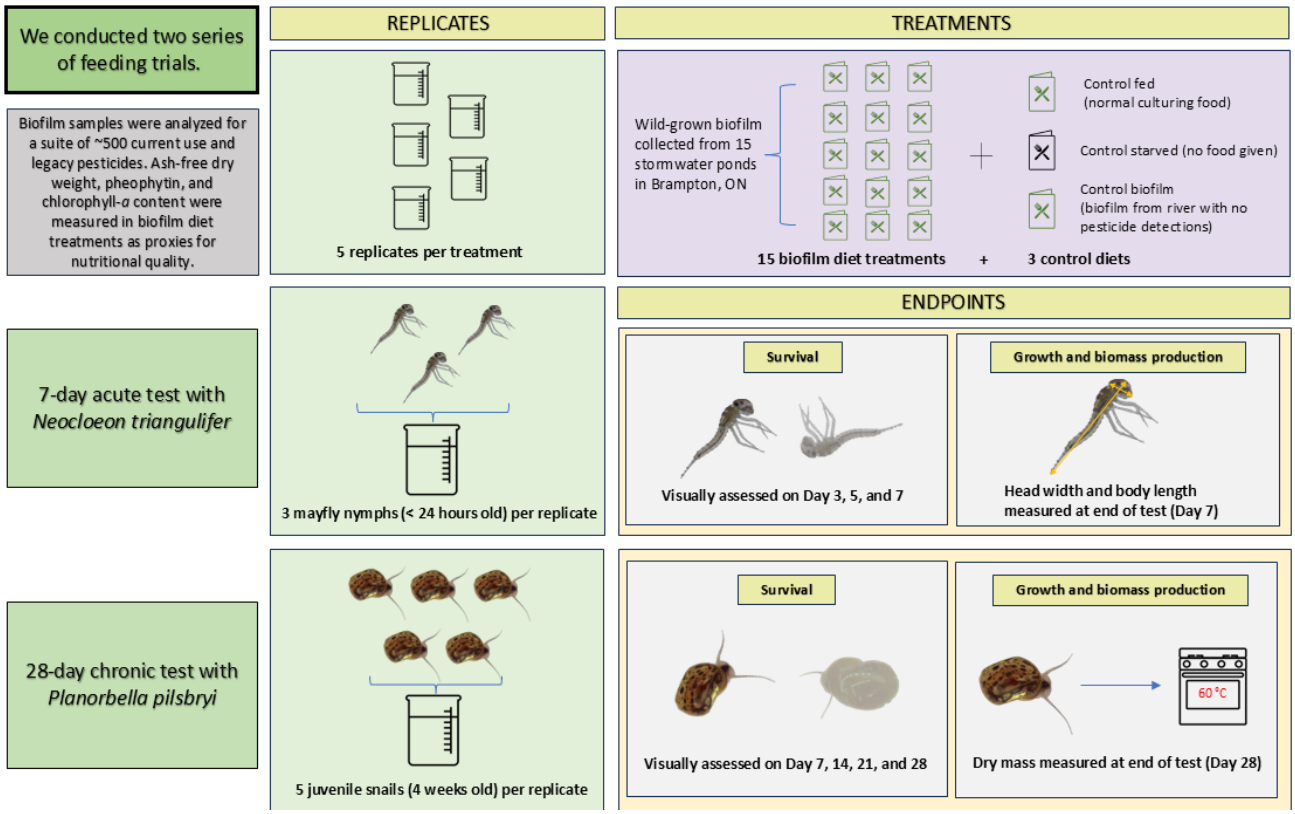


Figure 4-2. Study design of feeding trials conducted as a bioassay on the relative toxicity of biofilm collected from urban stormwater ponds (n = 15).

4.3.4 Dietary bioassay with *Planorbella pilsbryi*

Freshwater snails (class: Gastropoda) are also widely used to diagnose poor water quality in aquatic ecosystems, and their direct and indiscriminate feeding on biofilm signifies a clear trophic link through which contaminants may be passed (Oehlmann & Schulte-Oehlmann, 2003). For example, Friesen et al. (2017) demonstrated the link between bioconcentration of selenium (Se) in biofilms and accumulation in snails that fed on the biofilm. The File Ramshorn Snail, *Planorbella pilsbryi*, is also commonly used in laboratory exposure assays (e.g., Prosser et al., 2017; Osborne et al., 2020) and related species in the genus *Planorbella* have recently been used for dietary exposure trials with metals (Frankel et al., 2023).

We chose to conduct a chronic, 28-day exposure bioassay with *Planorbella pilsbryi* to complement the acute test with mayfly nymphs and because this species of pulmonate snails has been shown to be sensitive to chronic pesticide exposure at relatively low concentrations (Prosser et al., 2016). Table C-1 summarizes the test conditions. Adult egg-laying snails were obtained from a culture

maintained at the University of Guelph. This culture is fed organic spinach *ad libitum*, receives biweekly water changes, and is kept at 20 °C with a photoperiod of 16:8 light:dark. We randomly removed 200 adult snails with a mean shell length of 11.45 mm (± 1.49 mm) from the culture and placed them into glass containers for five days to allow for egg-laying. Then we transported egg masses to the Waterloo Wetland Lab at the University of Waterloo (Waterloo, Ontario) where they were aerated and allowed to hatch for four weeks. One week following the commencement of hatching, we administered organic spinach and changed the hatchery water once every three days.

We conducted the chronic dietary bioassay with juvenile (4-week-old) snails, using the same 15 test diets plus CS and CB treatments as the mayfly test. We also included a control fed (CF) diet in this trial comprising the preferred culturing diet: organic baby spinach. Each treatment (n = 18) was replicated five times in independent 250 mL test vessels, for a total of 90 test vessels, each containing five test organisms. Prior to the commencement of the test, we randomly assigned each test organism (n = 450) to a test vessel and allowed for three days of acclimation on the CF diet. From the remaining juveniles in the hatchery, we randomly selected and froze 20 individuals at −20 °C for initial growth measurements. The test vessels did not have lids; rather, a small sheet of plastic wrap was placed loosely over the top of each vessel to limit contamination while allowing for air circulation.

On Day 0, we removed any leftover spinach, changed the test water, and administered one of the 18 treatments. Each treatment, except for CS, was provided *ad libitum*. We fed the snails in the vessels on Days 0, 7, 14, and 21 of the chronic exposure trial. We completed a half-water change every other day, and a complete water change on Days 7, 14, and 21. We took water quality measurements (Table C-3) including temperature, dissolved oxygen, pH, and conductivity on Days 0, 7, 14, 21, and 28. We measured ammonia on Days 0, 7, 11, 14, 18, 21, 25, and 28 (Table C-4).

We visually tested for juvenile survival on Days 0, 7, 14, 21, and 28. Mortality was determined by the presence of an empty shell or the absence of an individual as sometimes empty shells are consumed by surviving snails of the test vessel.

Following survivorship assessments on Day 28, we froze each individual snail in a glass vial at −20 °C. We then dried the snails in aluminum micro weigh boats at 80 °C for 1 h and measured dry weight using a microbalance (Mettler Toledo MT WXTS3DU). Test endpoints were calculated using the following equation:

$$\mathbf{G_{snail} = DW_{snail} - ADW_{snail}}$$

Where DW_{snail} = the dry weight of each individual snail (mg); ADW_{snail} = the average initial dry weight (taken on day 0; n = 20) = 0.436 ± 0.0474 mg.

We used G_{snail} to calculate snail average growth and biomass production in the same method as G_{nymph}, where the average growth was calculated as the sum of the G_{snail} from the surviving snails in a replicate divided by the number of survivors, whereas biomass production is the sum of the G_{snail} in a replicate divided by the initial number of nymphs in each replicate. (i.e., 5).

4.3.5 Statistical analysis

All statistical analyses were conducted in R (version 4.2.2.) implemented with RStudio (version 2023.06.1). We used Spearman's rank correlation tests executed with the *cor.test* function in R base to explore how metrics of nutritional value (AFDW, chlorophyll-*a*, and pheophytin content) relate to the number of pesticide detections (i.e., pesticide burden) in biofilm diets and test endpoints. Similarly, we used the Spearman's test to relate pesticide burden to test endpoints. To evaluate differences in test endpoints (survival, growth, and biomass production) among treatment diets, we used a Kruskal-Wallis analysis of variance executed with the *kruskal.test* function from the *dplyr* package (https://www.rdocumentation.org/packages/dplyr/versions/1.0.10), followed by Dunnett's post hoc test executed with the *DunnettTest* function from the *DescTools* package (https://www.rdocumentation.org/packages/DescTools/versions/0.99.54) to compare the treatment diets to the controls. We chose nonparametric tests due to the non-normal distribution of residuals. For these tests, we rejected the null hypothesis when the *p*-value was < 0.05.

We investigated the relationships between contaminant concentrations in biofilm diets, biofilm nutritional metrics, and test endpoints for both mayflies and snails using nonmetric multidimensional scaling (NMDS). This type of analysis does not assume linear relationships, which is ideal for exploring pesticide concentration data where the assumption of bivariate linearity is violated and the 542 pesticides we tested for were below detection limits in most samples resulting in many zero values (McCune and Grace, 2001). We carried out the NMDS with pesticide concentrations and biofilm nutritional data from each site using the *metaMDS* function within the *vegan* package in R (https://www.rdocumentation.org/packages/vegan/versions/2.6-4). For this analysis, we excluded pesticide detections that were < MDL, and used the MDL limit value for detections that were over the MDL but < MQL. We used the function *decostand* along with the range argument in the *vegan* package to standardize all pesticide concentration and biofilm nutritional data to a value between 0 and 1 (Oksanen et

al., 2019). Using the *metaMDS* function, we used these values to calculate a Bray-Curtis dissimilarity matrix. We determined the optimal number of dimensions for the NMDS based on stress values below 0.2. Then, we used the function *envfit* in *vegan* with 999 permutations to fit the mayfly and snail test endpoints onto the NMDS ordination of the pesticide and nutritional value dissimilarity matrix (Oksanen et al., 2019). The test endpoints were reasonably correlated ($r^2 > 0.1$) with at least one NMDS axis. We visualized the ordination using the *ggplot* function in the *ggplot2* package (Wickham, 2016; https://www.rdocumentation.org/packages/ggplot2/versions/3.5.0).

4.4 Results and discussion

4.4.1 Analysis of biofilm test diets

4.4.1.1 Pesticides in biofilm

Fifteen pesticides in total were detected in the biofilm samples collected from the 15 stormwater ponds (i.e., the test diets). The detection and concentration of pesticides in biofilm collected from each site are reported in Table 4-1. Of these 15 pesticides, 13 had reported thresholds for immersion exposure in the databases we searched (CCME and ECOTOX), but none had dietary exposure thresholds for insects that would be relevant for our bioassays. The treatment diets ranged in pesticide burden, from as few as two to as many as eight pesticide detections per diet. This represents a gradient in pesticide contamination level (Table 4-1).

Table 4-1. Pesticide detection and concentrations (ng/g) in test diets (n = 15) consisting of biofilm collected from urban stormwater management ponds. Diets are listed in order of increasing number of pesticides detected. CF = control fed diet; CS = control starved diet; CB = control biofilm diet. DET = reported as a detection by the analytical laboratory but the contaminant concentration was below yet near the MDL. Pesticide properties, including CAS number, mode of action, and possible urban source, are found in Table C-5.

Diet	# of detections	Pesticides	Concentration (ng/g)
CF	0		
CS	0		
CB	0		
Site 14	2	Diuron Azoxystrobin	DET DET
Site 113	3	Diuron	DET

Diet	# of detections	Pesticides	Concentration (ng/g)
		Azoxystrobin	DET
		Thiabendazole	DET
Site 58	4	Diuron	DET
		Mecoprop	DET
		Azoxystrobin	DET
		Thiabendazole	DET
Site 85	4	2,4-Dichlorophenoxyacetic acid	<30*
		Diuron	DET
		Mecoprop	DET
		Carbendazim	DET
Site 174	4	2,4-Dichlorophenoxyacetic acid	DET
		Diuron	DET
		Azoxystrobin	DET
		Thiabendazole	DET
Site 188	4	Diuron	<20*
		Azoxystrobin	<20*
		Thiabendazole	DET
		Nicotine	40
Site 26	5	2,4-Dichlorophenoxyacetic acid	<30*
		Diuron	DET
		Azoxystrobin	DET
		Thiabendazole	DET
		Melamine	26
Site 49	5	Diuron	DET
		Azoxystrobin	<20*
		Imazalil	DET
		Thiabendazole	DET
		Nicotine	67
Site 56	5	Diuron	DET
		Hexaethylphosphoramide	710
		Azoxystrobin	35
		Thiabendazole	DET
		Nicotine	DET
Site 156	6	2,4-Dichlorophenoxyacetic acid	<30*
		Diuron	<20*
		Mecoprop	DET
		Azoxystrobin	32
		Thiabendazole	DET
		Nicotine	<20*
Site 46	7	Diuron	DET
		Azoxystrobin	<20*

Diet	# of detections	Pesticides	Concentration (ng/g)
		Carbendazim	DET
		Tebuconazole	<20*
		Thiabendazole	DET
		Thiophanate-methyl	<20*
		Nicotine	<20*
Site 51	7	2,4-Dichlorophenoxyacetic acid	DET
		Diuron	DET
		Mecoprop	DET
		Azoxystrobin	DET
		Carbendazim	<30*
		Thiabendazole	DET
		Nicotine	<20*
Site 109	7	Chlorpropham	33
		Diuron	DET
		Mecoprop	DET
		Hexaethylphosphoramide	130
		Azoxystrobin	DET
		Thiabendazole	DET
		Nicotine	<20*
Site 106	8	Diuron	DET
		Pendimethalin	51
		Azoxystrobin	<20*
		2-phenylphenol	DET
		Carbendazim	DET
		Tebuconazole	29
		Thiabendazole	DET
		Nicotine	47
Site 116	8	2,4-Dichlorophenoxyacetic acid	DET
		Diuron	DET
		Mecoprop	DET
		Hexaethylphosphoramide	610
		Azoxystrobin	<20*
		Imazalil	DET
		Thiabendazole	<10*
		Nicotine	<20*

*<MQL

The urban land use in the catchments contributing to stormwater ponds will be distinct from the types of land use in more commonly studied agricultural systems (e.g., Ijzerman et al., 2024). We expected that pesticides common in stormwater ponds would be registered for current use in Canada and

those uses would be typical of urban environments (e.g., construction or lawn care applications). Generally, this expectation was met. According to the Pesticide Product Information Database (PPID), most (11 of 15) detected pesticides are registered for use in Canada (https://pr-rp.hc-sc.gc.ca/ls-re/index-eng.php). The exceptions were imazalil, hexaethylphosphoramide (HEPA), melamine, and nicotine. Nicotine is not usually considered a pesticide by stormwater contaminant studies (e.g., Masoner et al., 2019; Wicke et al., 2021); however, it can be used as an insecticide and was included in the pesticide analysis due to its historical use from which many modern neonicotinoids are derived.

The herbicide diuron was detected in every biofilm test diet but was never above the limit of quantification. Diuron's mode of action operates by inhibiting photosystem II. It is approved in Canada for use in drainage and irrigation ditches (PPID), structures that often lead run-off into stormwater systems. The herbicides 2,4-Dichlorophenoxyacetic acid (2,4-D) and mecoprop, both synthetic auxins, were found in 40% of the test diets, and are approved for turfgrass management in Ontario (OMAFRA, 2017). The fungicide azoxystrobin was detected in 93% of the test diets, and above the limit of quantification in two of the test diets (32 and 35 ng g^{-1}). This fungicide inhibits respiration (PPDB) and is an ingredient in domestically available products for use on lawns, turf, and golf courses in urban areas (OMAFRA, 2017). Thiabendazole was detected in 87% of test diets, with no occurrences above the quantification limit. Thiabendazole is very versatile, being present in glues, adhesives, paints and textiles, with other applications in veterinary care and Dutch Elm Disease management (PPDB; PubChem). The fungicide carbendazim was detected in 27% of test diets. This fungicide is a byproduct of thiophanate-methyl degradation and has been found at high occurrences in Ontario's urban and agricultural surface waters (Metcalfe et al., 2016; Metcalfe et al., 2019). Carbendazim and thiophanate-methyl are commonly used for turfgrass and golf course maintenance (OMAFRA, 2017), and carbendazim is also used in construction products and paints and to control Dutch Elm Disease (Vincelli, 2004; Metcalfe et al., 2019). Nicotine was detected in 60% of samples; the improper disposal of cigarette butts and electronic cigarettes is thought to be the primary source of nicotine to urban surface waters (Green et al., 2014; Beutel et al., 2021).

We found immersion exposure toxicity guidelines for 13 of the 15 pesticides detected in biofilm samples when searching the CCME resource browser and the US EPA's ECOTOX database. However, these cannot be applied to assess toxicity risk for a dietary exposure route as the route of exposure can strongly influence the experienced toxicity of a contaminant. Currently, no appropriate toxicity thresholds or guidelines exist for the dietary exposure of freshwater organisms to pesticides. Consequently, we were

unable to evaluate whether the concentrations of pesticides detected in our biofilm samples constitute exceedances that could put aquatic biota at risk.

4.4.1.2 Nutritional analysis of biofilm

We used chlorophyll-*a*, pheophytin, and ash-free dry weight (AFDW) content as proxies for the nutritional quality of the biofilm test diets (Table 4-2). AFDW, which shows the fraction of the food that was digestible, was not correlated ($p = 0.487$) with the number of pesticides detected in the biofilm samples (referred to henceforth as the pesticide burden). Similarly, chlorophyll-*a* and pheophytin content were not significantly related to pesticide burden ($p = 0.524$ and $p = 0.0511$, respectively). Given the low p-value for the correlation between pheophytin content and pesticide burden, it may warrant further study on whether higher pesticide burdens in biofilm lead to a slower chlorophyll-*a*-to-pheophytin breakdown or a faster breakdown of pheophytin (and thus slower accumulation rates).

Table 4-2. Nutritional quality analysis of biofilm test diets which are biofilm samples collected from 15 stormwater pond sites, one river site (CB = control biofilm), and the control fed (CF) diet composed of *Navicula* sp. Treatments are listed in order of increasing pesticide burden (reported in Table 4-1).

Treatment	Chlorophyll-*a* (µg cm^{-2})	Pheophytin (µg cm^{-2})	Chlorophyll-*a* :pheophytin ratio	AFDW (mg cm^{-2})
CF	0.922	3.723	0.248	0.473
CB	0.899	7.801	0.115	0.497
14	0.252	0.271	0.930	1.646
113	0.484	0.831	0.582	0.287
58	1.070	0.209	5.120	2.419
85	0.216	0.460	0.470	0.335
174	0.506	0.604	0.838	0.584
188	0.060	0.564	0.106	3.316
26	0.784	0.881	0.890	0.779
49	1.358	0.610	2.226	0.735
56	0.579	0.207	2.797	0.760
156	0.158	0.150	1.053	2.062
46	0.063	0.083	0.759	0.346
51	0.239	0.070	3.414	0.756
109	0.490	0.278	1.763	0.635
106	0.830	0.629	1.320	0.626
116	0.537	0.312	1.721	1.204

The ratio of chlorophyll-*a* to pheophytin content was significantly ($p = 0.0195$) and positively (rho = 0.560) correlated to pesticide burden, meaning a greater proportion of living phototrophic microbial tissue likely was present in biofilms with a higher number of pesticide detections. This relationship is counterintuitive and deserves further inquiry. However, although this metric may be useful in determining the health of the biofilm communities themselves, there is currently no evidence supporting its use as an indicator for nutritional value in the context of biofilm-grazing invertebrate diets.

4.4.2 Acute bioassay with *N. triangulifer*

4.4.2.1 Survival

We confirmed 100% survival of all organisms in the three control diets after 24 h. Both the control fed (CF) and control biofilm (CB) treatments had survival rates exceeding the standard (> 80%; Weaver et al., 2015) upon completion of the test (Figure 4-3A; Table C-6). All individuals in each replicate of the control starved (CS) treatment groups had 0% survival on Day 3, indicating effects on survival in the treatment groups were more likely due to consumption of the biofilm diets, rather than aversion to the biofilm and consequent starvation. Survival in the CF and CB treatments were not significantly different ($p = 0.855$) from each other, and both controls had significantly ($p < 0.05$) higher survival from all test diets except for those collected from sites 85, 109, and 156 (Table C-6). This represents a major reduction in the mayfly survival of 80% of biofilm test diets compared to the CF and CB control diets. Biofilms collected from stormwater ponds clearly impair mayfly survival, perhaps by being nutritionally inadequate (e.g., with insufficient levels of diatoms which provide essential fatty acids and whose abundances are known to be altered by water quality (Tien et al., 2009)) or by exposing nymphs to toxic substances, or through a combination of these two mechanisms. Without further evaluation of the quality of the biofilm as a food source, we cannot differentiate whether the toxicological effects experienced by invertebrates following consumption of these diets are due to reduced nutritional value, high contaminant content, or a combination of both factors. Evaluating the protein content (e.g., Ledger and Hildrew, 1998) or caloric density of the diets may provide additional insight into the effects of variable diet quality on test subjects.

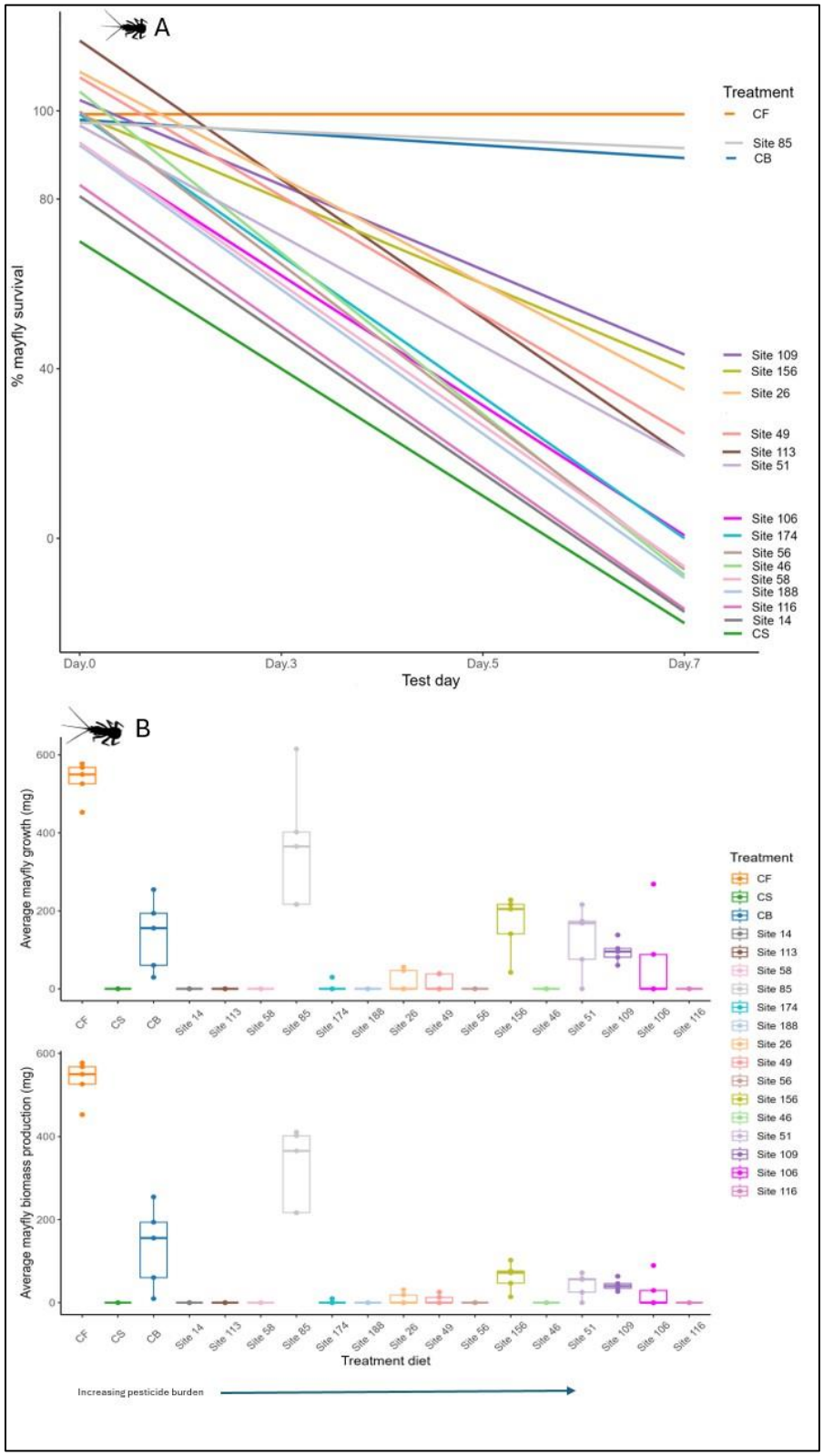

A
% mayfly survival
100
80
40
0
Day.0
Day.3
Day.5
Day.7
Test day
Treatment
CF
Site 85
CB
Site 109
Site 156
Site 26
Site 49
Site 113
Site 51
Site 106
Site 174
Site 56
Site 46
Site 58
Site 188
Site 116
Site 14
CS
B
Average mayfly growth (mg)
Average mayfly biomass production (mg)
600
400
200
0
CF
CS
CB
Site 14
Site 113
Site 58
Site 85
Site 174
Site 188
Site 26
Site 49
Site 56
Site 156
Site 46
Site 51
Site 109
Site 106
Site 116
Treatment diet
Increasing pesticide burden

Figure 4-3. (A) Average mayfly nymph survival rate across the 7-day test period. Survival rate is represented by the slope of the linear regression describing the relationship between test day and the percent of mayfly nymphs surviving. Survival was assessed at four observation points. (B). Average mayfly growth (µm) and average mayfly biomass production (µm), both measured at the end of the test. CF = control fed diet; CS = control starved diet; CB = control biofilm diet. Test diets are displayed in order of increasing pesticide burden, from left to right.

Seven test diets (from sites 188, 58, 46, 56, 113, 116, and 14) had 0% survival at the end of the test (Table C-6). These diets represent a wide range in pesticide burden, with the number of detections ranging from 2 to 8. We confirmed there was no significant Spearman correlation between pesticide burden and survival for this test ($p = 0.564$). The wide range in mayfly survivorship, regardless of treatment diet, suggests that factors other than pesticide burden, such as the presence of other contaminants in the diets or varying microbial community compositions of the biofilms, are driving the reduced survival. Alternatively, some pesticides may simply be more toxic than others, and the significance of their occurrences are obscured by the presence of less-toxic substances.

The survival rate, calculated from the average survival for each treatment at Days 0, 3, 5, and 7, is represented by lines of best fit in Figure 4-3A. Compared to a similar analysis of mayfly nymph survival rate by Ijzerman et al. (2023) when fed biofilms from agricultural streams, the survival rate of seven of our treatments is steeper due to the mortality of all organisms by Day 7. This may indicate that biofilm grazers living in urban ponds may experience more dietary stress (either from contaminated diets or inadequate nutrition) than those living in agriculturally-impacted streams, despite lower exposures to pesticides.

4.4.2.2 Growth and biomass production

The CF (mean = 534.8 ± 68.5 µm) and CB (mean = 155.5 ± 112.9 µm) treatments did not have significantly different average growth from each other ($p = 0.195$; Figure 4-3B and Table C-7). The CF treatment had significantly higher average nymph growth than all biofilm treatments except for test diets collected from sites 109, 156, 51, and 85 (which also had high survival on par with the CF and CB treatments), revealing reduced growth in 73% of test diets. The CB treatment had significantly higher average nymph growth than all test diets except for those collected from sites 106, 109, 156, 26, 51, and 85, revealing reduced growth in 60% of test diets. In addition to the seven treatments that resulted in 0% survival (and therefore 0% growth), sites 174 and 49 had reduced growth compared with the control diets.

Mayfly growth is therefore likely impaired by consumption of biofilm from stormwater ponds, which may lead to delayed or inhibited development, emergence, and reproduction. Such effects at the organismal-level can cascade into wider population-level impacts. For example, lower growth could mean fewer mayflies survive into adulthood, producing fewer offspring to maintain populations.

The CF (mean = 534.8 ± 49.8 µm) and CB (mean = 134.8 ± 99.2 µm) diets also did not have significantly different average biomass production from each other ($p = 0.285$; Figure 4-3B and Table C-7). The CF treatment had significantly higher average biomass production than all test diets except for those from sites 109, 156, and 85, representing reduced biomass production in 80% of treatments, while the CB treatment had significantly higher average biomass production for all test diets except for those from 106, 109, 156, 51, and 85, representing reduced biomass production in 67% of test diets. In addition to the seven treatments that resulted in 0% survival (and therefore 0% biomass production), sites 174, 26, 49 had reduced biomass production compared with the control diets. Reduced biomass production of a species feeding on biofilm from a stormwater pond could alter food web relationships; for example, predators of mayflies may need to shift their foraging behaviour to other food sources.

We did not find significant correlations between pesticide burden and growth ($p = 0.981$) or biomass production ($p = 0.683$). Because survival in nearly half (7 out of 15) of the test diets was 0% by the end of the test, it is possible that the nymphs at this early life stage were in fact too sensitive, and that using a longer test with diluted diets or with older nymphs could provide enhanced resolution of the effects of consuming stormwater pond biofilm on their growth and biomass production.

4.4.2.3 Correlations between test endpoints and nutritional quality

There were no significant Spearman correlations between mayfly survival and diet nutritional quality (i.e., AFDW ($p = 0.382$), chlorophyll-*a* content ($p = 0.263$), or pheophytin content ($p = 0.0725$)). Similarly, there were no significant correlations between diet nutritional quality metrics and mayfly growth (AFDW ($p = 0.394$), chlorophyll-*a* content ($p = 0.394$), and pheophytin content ($p = 0.152$)) or biomass production (AFDW ($p = 0.380$), chlorophyll-*a* content ($p = 0.358$), and pheophytin content ($p = 0.127$)). As already articulated, our metrics used for evaluating nutritional quality are simple indicators and do not take into account the specific nutritional needs of mayfly nymphs of this species. A closer examination of nutritional requirements (e.g., diatom content) and a diverse set of metrics of measuring the nutritional quality of biofilms is warranted to investigate potential indirect effects of pesticide exposure on mayflies via reduced nutritional value of their biofilm food.

4.4.3 Chronic bioassay with *P. pilsbryi*

4.4.3.1 Survival

We confirmed 100% survival of all organisms in the three control treatment diets after 24 h. Survival rates of both the control fed (CF) and control biofilm (CB) treatments remained at 100% upon completion of the test (Figure 4-4A; Table C-8). On Day 21, all individuals in each replicate of the control starved (CS) treatment groups were deceased, indicating aversion to consumption of biofilm diets likely was not the main driver of effects on survival in the treatment, as complete mortality was not observed in any fed treatments.

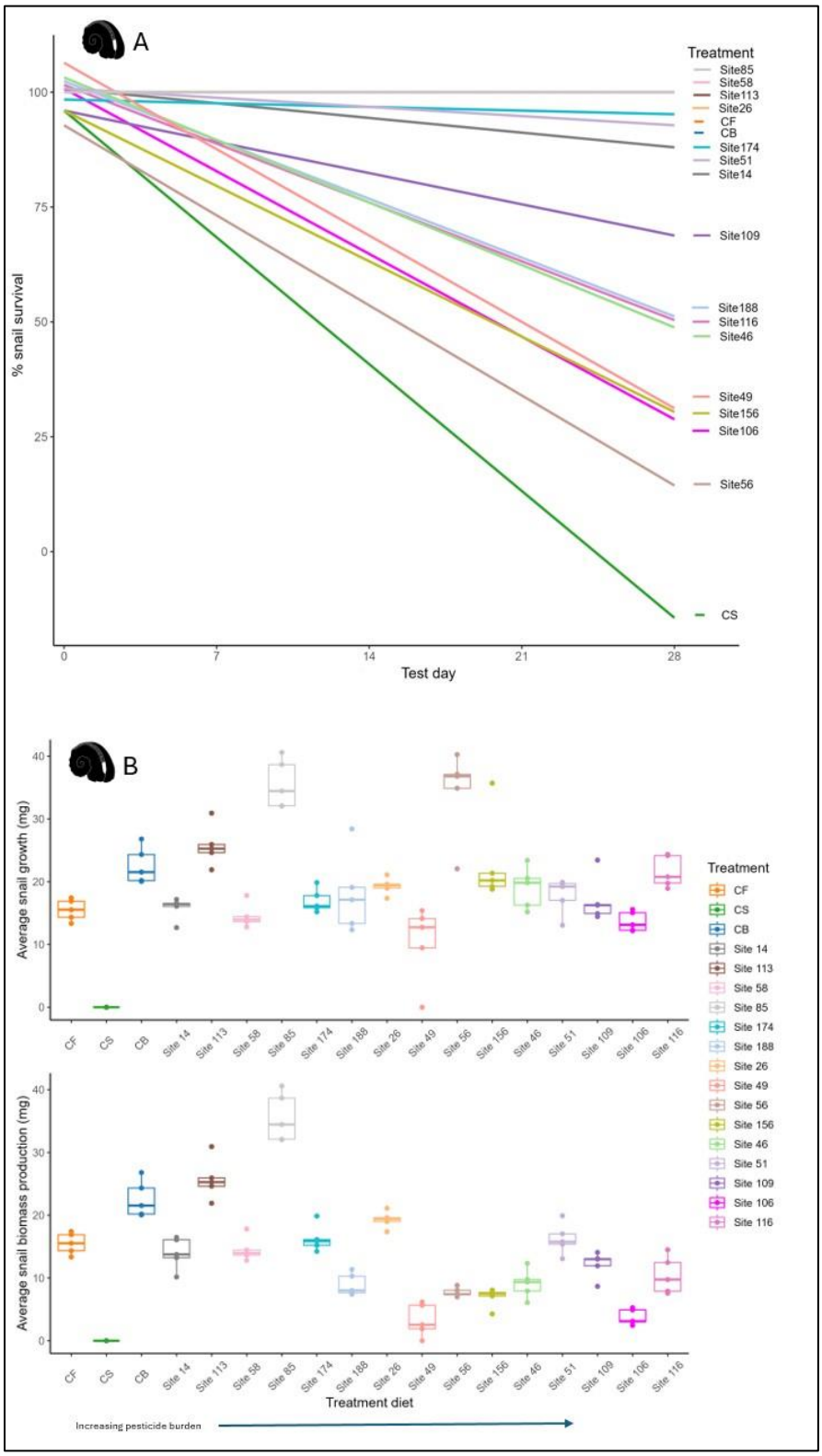

A
% snail survival
Test day
Treatment
Site85
Site58
Site113
Site26
CF
CB
Site174
Site51
Site14
Site109
Site188
Site116
Site46
Site49
Site156
Site106
Site56
CS
B
Average snail growth (mg)
Average snail biomass production (mg)
Treatment diet
Increasing pesticide burden
Treatment
CF
CS
CB
Site 14
Site 113
Site 58
Site 85
Site 174
Site 188
Site 26
Site 49
Site 56
Site 156
Site 46
Site 51
Site 109
Site 106
Site 116

Figure 4-4. (A) Average snail survival rate across the 28-day test period. Survival rate is represented by the slope of the linear regression describing the relationship between test day and the percent of snails surviving. Survival was assessed at five observation points. (B) Average snail growth (mg) and average snail biomass production (mg) at the end of the test period. Sites are ordered from lowest to highest pesticide burden. CF = control fed diet; CS = control starved diet; CB = control biofilm diet. Test diets are listed from left to right, in order of increasing pesticide burden.

The CF and CB treatment diets were not significantly different (all replicates had 100% survival in both treatments; p = 1.00). Average survival was also high (ranging 88–100%) in test diets from sites 58, 51, 113, 174, 14, 26, and 85. The CF and CB treatments were significantly different from test diets from sites 106, 116, 156, 188, 46, 49, and 56. Therefore, there was a significant reduction in the average survival of 53% of stormwater pond test diets compared with the fed controls. This agrees with the results presented in Section 4.4.2.1, but notably the stormwater ponds from which biofilm diets led to the significantly reduced survival of snails are not exactly the same set of ponds from which biofilm diets resulted in the lowest survival of mayflies. The reasoning behind this disagreement is discussed later in Section 4.4.5.

We found a negative (rho = − 0.637) and significant (p = 0.00597) correlation between pesticide burden and average snail survival, meaning higher survival was observed in treatments with lower pesticide burden. This might be the result of direct toxicity to the pesticides in the biofilm consumed by the snails, but it could alternatively or additively be due to some change in the nutritional value of the biofilm associated with the pesticide exposure which is not captured by our gross measures of biofilm quality, such as the chlorophyll-*a* content and the ash-free dry weight of the biofilm diets. Unfortunately, our bioassay does not allow us to separate these possible mechanisms. A third possible mechanism to explain the observed reduction in survival of the snails consuming the biofilm from the stormwater ponds compared to the control biofilm or control spinach diets is that the stormwater ponds with higher pesticide burden may also coincidentally contain some other, unmeasured contaminant. For example, stormwater ponds with more pesticides in them may also have more pharmaceuticals or heavy metals or organic pollutants and these may be directly responsible for the reduced snail survival. Confirming the mechanism of toxicity from a bioassay would require additional testing with pesticide-spiked diets, but the correlation between average snail survival and biofilm pesticide burden indicates that such testing is warranted.

4.4.3.2 Growth and biomass production

Average snail growth was significantly higher in the CB treatment (mean = 22.58 ± 2.92 mg) compared to the CF treatment (p = 0.0214; mean = 15.51 ± 1.70 mg) and CS treatment (p = 0.0000994; mean = 0 ± 0 mg; Table C-9 and Figure 4-4B). This suggests that spinach may lack some essential nutrients that are more abundant in biofilm, the natural diet of these snails in the wild, however more research is needed to better understand the content and effects of these two diets on juvenile snail development.

Mean growth was similar among 10 (sites 46, 49, 58, 188, 109, 51, 14, 174, 106, and 26) out of 15 of the test diets (i.e., 67%) compared with the CF treatment. Only sites 56, 156, 113, 116, and 85 had significantly lower growth than the CF treatment, representing a reduction of growth in 33% of test diets. Unlike snail survival, we observed no significant relationship between pesticide burden and mean snail growth (p = 0.850). In fact, the second highest mean growth value was observed in the test diet collected from site 56 (34.23 mg), which paradoxically had a survival rate of only 24%. By providing surplus food in each test vessel, we attempted to control the effect that decreased survival could have on competition for food, although reduced competition for foraging space may have benefited surviving snails as snail growth is known to be negatively correlated to snail density (Hertonsson et al., 2008). As another possible explanation, we observed that snails which died were sometimes consumed by the surviving snails sharing the same test vessel. This can provide additional nutrients or an alternative food source to the test diet, affecting our measurement of mean growth. Consequently, we consider mean growth an unreliable measure of the effects of pesticide-contaminated biofilm consumption on snail fitness.

Biomass production, on the other hand, considers both the growth and survival of the test organisms, and thus better accounts for the effects of mortality on snail growth in this experiment. We observed a negative (rho = − 0.536) and significant (p = 0.0266) relationship between pesticide burden and snail biomass production. Snail biomass production was not significantly different between the CF (mean = 15.51 ± 1.70 mg) and CB (mean = 22.58 ± 2.92 mg) treatments (p = 0.231; Table C-9 and Figure 4-4B). Both controls were significantly higher than the CS treatment (p = 0.000816 and 0.00000547, respectively). The CB treatment had significantly higher biomass production than eight (sites 46, 49, 56, 106. 109, 116, 156, and 188) of the 15 test diets (i.e., 53%), while the CF treatment only had significantly higher biomass production than four (sites 49, 56, 106, and 156) of the 15 test diets (i.e., 27%). No significant difference in biomass production was observed in test diets from sites 58, 51, 14, 174, 113, 85, and 26 compared to the CF and CB treatments, although notably the average biomass production observed in snails fed biofilm from site 113 (mean = 25.74 ± 3.28 mg) and site 85 (mean = 35.57 ± 3.89

mg), both of which had low pesticide burden, was actually higher than in the CB and CF treatments. The observed reduction in biomass production in over half of the stormwater pond test diets compared with the clean river biofilm diet underscores the negative effect of biofilm contamination on snail development. A reduction in the available biomass of snails could impact food-web dynamics (Brönmark and Weisner, 1996) and alter the development of the biofilm crop (Lawrence et al., 2002).

In comparison to the results presented in Section 4.4.2.2., a smaller proportion of biofilm test diets resulted in decreased growth and biomass production compared with control diets. As with survival, the stormwater ponds from which biofilm diets led to the significantly reduced growth and biomass production of snails are not exactly the same set of ponds from which biofilm diets resulted in the lowest growth and biomass production of mayflies. We discuss this further in Section 4.4.5.

We recommend using survival and biomass production as useful endpoints with clear ecological implications regarding risks to aquatic biota feeding on biofilm in the stormwater ponds, rather than growth measurements which may only help predict population-scale effects and can be confounded with survival.

4.4.3.3 Correlations between test endpoints and nutritional quality

There were no significant correlations between snail survival and biofilm nutritional quality (i.e., AFDW ($p = 0.228$), chlorophyll-*a* content ($p = 0.687$), or pheophytin content ($p = 0.0964$)). We also did not find significant correlations between diet nutritional quality metrics and snail growth (AFDW ($p = 0.467$), chlorophyll-*a* content ($p = 0.0585$), and pheophytin content ($p = 0.666$)) or biomass production (AFDW ($p = 0.0677$), chlorophyll-*a* content ($p = 0.884$), and pheophytin content ($p = 0.188$)). These results do not necessarily indicate that contamination has a stronger influence on snail survival and biomass production than the quality of the diet. Rather, our selected metrics for analyzing nutritional quality do not seem to be related to these endpoints, but other metrics (e.g., caloric density, mineral content) may be more influential on snail health.

4.4.4 Qualitative overview of test results using NMDS

To strengthen our understanding of the bioassay test results, we also conducted a nonmetric multidimensional scaling (NMDS) analysis using the pesticide concentrations. Although the pesticide burden allowed us to include pesticides that were below their quantification limits in our correlation tests, visualizing the concentration of pesticides allows us to determine whether their concentrations are

correlated with test endpoints. This alignment might indicate potential drivers of toxic responses in the test organisms. A quantitative interpretation of concentration data is difficult due to the lack of research into chronic dietary exposures in mixtures. More, a pesticide present at a higher concentration may have a greater impact than several pesticides detected at trace levels, however, some pesticides may be highly toxic at a low concentration (e.g., Raby et al., 2018), while others may be relatively nontoxic at a high concentration (e.g., Nebeker and Schuytema, 1998). As such, we preface our qualitative analysis by calling for more research on environmentally-relevant mixture toxicities.

The NMDS illustrates pesticide concentrations, biofilm nutritional quality, and test diets collected from stormwater pond sites, with test endpoints shown in yellow font for the mayfly test and in red font for the snail test (Figure 4-5). The optimal ordination solution for the NMDS had a fair fit with two dimensions, a final stress of 0.178 following 93 iterations. This solution explained 61.7% of the total variance: 44.4% on NMDS1 and 17.0% on NMDS2.

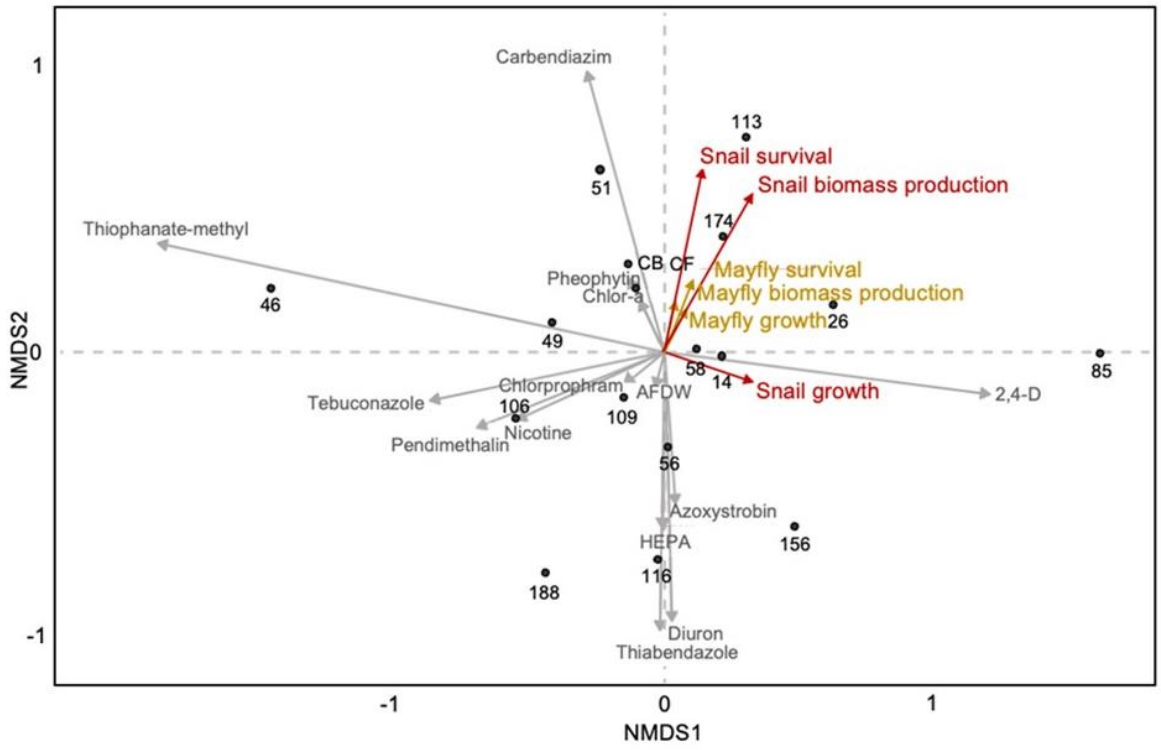


Figure 4-5. Nonmetric multidimensional scaling plot (stress = 0.178) of pesticide content and nutritional quality of biofilm diets used in bioassay tests. Survival, mean growth, and mean biomass production are averages of measured endpoints. Yellow font indicates a mayfly test endpoint; red

font indicates a snail test endpoint. Field sites where treatment diets were collected are noted with dots and labeled in black font. Chlor-*a* = chlorophyll-*a* content; AFDW = ash-free dry weight content; CB = control biofilm; CF = control fed.

The snail and mayfly endpoints showed a high degree of correlation, generally aligning positively with both NMDS1 and NMDS2 scores. Snail mean growth was an exception, for the reason already discussed (Section 4.4.3). The concentration of pesticides was not as collinear as reported from a study of agricultural streams (Ijzerman et al., 2023), but concentrations of certain groups of pesticides tended to be correlated. For example, thiabendazole, diuron, HEPA, and azoxystrobin were all negatively correlated with NMDS2 scores and with the concentration of carbendazim (Figure 4-5). Somewhat independently and aligned on NMDS1, the concentration of 2,4-D was negatively correlated with the concentration of a group of pesticides including chlorprophram, nicotine, pendimethalin and tebuconazole (Figure 4-5).

Based on the inverse correlation between most test endpoints and the concentration of eight of the pesticides, we elected to reexamine the correlation between survival rate, growth, and biomass production of snails and mayflies with pesticide burden, where we constrained pesticide burden to these eight pesticides. Our rationale was that the presence of pesticides which are typically less toxic to invertebrates (based on CCME reports), such as 2,4-D and carbendazim, could introduce noise that obscured the effect of pesticide burden on test endpoints. These eight pesticides included 1) thiabendazole, 2) diuron, 3) HEPA, and 4) azoxystrobin, which were more strongly associated with NMDS2 scores. We also included 5) chlorprophram, 6) nicotine, 7) pendimethalin, and 8) tebuconazole, which were more strongly loading on NMDS1 (results presented in Table C-11). This refinement of pesticide burden increased the statistical significance and strength of its correlations with snail survival ($p = 0.000204$; rho = −0.783) and biomass production ($p = 0.00158$; rho = −0.705), but our remaining endpoints remained uncorrelated with pesticide burden.

4.4.5 Dietary bioassays can indicate toxicity risk

In this study, we used dietary bioassays carried out with biofilm collected from urban stormwater ponds to investigate the toxicological effects of dietary exposure to biofilm contaminated with environmentally relevant mixtures of chemicals, including pesticides. By culturing the biofilm in stormwater ponds and then exposing aquatic invertebrates to it through dietary bioassays carried out in a controlled laboratory environment, we were able to separate the effects of consuming this contaminated biofilm from other exposure pathways, such as contact with water or sediment. Additionally, we eliminated predation- or

hydrologically-mediated effects that might influence the survival or growth of aquatic invertebrates in situ that would confound field surveys of invertebrate communities in stormwater ponds. With the dietary bioassay, we can infer that any effect on test species survival or growth is attributable to consuming the contaminated biofilm.

These tests were the first known feeding bioassays using biofilm cultivated in stormwater ponds and represent an important contribution to the development of novel ecotoxicological methods. As with all bioassays, these dietary bioassays screen for potential hazardous chemicals. The survival and growth of our test species were clearly reduced when fed biofilm from stormwater ponds compared to biofilm from a cleaner environment. For snails, their survival rate and biomass production were also negatively correlated with pesticide burden of the biofilm, indicating that pesticides may be playing a toxicological role, although we cannot rule out potential toxicity from other contaminants that we did not test for. The presence of pesticides may also indicate the presence of other associated pesticide additives that are known to be toxic to bacteria and algae (van de Merwe et al., 2018). Using field-cultured biofilms containing mixtures of contaminants at ambient concentrations that reflect the range of real-world conditions, we can provide some insight into the risk faced by occupants of common urban habitats. Dietary bioassay methods could make excellent additions to risk assessment frameworks that improve the interpretation of environmental monitoring results.

We observed variability in the responses of our two selected test species to contaminated biofilm from the stormwater pond sites. For example, biofilm diets from sites 188, 46, 56, and 116 resulted in lower survival in both mayflies and snails, however where test diets from sites 58, 113, and 14 had 0% mayfly survival by the end of the test, these same diets resulted in survival between 80 and 100% in snails. Growth and biomass production were higher for both test species when fed diets from sites 109, 51, and 85, and were lower when fed diets from sites 56, 113, 116, and 49. However, opposing results were observed from test diets from sites 156 and 85, where growth and biomass production was higher in mayflies and lower in snails, and in test diets from sites 46, 58, 188, 14, 174, and 26, where growth and biomass production was lower in mayflies and higher in snails). This variability may be the result of differing feeding behaviours, since mayflies are active and mobile in their search for food and may exhibit more selective behaviour than the more sedentary feeding activity of snails (e.g., preferential feeding behaviour was demonstrated by Beck et al. (2019) in the mayfly genus *Rhithrogena*). Additionally, both species are likely to have different nutritional requirements, as reflected by the different standardized culturing diets: lipid and protein-rich diatoms for mayfly nymphs (Weaver et al.,

2015) and fibre-rich spinach for snails (Prosser et al., 2016), although our results indicate that snails may benefit from a more diverse diet. More, as each test species was subjected to a different duration of exposure, the effects of contaminant toxicity or nutritional deficiency had varying lengths of time within which to manifest. Finally, there may be countless differences in the sensitivities of the test species to the contaminants (known and unknown) present in their diets.

Despite these distinctions in feeding behaviour, duration of exposure, and nutritional requirements, and the variability observed in treatment-specific responses, the test endpoints of both species generally aligned when visualized in the NMDS plot. This alignment, despite test species' differences, broadens and strengthens our conclusion that sensitive biofilm-consuming organisms living in stormwater ponds are likely suffering from previously unrecognized dietary exposure to contaminants, and we recommend further research to ascertain what contaminants might be responsible for the observed effects. We also recommend future research address the critical lack of data supporting the development of dietary ecological thresholds and prioritize the development and integration of dietary bioassay methodologies into ecological risk assessments.

4.5 References

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Chapter 5: An Urban Stormwater Contaminant Signature: Defining priority contaminants for urban stormwater research

5.1 Overview

Stormwater ponds (SWPs) are a common feature of urban landscapes, designed to manage runoff and reduce flooding. Increasingly, they are also recognized as seminatural habitats supporting aquatic biodiversity. However, SWPs receive complex mixtures of contaminants from surrounding urban areas, and the extent of contamination within the ponds themselves remains underexplored. Most studies have focused on outflows or a narrow set of targeted analytes, limiting our understanding of the exposure risks for organisms residing within these systems. To address this gap, we assessed contaminant profiles in 21 SWPs across a highly urbanized city in Ontario, Canada, using three complementary sampling approaches: time-integrated water samples, biofilm on artificial substrates, and organic diffusive gradients in thin films (o-DGTs). Across all sites, we detected 200 organic compounds, including pesticides, pharmaceuticals, industrial chemicals, and compounds, linked to vehicles and infrastructure. Additionally, we documented widespread chloride and fecal contamination and elevated levels of traffic-related metals in biofilms. From these data, we identified a set of frequently detected and environmentally relevant contaminants, which we term the *urban stormwater contaminant signature* (USCS). This proposed list may support the development of targeted monitoring strategies and help focus future research on mixture toxicity and risk to aquatic biota. Given the apparent ecological role of SWPs and the range of stressors they contain, assessing cumulative exposures is critical for understanding the potential impacts of urban runoff on resident organisms.

5.2 Introduction

Chemical pollution is a pervasive and escalating threat to global biodiversity, contributing significantly to ecological degradation across a wide range of environments (Groh et al. 2022; Sigmund et al. 2023; Osukoya et al. 2024). Globally, the number of registered chemicals and commercial mixtures is well over 350,000 (Wang et al. 2020). As shown in Chapters 2 and 3, stormwater management ponds (SWPs) are receiving bodies for chemical pollution from wet-weather discharge in urban areas. These highly engineered hydraulic features integrate many contaminants from the surrounding catchment prior to their release into natural habitats, making them an excellent focal point for environmental monitoring in urban areas (Marques et al. 2024). SWPs can act as a barometer of contaminant exposure in urban landscapes

because they accumulate contaminants from their sewersheds over spatial and temporal variability in concentration and occurrence (Gallagher et al. 2011; Tixier et al. 2011; Flanagan et al. 2021).

For the purpose of our study, we refer to the term stormwater as water from rain, melting snow, or outdoor residential discharge (e.g., car washing, lawn watering) that runs off impervious surfaces into the municipal storm sewer system. Stemming from an assortment of sources, diverse contaminants found in urban stormwater runoff can include organic contaminants, such as pharmaceuticals, pesticides, and biocides (Masoner et al. 2019); nutrients, such as phosphorus and nitrogen (Pamuru et al. 2022); metals and metalloids (Birch 2024); salt (Marsalek 2003); and bacterial contamination (Tiefenthaler et al. 2011). This assortment of contaminants is distinct from expected contaminant profiles in agricultural lotic and lentic surface waters (Gómez et al. 2012; Khatri and Tyagi 2015), and the overall toxicological risk that this mixture presents to biota living in urban environments is a complex, multifaceted area requiring more research (Tang et al. 2013; Young et al. 2018; Popick et al. 2022). In this Chapter, we present an approach to characterizing that risk through an integrated assessment of contaminants in stormwater ponds located in a highly urbanized landscape. We seek to identify the common chemicals that co-occur and propose the term *urban stormwater contaminant signature* (USCS). The definition of a USCS benefits water quality monitoring and management in urban centers, including guiding source tracking and identifying priority contaminants for ecotoxicological investigations.

A contaminant signature is a distinct combination of chemical contaminants associated within a specific context. Contaminant signatures can be used as “fingerprints” to help identify sources of contamination and their contributions (Plumb 2004; Du et al. 2020; Fennell et al. 2021; Kaown et al. 2021) and to provide a basis for generalizations. Contaminant signatures have been developed for pesticides in urban streams across the United States (Nowell et al. 2021), metals in an urban river in Ukraine (Vystavna et al. 2013), and fecal pollution in Lake Michigan's urbanized coast (Newton et al. 2013). Predictable contaminant profiles have been found in other stormwater features (e.g., storm drains; Du et al. 2020; Gasperi et al. 2022). We define a UCSC as a characteristic mixture of contaminants found in stormwater runoff originating from urban areas. The USCS reflects the unique combination of contaminants introduced by human activities, infrastructure, and land use patterns within cities. Given the diversity of contaminants in urban areas, we determined that it would be useful to define a USCS to help focus future monitoring efforts of urban contamination in stormwater-impacted aquatic ecosystems on the most ubiquitous contaminants, inform an approach to predicting the toxic effect of this commonly

occurring mixture, and allow the comparison of contaminant signatures among regions to see how different urban areas might differ in their urban contaminant exposure profile.

Most assessments of contaminants in stormwater are focused on the outflows of SWPs and their potential impacts on receiving lakes and rivers (e.g., Koziel et al. 2019) or on other stormwater infrastructure (e.g., culverts and channels; Masoner et al. 2019). Consequently, research and monitoring on the level of contaminants in stormwater ponds proper are still limited. Five consecutive annual reviews of urban stormwater research found that studies tended to focus on a single contaminant type (e.g., metals, PCBs, PAHs, or pesticides), rather than surveying a broad suite of contaminant types (Vogel and Moore 2016; Moore et al. 2017, 2018; Rodak et al. 2019, 2020). A study of The International Stormwater Best Management Practices database and the National Stormwater Quality Database found a rich collection of observations of traditional water quality metrics, such as chloride and nutrient concentrations; however, reports of metal and organic contaminants were limited (Pamuru et al. 2022). Comprehensive approaches to water quality monitoring in SWPs are lacking. Yet understanding the concentration and occurrence of a broad range of possible contaminants in stormwater is necessary to evaluate the ecological risk posed to aquatic organisms that live in or are exposed to SWPs, given the diversity of potential toxicants released into urban environments (e.g., Masoner et al. 2019; Pamuru et al. 2022). With natural ponds and wetlands being replaced by SWPs at an alarming rate (Birch et al. 2022), SWPs now play an increasingly crucial role in supporting aquatic (Ferzoco and McCauley 2024) and terrestrial (Holtmann et al. 2017; Bishop et al. 2000) life as an unintentional urban habitat (McKercher et al. 2024). Conserving urban biodiversity depends on monitoring all habitat, conventional or not, where life resides.

We conducted an integrated assessment of stormwater pond water quality with three complementary sampling approaches: composite water grab sampling, biofilm sampling from artificial substrates, and passive sampling using organic diffusive gradients in thin films (o-DGT) samplers. By sampling from three different substrate types in two different analytical labs, we were able to assess a holistic exposure profile by targeting a range of potential exposure pathways, as the presence of contaminants in the biofilm exposes aquatic organisms through their diets while the presence of contaminants in the water exposes biota through contact with their skin or via their gills. We measured 542 legacy and current-use pesticides in composite water and biofilm samples and 491 urban-use contaminants in o-DGT samplers. We analyzed 20 metal and metalloids in biofilm samples. We also measured standard water quality parameters, including nutrient concentrations, salinity, bacterial coliforms, dissolved oxygen (DO), and total suspended solids (TSS). We used the Canadian Council of

Ministers of the Environment (CCME) Canadian Water Quality Guidelines (CWQG) for the Protection of Aquatic Life to contextualize the quality of the stormwater. The research took place at 21 SWPs in a highly urbanized city representing the effects of present-day rapid suburban expansion on stormwater runoff.

5.3 Methods

5.3.1 Study area

We surveyed 21 stormwater ponds in Brampton, Ontario, Canada (Figure D-1), one of the most rapidly expanding cities in Canada (Statistics Canada 2021). Brampton is a representative mix of urban, suburban, light industry, and highway land uses for a growing North American municipality. The ponds range in size from 1051 to 3686 m^2 (mean = 2272, stdev = 851 m^2; McIsaac 2022). The impervious cover within 300 m of each pond ranges from 10.9% to 55.2%, representing a gradient of urbanization (McIsaac 2022; please refer to Appendix D for location and descriptions of each pond). These ponds are wet ponds, meaning they are designed to hold water throughout the year. We selected ponds that were at least 10 years old and had not been dredged in the past decade to prevent age or recent maintenance serving as confounding variables.

5.3.2 Sampling methods

We sampled three media types (water, biofilms, and o-DGTs) for various characteristics of contamination, as summarized in Table 5-1 and described in the following sections. The survey period took place over the course of nine weeks, from May 25 to July 25, 2022.

Table 5-1. Overview of sampling methods used to survey contamination of urban stormwater ponds (SWPs) in Brampton, Ontario. All analyses were carried out on samples from 21 stormwater ponds, unless otherwise noted.

Sampling media	Method	Analyses
Water	1.35 L composite of nine 150 mL surface water grab samples taken weekly over a nine week period	• 542 current-use and legacy pesticides • Nutrients • *E. coli* and coliform bacteria • Total suspended solids, dissolved oxygen, and chloride

Biofilm	300 – 500 g (wet weight) composite samples from biofilm cultured on artificial substrates for nine weeks	• 542 current-use and legacy pesticides • 20 metals and metalloids (in 15 ponds)
o-DGT samplers	Two 27-day deployments of two duplicates	• 491 urban contaminants (including pesticides, pharmaceuticals, and other anthropogenic chemicals)

5.3.2.1 Water sampling

Water sampling and water quality surveys were completed once in the summer of 2022 and the collected data have been previously investigated in prior Chapters and publications (Izma et al. 2024a, 2024b, 2025) to address other, distinct objectives. Detailed sampling methods are described in these previous Chapters and publications; here we provide a brief summery. Over the nine week survey period, we collected weekly 150 mL of surface water grab samples to compose a time-integrated composite sample of 1.35 L from each pond. Each composite sample was stored frozen at −20°C until submission to the Agriculture and Food Laboratory (AFL) (ISO/IEC 17025 accredited) in Guelph, Ontario.

We took weekly measurements of temperature (°C) and conductivity (mS cm^{-1}) using a Hach HQ1140 Portable Conductivity/TDS Meter (Hach Sales and Services LP, London, Canada) and DO (mg L^{-1}) using a YSI ProSolo ODO Optical Dissolved Oxygen Meter (Xylem Inc., Miami, FL) at three points in the open water at ~20 cm deep. Each week, we took 50 mL of surface water grab samples from a depth of ~20 cm and placed them within a high-density polyethylene Nalgene bottle to measure TSS (mg L^{-1}) gravimetrically and chloride ion concentration (Cl^-; mg L^{-1}) colorimetrically, using a Chloride Checker HC (Hannah Instruments, Woonsocket, RI).

We submitted a separate, one-time water grab sample from each site to ALS Laboratories in Waterloo, Ontario, for fluorometric determination of ammonia (NH_3), ion chromatographic determination of nitrate (NO_3) and nitrite (NO_2), and calorimetric analysis of orthophosphate (PO_4). These samples were taken during the week of June 21–27, 2022. No precipitation was recorded at the nearest weather station from June 21 to 26, and total rainfall for June 27 was 0.4 mm (Figure D-2). To describe bacterial contamination, we collected water grab samples from each site on June 8, 2022, following a precipitation event on June 7 (total rainfall was measured at 12.6 mm at the nearest weather station; Figure D-2), and submitted them to AFL for plate-count analysis of total coliform bacteria and *Escherichia coli*.

These water chemistry parameters are summarized in Table D-1. Results of all measured water chemistry parameters can be found in the Figshare repository linked in Appendix D and are summarized in Table D-2.

5.3.2.2 Biofilm sampling

Biofilm sampling was completed once in the summer of 2022 and the collected data have been previously investigated in prior Chapters and publications (Izma et al. 2024a, 2024b, 2025) to address other, distinct objectives. At each SWP site, we cultured biofilm in situ, as described in more detail in Chapters 2 and 3. Briefly, we used 10 acrylic plates (20.2 × 44.4 cm) as standard substrates on which to grow biofilm in the ponds. We suspended these on floating racks ~10 cm below the surface of the water for 54 days to allow for maximum biofilm growth (Figure D-3). Upon retrieval, we scraped each side of each plate into a composite sample for each SWP. We then freeze-dried 300–500 g (wet weight) of each composite sample using a FreeZone I Labconco benchtop freeze dryer (Labconco Corporation, Kansas City, MO) prior to submission to AFL for pesticide analysis on all 21 biofilm samples. An aliquot of freeze-dried biofilms was taken from 15 samples with sufficient biomass, as well as a sample from a reference site, and sent to Biotron at the University of Western Ontario in London, Ontario, for analysis of 20 metals and metalloids. The reference site was the Batteaux River (44.48831889, −80.16709086), near Collingwood, Ontario. This river flows through undeveloped land and represents a relatively pristine environment (e.g., no pesticides were detected in these biofilm samples; Ijzerman et al. 2024). We sampled biofilms at this reference site under the same field sampling protocol as for the SWP sites, using similar acrylic plates as artificial substrates deployed over an eight week deployment.

5.3.2.3 o-DGT Sampling

Passive sampling was completed once in the summer of 2022 and the collected data have been previously investigated in Chapter 2 to address a different objective. Detailed methods have been described in Chapter 2; here we provide a summary. We conducted two consecutive 27-day deployments of two duplicate o-DGT (DGT Research Inc., UK; LSND-AT with PTFE membranes; exposed area = 3.14 cm^2) samplers in each pond, aligned with the water and biofilm sampling regimes. Each sampler pair was mounted on custom-built holders (Figure D-4) and attached to the biofilm sampler floating rack to suspend them at consistent depths (~20 cm) in the water column during deployment. A travel blank was used for each day of deployment and collection. After collection, samplers were wrapped in clean aluminum foil, transported on ice, and stored frozen at −20°C until processing.

5.3.3 Analysis

5.3.3.1 Organic chemical analysis

5.3.3.1.1 Water and biofilm analysis

All analyses of water and biofilm samples were performed at the Agriculture and Food Lab of the Laboratory Service Division at the University of Guelph (ISO/IEC 17025 accredited). Methods of these analyses are described in Chapters 2, 3, and 4. Samples were analyzed for 542 current-use and legacy pesticides using multi-residue liquid chromatography/electrospray ionization–tandem mass spectrometry (LC/ESI–MS/MS) and gas chromatography–tandem mass spectrometry (GC–MS/MS). A list of all chemical analytes from all pesticide screens, along with their detection and quantification limits, can be found the Figshare repository, linked in Appendix D.

5.3.3.1.2 o-DGT analysis

o-DGT samplers were extracted and analyzed via previously described methodologies in Chapter 2. Briefly, the samplers were thawed and disassembled, and the resin gels were sequentially extracted with methanol in 15-mL polypropylene centrifuge tubes (Corning reference 430052). Analyses for 491 contaminants (including insecticides, herbicides, fungicides, pharmaceuticals, and other anthropogenic chemicals) were conducted by Agriculture and Agri-Food Canada (AAFC; London, ON, Canada) using liquid chromatography–tandem mass spectrometry (LC–MS/MS) with a Vanquish Duo high-performance liquid chromatograph (HPLC) coupled to a Thermo Fisher Scientific Altis triple-quadrupole mass spectrometer. Details of the target analytes, including their limits of detection (LODs), limits of quantification (LOQs), multiple reaction monitoring (MRM) transitions, and retention times, are available in the Figshare repository linked in Appendix D. LODs were calculated based on guidelines from the International Council for Harmonisation (ICH), accessed in 2019. Quantitative analysis was performed using Thermo Fisher Scientific TraceFinder 5.0 software.

5.3.3.2 Metal analysis

Freeze-dried biofilm samples from 15 SWP sites (only 15 of the 21 SWP sites contained sufficient mass for analysis) and one reference site were analyzed by Biotron Laboratory, an ISO/IEC 17025–accredited laboratory. Samples were first microwave acid-digested following EPA Method 200.8 (USEPA 1994). Analysis was completed by inductively coupled plasma mass spectrometry (ICP-MS) using Agilent

7700x. Na, Mg, Al, Ti, V, Cr, Mn, Fe, Co, Ni, Cu, As, Sr, Mo, Cd, Sn, Ba, Pb, and Zn were analyzed using the He Gas method, and Se was analyzed using the No Gas method. The coefficient of determination (R^2) was > 0.9980. The recovery of quality control samples was within 15% of the expected value. The recovery of matrix spike and matrix spike duplicates was within 30% of the known value, and the relative percentage difference in these spikes was within 20% of the control limit. The relative percentage difference in sample duplicates was within 20% of the control limit. The method detection limits (MDL) and method recovery limits (MRL) are available in the Figshare repository, linked in Appendix D. The MDL and MRL are calculated once every year to comply with EPA MDL revisions (USEPA 2016).

5.3.4 Detection frequencies

We define a detection as a quantified amount of a compound above the level of detection in a matrix. For o-DGTs samplers, a detection is an occurrence above the LOD in at least one of the four o-DGT samplers deployed in each stormwater pond. A detection represents an occurrence of a compound at any time point during the deployment period; thus, measured quantities above the LOD in more than one sampler per pond is still defined overall as a singular detection of that compound for that particular site. Detection frequencies were calculated by dividing the number of detections of a compound by the number of sampling sites (n = 21) and multiplying by 100%. Our criteria for inclusion within the proposed USCS was a detection frequency of 80% or higher, representing detection in at least 17 of the 21 SWPs, to encapsulate contaminants with widespread occurrences. This arbitrary threshold was chosen to align with similar studies characterizing contaminant signatures (e.g., Nowell et al. 2021; Peter et al. 2018), although alternative approaches to formally identify common toxicological mixtures exist (e.g., Scott et al. 2013; Loken et al. 2023).

5.4 Results and discussion

5.4.1 Breadth of contaminants in urban stormwater ponds

Our results reveal a complex mixture of urban contaminants across the three matrices and evidence that water quality is impaired and may present a risk to aquatic biota (as tested in Chapter 4; Izma et al. 2024b). SWPs are engineered primarily to manage runoff quantity, accumulate and settle excess sediment, and improve downstream water quality; however, they are increasingly recognized as *de facto* urban aquatic habitats (Hassall and Anderson 2015; Moore and Hunt 2012). This dual role creates tension

between their intended function as treatment infrastructure and their unintended role as ecological space, one that often lacks the physical or chemical conditions needed to sustain aquatic life, such as periodic draining and dredging to maintain storage volume. Despite not being designed for habitat provision, SWPs support diverse biotic communities (Ferzoco and McCauley 2024), making it important to assess the contaminants present and consider their implications for aquatic health.

Below, we discuss the results and implications of each contaminant group, starting with commonly measured water quality parameters like chloride concentration, TSS, and nutrients, before discussing the metals and metalloids and organic contaminant categories more unique to urban environments, specifically stormwater pond water quality.

5.4.1.1 Chloride

Composite water samples had mean chloride concentrations ranging from 61 mg L^{-1} to above the upper method quantification limit (MQL; 200 mg L^{-1}; Table D-2), and mean concentrations in 17 of the 21 SWPs surpassed the CWQG long-term exposure guidelines for the protection of aquatic life (120 mg L^{-1}; CCME 2011). Existing monitoring in Ontario via the Provincial Stream Water Quality Monitoring Network has also observed chloride levels in urban waters exceeding the chronic CWQG guideline since the year 2000 (Sorichetti et al. 2022). Not only do elevated chloride concentrations (i.e., above the CCME guidelines) pose direct toxicological risks (Marsalek 2003; Hassell et al. 2006; McIsaac 2022), but also excessive amounts of chloride in ponds can create density gradients that inhibit mixing and limit aeration (Szklarek et al. 2022), leading to a lack of oxygen and high chloride levels in the bottom layers of the pond. These conditions can in turn alter chemical dynamics in the benthic region, for example, causing the leaching of metals (Marsalek 2003). This is an excellent example of the value of considering chemical mixtures rather than just single contaminants or contaminant classes in isolation: The presence of chloride may modify the bioavailability or toxicity of other contaminants (Hall and Anderson 1995).

5.4.1.2 TSS

Stormwater runoff is a major source of TSS into other aquatic systems; thus, one of the core objectives of stormwater management in Ontario is the 80% removal efficiency of TSS within SWPs prior to release (OME 2003). Suspended solids accumulate from construction sites, road dust, soil erosion, sediment erosion from fast flows, deposition, and litter (USEPA 2025). The turbidity caused by increased TSS levels can inhibit algal and submerged vegetation growth, and excess deposition of foreign sediments can

smother important habitat for benthic invertebrates and bottom-dwelling fish (Horner et al. 1994). These fine particulates may also serve as a sink for other contaminants, such as metals (Herngren et al. 2005) and fecal coliforms (Murray et al. 2001). Mean TSS concentrations at our ponds ranged from 7.3 to 135.4 mg L^{-1}, averaging 38.6 mg L^{-1} across all sites (Table D-2). This range fits into the lower end of the observed distributions reported in the international and national stormwater quality databases summarized by Pamuru et al. (2022) and in the Ontario Stormwater Management Planning and Design Manual (OME 2003). Guidelines for TSS in Canada are designed for stream systems and are based on background levels from reference sites (CCME 2002), which may not be appropriate for SWPs; therefore, we are unable to place our measured TSS concentrations into an ecotoxicological context.

5.4.1.3 DO

As receivers of thermal pollution (Sabouri et al. 2016), SWPs may be particularly susceptible to low-oxygen (hypoxic) conditions because DO concentrations are inversely related to water temperature (Boyd and Boyd 2020). Across the 9-week sampling period, mean daytime DO levels within the ponds (measured at depths of ~20 cm from the water surface between 10:00 and 16:00 h) ranged from 2.87 to 14.1 mg L^{-1} (Table D-2). Three ponds had means below 6 mg L^{-1}, the CWQG threshold for early-life stage warmwater organisms (CCME 1999). Although SWPs are not designed to support oxygen-sensitive taxa, many become colonized by aquatic biota. It is important to note that DO in shallow waters can fluctuate diurnally, especially in the presence of submersed aquatic vegetation (Boyd and Boyd 2020), of which there is commonly an abundance in SWPs. The concentration of DO could reach daily minima late at night when respiration proceeds but photosynthesis is absent, making our daytime observations a conservative assessment of ecological risk. There is evidence that some organisms, such as chironomids and snails, can acclimate or adapt to chronically low oxygen levels (CCME 1999); however, the presence of other stressors in hypoxic conditions can be confounded (Lowell and Culp 1999).

5.4.1.4 Nutrients

Ammonia (NH_3), nitrate (NO_3), nitrite (NO_2), and phosphate (PO_4) concentrations in the SWP water were generally found to be low, with most NO_3, NO_2, and PO_4 concentrations being below detection limits (Table D-2). Only one site exceeded the CWQG values for NH_3 for freshwaters (1.54 mg L^{-1}; CCME 2010) with a concentration of 2.50 mg L^{-1}. Governmental stormwater policies often aim to reduce nutrient loading to prevent eutrophication in sensitive downstream waters (e.g., OME 2003). Our findings suggest generally effective nutrient removal, but these one-time samples may not capture seasonal variability or

storm-driven spikes. During rainfall events, nutrients can originate from pet waste, fertilizers, detergents, and decomposing vegetation washed into SWPs (USEPA 2023).

5.4.1.5 Bacterial contaminants

Fecal indicator bacteria such as total coliforms and *E. coli* were detected in all ponds. Nineteen ponds exceeded 1000 cfu 100 mL^{-1} for total coliforms and 13 exceeded 235 cfu 100 mL^{-1} for *E. coli* - benchmarks used for recreational waters (Government of Ontario 1994; Government of Canada 2023). Samples from multiple ponds exceeded the upper analytical limits for these bacterial contaminants. While SWPs are not designated for swimming, they are often situated near parks and green spaces where incidental human exposure may occur. Bacterial contamination is primarily a public health concern rather than a direct toxic threat to aquatic organisms, yet may result in indirect ecological effects. For example, high bacterial loads can result in increased biological oxygen demand, reducing oxygen levels in the water (USEPA 2012). Feces from pets, birds, or other wildlife are likely sources of contamination (Staley et al. 2018; Beaudry 2019); however, contamination of stormwater with human sewage from leaking wastewater infrastructure is well documented in the literature and cannot be ruled out as a source of microbial contaminants in stormwater ponds (e.g., Sercu et al. 2011; Sauvé et al. 2012; Sidhu et al. 2013; Fairbairn et al. 2018; Masoner et al. 2019). Given the ambiguity in recreational use and ecological exposure, microbial contamination warrants further monitoring.

5.4.1.6 Metals and metalloids

All 20 analyzed metals and metalloids were found in every SWP biofilm sample (n = 15). Traffic-associated metals (Ba, Cr Co, Cu, Pb, Mn, Ni, Se, Sr. and Zn; as described by Hjortenkrans et al. 2006 and Wang et al. 2021) were higher in biofilm samples from at least half of ponds compared with the reference site. Traffic pollution, from both emission and non-emission sources, is an important contributor of heavy metals to stormwater (Czemiel Berndtsson 2014), especially in car-dominated cities like the study city Brampton. Nine of the 15 SWP sites were within 300 m of arterial roads, however whether they are included in the engineered sewershed drainage basin of the sites is unknown. Busy arterial roads (> 5000 cars per day) can contribute higher levels of heavy metals than highways, due to factors such as starting and stopping in traffic congestion and at streetlights, which results in increased shedding of tire and brake materials (Huber et al. 2016).

For seven of the 20 metal analytes (Na, Mn, Cu, Zn, As, Mo, Ba), concentrations in biofilm samples were higher at every single stormwater pond site than at a reference stream. Guidelines do not exist for dietary exposure to these metals through biological materials, thus inhibiting our ability to assess the potential effects of consumption of metal-contaminated biofilms. The development of appropriate thresholds for this type of exposure is warranted. A preliminary assessment of the effects of chronic dietary uptake of Cd by *Hyalella azteca* via periphyton found that dietary Cd contributed to decreased survival and that this type of exposure was more responsible for increased Cd body concentration than through water exposures (Golding et al., 2011a, 2011b).

Metal profiles can reflect distinctive urban source signatures - vehicle wear, construction materials, and possibly building runoff (e.g., Zn from roofs; Wicke et al. 2022). Instead of focusing on threshold exceedances, which are limited in their applicability to biofilm or dietary exposures, these contaminants are better understood as indicators of urban influence and mechanisms for source tracking. Their presence is also an important consideration for exploring mixture effects, discussed further in Section 5.4.3.

5.4.1.7 Organic contaminants

Across the three sampling matrices, we found a total of 200 organic contaminants, including 67 pharmaceuticals, 51 herbicides, 37 fungicides, 26 insecticides, and 20 other compounds associated with building materials, traffic, or other consumption and metabolic waste products (e.g., caffeine and its breakdown product paraxanthine). In Chapter 2, we compared o-DGTs, composite water samples, and biofilm samples, and concluded that combining all three matrices yielded valuable complementarity in contaminant detection. Importantly, o-DGTs provided lower detection limits than the other two matrices; however, between-duplicate discrepancies across sites revealed that analyte quantifications for o-DGTs were not reliable (see Chapter 2). Consequently, herein, we limit our discussion of organic chemicals in all three matrices to presence/absence results only. The number of organic compounds detected at each site ranged from 45 to 138, indicating SWPs are exposed to a large assortment of contaminants. Chemicals known to have potential ecotoxicological consequences are present in these mixtures, such as imidacloprid which can be detrimental to mayfly nymphs (Raby et al. 2018; Macaulay et al. 2021), however reliable concentration data is required to define their toxicity. Some of these chemicals are also known to bioaccumulate in aquatic environments (Fernandes et al. 2020), potentially prolonging the duration of exposure. The pesticides atrazine and azoxystrobin, omnipresent in our SWPs, can induce

toxic effects to non-target organisms at environmentally relevant concentrations (Khoshnood 2024; Kunz et al. 2017). Further, the assortment of pesticides and coating preservatives sourced from building materials include both hazardous substances to aquatic biota, such as carbendazim (Jiang et al. 2015), and substances with little or no available ecotoxicological information, such as sebacic acid. Alarmingly, there is a critical lack of relevant exceedance guidelines for organic contaminants for the protection of aquatic life (e.g., of the 114 pesticides detected in our samples, only 11 [atrazine, bromacil, bromoxynil, carbaryl, dicamba, tebuthiuron, simazine, metolachlor, metribuzin, MCPA, and imidacloprid] have CWQG values). This data gap severely limits ecotoxicological interpretation. We discuss the sources and detection frequencies of pesticides, pharmaceuticals, and other human-associated contaminants in the following two sections.

5.4.1.7.1 Pesticides

We detected 114 pesticides (including herbicides, fungicides, and insecticides) in our SWPs, many of which could be attributed to uses in landscaping. In Ontario, golf courses, sports turf, and transportation corridors are exempt from the Cosmetic Pesticide Ban, implemented in 2009, allowing intensive use of herbicides, insecticides, and fungicides in those areas (Government of Ontario 2009). Turfgrass management may contribute a disproportionate amount of pesticide releases to urban waters, as application rates are estimated to be significantly higher than on agricultural land (Schueler 2000). Levels of carbendazim in urban surface waters were previously found to be associated with the number of golf courses in two urban catchments in Ontario (Metcalfe et al. 2016). From visual assessment of aerial imagery of our SWPs, we can identify golf courses and sports fields in proximity to our study sites; however, as we did not have full access to the sewershed boundaries for the SWPs, we were unable to verify whether or not our SWP sewersheds actually contained golf courses or sports turf.

In addition to their use on lawns and turfgrass, herbicides are also used to control unwanted vegetation in private and public grounds. Some, such as 2,4-D and mecoprop, are sold at hardware stores for use in residential landscaping (despite their ban for cosmetic use), while others, such as prometon, are often applied directly to municipal grounds, such as rights-of-way and roadside ditches (AERU 2024), facilitating their entry into the stormwater system (Table 5-2).

Table 5-2. The most frequently detected organic compounds found in the 21 stormwater pond (SWP) sites (detection frequency = 81–100%), listed in order of decreasing detection frequency. All chemical properties and source information was retrieved from NCBI's PubChem unless noted otherwise.

Compound name	CAS number	Use	Possible urban source	Mode of action	Detection frequency (%)	Matrix detected in
2,4-D	94-75-7	Herbicide	Lawn/turf care	Synthetic auxin [d]	100	Water, biofilm, o-DGTs
4-Methyl-1H-benzotriazole	29878-31-7	Industrial (UV stabilizer)[a]	Building or other outdoor materials	Unknown but likely enzyme-related	100	o-DGTs
6PPD-quinone	2754428-18-5	Traffic-related (Component of vehicle tires)	Tire-wear	Species-dependent, often altering enzyme activity [b]	100	o-DGTs
Atrazine	1912-24-9	Herbicide	Lawn/turf care	Photosystem II inhibitor [d]	100	o-DGTs
Azoxystrobin	131860-33-8	Fungicide	Lawn/turf care	Respiration inhibitor [d]	100	Biofilm, o-DGTs
Caffeine	58-08-2	Household (psychoactive stimulant, diuretic, respiratory and cardiac stimulant)	Unknown	Unknown	100	o-DGTs
Carbendazim	10605-21-7	Fungicide	Lawn/turf care; building materials (paints, textiles, caulks, concrete, etc.)	Mitosis and cell division inhibitor [d]	100	Water, biofilm, o-DGTs
Chlorantraniliprole	500008-45-7	Insecticide	Lawn/turf care	Ryanodine receptor modulator [d]	100	o-DGTs
Clomazone	81777-89-1	Herbicide	Unknown	Chlorophyll and carotene synthesis disruptor [d]	100	o-DGTs

Compound name	CAS number	Use	Possible urban source	Mode of action	Detection frequency (%)	Matrix detected in
DEET	134-62-3	Household (insect repellant)	Direct release	Neurotoxin	100	o-DGTs
Desethylatrazine	6190-65-4	Herbicide (metabolite)	Lawn/turf care	Photosystem II inhibitor	100	o-DGTs
Desisopropylatrazine	1007-28-9	Herbicide (metabolite)	Lawn/turf care	Photosystem II inhibitor	100	o-DGTs
Fluopyram	658066-35-4	Fungicide	Lawn/turf care [c]	Succinate dehydrogenase inhibitor [d]	100	o-DGTs
Hydroxyatrazine	2163-68-0	Herbicide (metabolite)	Lawn/turf care	Photosystem II inhibitor	100	o-DGTs
MCPA	94-74-6	Herbicide	Lawn/turf care	Synthetic auxin [d]	100	Water, biofilm, o-DGTs
Mecoprop	93-65-2	Herbicide	Lawn/turf care	Synthetic auxin [d]	100	Water, o-DGTs
Metalaxyl	57837-19-1	Fungicide; wood preservative	Lawn/turf care; construction and building materials	Fungal nucleic acid synthesis disruptor [d]	100	o-DGTs
Metolachlor	51218-45-2	Herbicide	Lawn/turf care	Cell division inhibitor [d]	100	o-DGTs
Mirtazapine	85650-52-8	Pharmaceutical (antidepressant)	Unknown	Unknown	100	o-DGTs
Penflufen	494793-67-8	Fungicide	Unknown (agricultural)	Succinate dehydrogenase inhibitor [d]	100	o-DGTs
Propazine	139-40-2	Herbicide	Greenhouses [d]	Photosystem II inhibitor	100	o-DGTs
Propiconazole	60207-90-1	Fungicide; wood preservative	Lawn/turf care; construction and building materials.	Demethylation inhibitor	100	o-DGTs

Compound name	CAS number	Use	Possible urban source	Mode of action	Detection frequency (%)	Matrix detected in
Pydiflumetofen	1228284-64-7	Fungicide	Lawn/turf care; greenhouses	Succinate dehydrogenase inhibitor [d]	100	o-DGTs
Simazine	122-34-9	Herbicide; algicide	Lawn/turf care; direct release	Photosystem II inhibitor [d]	100	o-DGTs
Tris(2-chloroethyl) phosphate (TCEP)	5961-85-3	Industrial (flame retardant)	Unknown	Unknown	100	o-DGTs
Tebuconazole	107534-96-3	Fungicide; wood preservative	Lawn/turf care; construction and building materials	Sterol biosynthesis inhibitor [d]	100	Biofilm, o-DGTs
Tebufenozide	112410-23-8	Insecticide	Urban forestry	Ecdysone receptor agonist [d]	100	o-DGTs
Thiabendazole	148-79-8	Fungicide; Pharmaceutical (anthelminthic agent); Household (antimicrobial in cosmetics)	Urban forestry	Compromises cytoskeleton via selective interaction with ß-tubulin [d]	100	Biofilm, o-DGTs
Bentazon	25057-89-0	Herbicide	Lawn/turf care	Photosystem II inhibitor [d]	95	Water, o-DGTs
Carbamazepine-10,11-epoxide	36507-30-9	Pharmaceutical (antiepileptic)	Unknown	Unknown	95	o-DGTs
Diuron	330-54-1	Herbicide (algicide)	Building/construction materials (paints/plasters/coatings)	Photosynthesis inhibitor [d]	95	Water, biofilm, o-DGTs
Fluxapyroxad	907204-31-3	Fungicide	Lawn/turf	Succinate dehydrogenase inhibitor [d]	95	o-DGTs

Compound name	CAS number	Use	Possible urban source	Mode of action	Detection frequency (%)	Matrix detected in
Imazethapyr	81335-77-5	Herbicide	Unknown (agricultural)	Plant amino acid synthesis inhibitor [d]	95	o-DGTs
Lidocaine	137-58-6	Pharmaceutical (anesthetic)	Unknown	Unknown	95	o-DGTs
Prometon	1610-18-0	Herbicide	Lawn/turf care; maintenance of railways and right-of-ways	Photosynthesis inhibitor [d]	95	o-DGTs
Sebacic-acid	111-20-6	Industrial (corrosion inhibitor, adhesive, paint additive, binder, etc.)	Building and construction materials; traffic-related (lubricant additive)	Not found	95	o-DGTs
Triclopyr	55335-06-3	Herbicide	Lawn/turf care; urban forestry.	Synthetic auxin [d]	95	Water
Clothianidin	210880-92-5	Insecticide	Urban forestry [e]; domestic pet care; domestic pest control	Nicotinic acetylcholine receptor competitive modulator [d]	90	Water, o-DGTs
Flupyradifurone	951659-40-8	Insecticide	Unknown (agricultural)	Nicotinic acetylcholine receptor competitive modulator [d]	90	Water, o-DGTs
Mefentrifluconazole	1417782-03-6	Fungicide	Unknown (agricultural)	Sterol biosynthesis inhibitor [d]	90	o-DGTs
Paclobutrazol	76738-62-0	Herbicide (plant growth regulator)	Lawn/turf care [f]	Disruption of gibberellic acid production and abscisic acid destruction [e]	90	o-DGTs
Paroxetine	61869-08-7	Pharmaceutical (antidepressant)	Wastewater contamination	Unknown	90	o-DGTs

Compound name	CAS number	Use	Possible urban source	Mode of action	Detection frequency (%)	Matrix detected in
Piperonyl-butoxide	51-03-6	Insecticide (synergist)	Domestic pest control	P450-dependent monooxygenase inhibitor [d]	90	o-DGTs
Imidacloprid	138261-41-3	Insecticide	Urban forestry [e]; domestic pet care; domestic pest control	Nicotinic acetylcholine receptor competitive modulator [d]	86	Water, o-DGTs
Dimethenamid	87674-68-8	Herbicide	Unknown (agricultural)	Mitosis and cell division inhibitor [d]	81	o-DGTs
Pyraclofos	89784-60-1	Insecticide	Unknown	Acetylcholinesterase inhibitor [d]	81	o-DGTs

[a]Khare et al., 2023. Please note that 4-methyl-1H-benzotriazole is synonymous with 4-methylbenzotrioazole.

[b]Bohara et al., 2024.

[c]Health Canada, 2016.

[d]AERU, 2024.

[e]Health Canada, 2021.

[f]Health Canada, 2023.

Insecticides have limited permitted uses in outdoor urban spaces. Clothianidin, for example, is permitted for indoor use to control domestic pests (PMRA 2016) but may enter runoff through improper application or disposal practices. Clothianidin (90% detection frequency) and imidacloprid (86% detection frequency) are both permitted in Ontario for use in urban forestry, in horticulture, and in plant nurseries (OMECP 2014) and are evidently widely detected in urban stormwater sampled in our study (Table 5-2). Imidacloprid is also a common ingredient in ingestible and topical tick and flea medications for domestic pets (Anadón et al. 2025) and may have direct inputs into stormwater given pets are frequently walked in the green spaces adjacent to SWPs. Tebufenozide (100% detection frequency) is applied in urban forestry initiatives to treat areas affected by caterpillar pests (Natural Resources Canada 2024; e.g., spongy moths *Lymantria dispar*; Linnaeus, 1758).

Fungicides, such as azoxystrobin and tebuconazole, often have dual uses in city spaces. In addition to their use on turfgrass and lawn care, these fungicides are added to paints and preservative coatings on urban structures, like roofs and building facades, and can leach into stormwater during rain events (Wittmer et al. 2010; Bollmann et al. 2014). The need for long-term protection means these coatings can be applied throughout the year, extending the potential exposure window for receiving water bodies. Thiabendazole (100% detection frequency) is used in urban forestry, for example to combat Dutch elm disease (NCBI 2024).

Numerous information gaps exist on the registrations and permitted urban uses of our commonly detected pesticides in Canada. For example, we could not find pesticide registrations for pyraclofos or prometon by Health Canada. Flupyradifurone, imazethapyr, and dimethenamid are only registered for use in agricultural areas in Canada. Although several of our SWP sites are located in suburban neighborhoods adjacent to agricultural lands, the water delivered to the SWP sites is assumed to be delivered only from catch basins within each pond's sewersheds. The high occurrences of these agricultural pesticides in urban areas (Table 5-2) are unlikely to be driven by spray-drift, as application guidelines enforced by Health Canada aim to limit mobilization into surrounding environments (e.g., imazethapyr cannot be sprayed by air and requires buffer zones to prevent drift to areas of human habitation; PMRA 2024). Sometimes, a substance can be subject to a ban under one use (e.g., as a pesticide), but be permitted for use under a different application (e.g., as a coating protector) (Drakvik et al. 2020). Pesticide monitoring in surface waters is often based on application trends, yet contaminants with unreported applications in urban catchments are evidently prominent. Defining contamination exposure necessitates a more unbiased approach that does not rely on accurate reporting of chemical sales and applications, given the extent of unknown or dual sources in an urban landscape.

5.4.1.7.2 Pharmaceuticals and other consumption and metabolic waste products

With the exception of topical veterinary medications, the vast majority of pharmaceuticals detected have no obvious natural route into the stormwater systems. In theory, compounds associated with human waste should only be found in wastewater due to the separation of stormwater and wastewater systems and regulations protecting public and environmental health (e.g., combined sewer–stormwater systems were banned in Ontario in 1985; Government of Ontario 2016). However, urban stormwater is clearly widely contaminated with human wastewater products, a phenomenon also documented by Masoner et al. (2019) in their survey of stormwater contamination across the United States and by Kang et al. (2024) in an industrial and urbanized Korean watershed. Contamination could occur when aging infrastructure results in leaky sewage pipes, which are often near stormwater drainage pipes, or when infrastructure is improperly built, such as with illegal junctions between the wastewater and stormwater networks (Panasiuk et al. 2015; Yin et al. 2019). Caffeine (100% detection frequency), and its breakdown product paraxanthine (62% detection frequency), could be a source tracker for human waste contamination due to sewer leakages, as caffeine concentrations in the stormwater system increase with sewer system age (Ozaki et al. 2024). The prevalence of the antidepressants mirtazapine (100% detection frequency) and paroxetine (90% detection frequency) in our ponds could also indicate contamination with sewer lines, which has been reported in other urbanized areas (e.g., Sercu et al. 2011; Sauvé et al. 2012; Sidhu et al. 2013; Fairbairn et al. 2018; Olds et al. 2018; Masoner et al. 2019; Kang et al. 2024). This is an area requiring attention for the management of water quality in these systems.

Even fewer ecological guidelines are available for pharmaceuticals and other human-associated contaminants than with pesticides, which are often tested prior to registration (e.g., carbamazepine, an antiepileptic drug, is the only pharmaceutical with environmental quality guidelines by the CCME). Thus, we were unable to systematically compare our findings to published guidelines. It is important to note, however, that even without exceedances, chronic exposures to low levels of a diverse mixture of contaminants is concerning given we know very little about the potential additive, synergistic, or antagonistic effects of exposures to such complex mixtures (Laetz et al. 2015; Martin et al. 2021).

5.4.2 An USCS: Identifying the common mixture

An USCS identifies the overlap in contaminant profiles in an urban setting, enabling a description of a common set of urban contaminants for targeted monitoring and more focused research. We found 28 organic contaminants in every single SWP site in our study (listed in Table 5-2, along with relevant

source and toxicological information). We also found 18 additional organics present in over 80% of SWPs, which met our stringent criteria for inclusion in our proposed signature (Table 5-2). These co-occurring contaminants may comprise a regional USCS which could aid in targeted monitoring of urban contamination of freshwater resources. Within the USCS, there are 19 herbicides or herbicide metabolites, 11 fungicides, six insecticides, four pharmaceuticals, three industrial products, a tire-wear substance, an insect repellant, and caffeine—a stimulant. To this collection of organic contaminants, we also add chloride, TSS, bacterial contaminants, and traffic-associated metals (Ba, Ch, Co, Cu, Pb, Mn, Ni, Se, Sr, and Zn) to compose a USCS reflective of our findings (Figure 5-1); we found these stressors of toxicological concern to be consistently elevated across sites and propose them as useful additions to the USCS. We note that not all contaminants relevant to SWPs (e.g., polycyclic aromatic hydrocarbons, which are associated with traffic activity, or polychlorinated biphenyls, which are associated with industrial manufacturing) were included in our analyses. This is a limitation of our study, and we encourage future researchers to further develop the utility of the USCS by assessing the candidacy of additional urban-use contaminants.

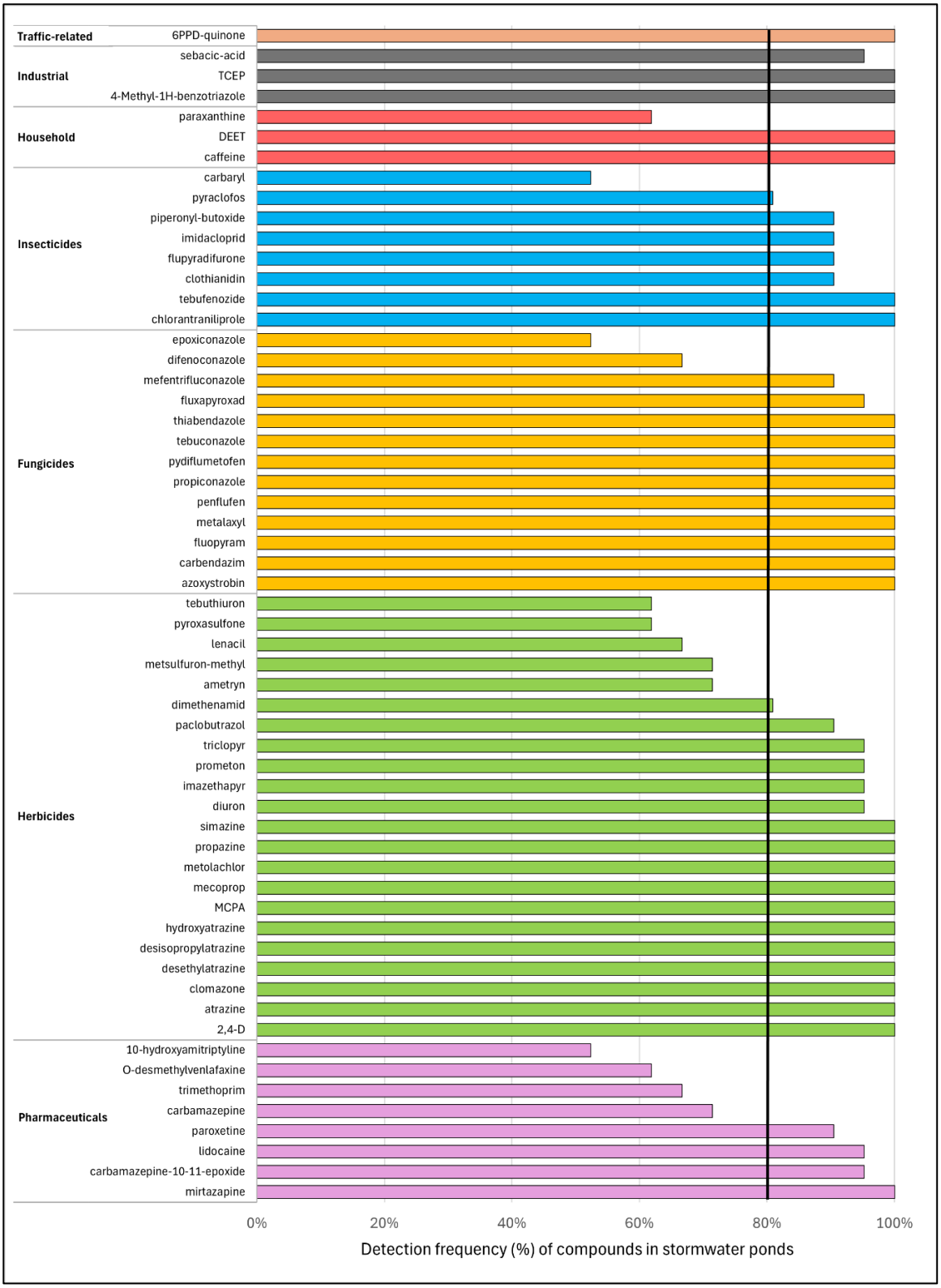

Traffic-related
6PPD-quinone
Industrial
sebacic-acid
TCEP
4-Methyl-1H-benzotriazole
Household
paraxanthine
DEET
caffeine
Insecticides
carbaryl
pyraclofos
piperonyl-butoxide
imidacloprid
flupyradifurone
clothianidin
tebufenozide
chlorantraniliprole
Fungicides
epoxiconazole
difenoconazole
mefentrifluconazole
fluxapyroxad
thiabendazole
tebuconazole
pydiflumetofen
propiconazole
penflufen
metalaxyl
fluopyram
carbendazim
azoxystrobin
Herbicides
tebuthiuron
pyroxasulfone
lenacil
metsulfuron-methyl
ametryn
dimethenamid
paclobutrazol
triclopyr
prometon
imazethapyr
diuron
simazine
propazine
metolachlor
mecoprop
MCPA
hydroxyatrazine
desisopropylatrazine
desethylatrazine
clomazone
atrazine
2,4-D
Pharmaceuticals
10-hydroxyamitriptyline
O-desmethylvenlafaxine
trimethoprim
carbamazepine
paroxetine
lidocaine
carbamazepine-10-11-epoxide
mirtazapine
0%
20%
40%
60%
80%
100%
Detection frequency (%) of compounds in stormwater ponds

Figure 5-1. Detection frequencies (%) of organic compounds found in 21 stormwater ponds in Brampton, Ontario. Compounds were detected in either water, biofilm, or o-DGT samples, or a combination of these matrices, as noted in Table 5-2. Only organic compounds with detection frequencies over 50% are shown. The vertical black line denotes the threshold of 80% detection frequency, above which contaminants meet the criteria for inclusion in the urban stormwater contaminant signature (USCS).

The complete breadth of our analyses was informative, with hundreds of possible contaminants screened for across three sampling matrices. However, this approach is very expensive and not one we could reasonably recommend for future monitoring programs, as all possible contaminants cannot be realistically screened for across temporal and spatial scales. Hence, the group of ubiquitous contaminants we identify here in our USCS is a useful tool to inform target analytes for monitoring in urban areas with similar catchment characteristics and reveals key areas for source control management. Additionally, future ecotoxicological research into chemical mixtures could better capture the cumulative effects of urban contamination by focusing on the contaminants in the USCS (Figure 5-2).

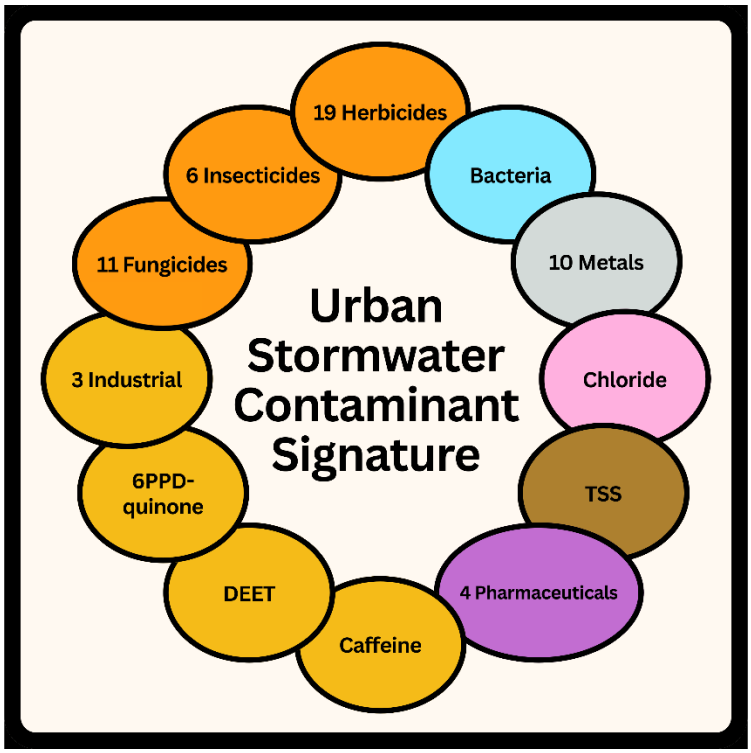


Figure 5-2. An urban stormwater contaminant signature, as described by our characterization of stormwater ponds in Brampton, Canada. Contaminants are grouped by type: pesticides (orange), bacterial contaminants (blue), metals (gray), chloride (pink), sediment (brown), pharmaceuticals (purple), and other anthropogenic consumables (yellow).

A key limitation of our study lies in the use of a targeted approach to chemical analysis. Stormwater contamination is highly complex, with numerous and variable sources influenced by ongoing changes in product formulations, application practices, and land use (Gasperi et al. 2022; Flanagan et al. 2025). As a result, it is challenging to comprehensively characterize the full range of contaminants present. Targeted analytical methods, while useful for detecting known compounds, inherently exclude unknown or emerging substances and their transformation products, introducing a bias in the assessment of chemical exposure (Gasperi et al. 2022). In some cases, non-target analyses can be coupled with targeted screenings to increase comprehensiveness (Gasperi et al. 2022; Kang et al. 2024) and address analyte selection bias.

The USCS of our study area indicates that most omnipresent organic contaminants have uses in turfgrass and lawn care, structure coatings, or may indicate possible human waste contamination (Table 5-2). Previous characterizations of organic pollutants in stormwater have been comparable to occurrences in wastewater treatment plant effluent (Launay et al. 2016; Masoner et al. 2019), and the presence of several human waste-associated compounds (e.g., caffeine, mirtazapine) in all or nearly all SWP sites in our study (Table 5-2) cannot be solely attributed to wash off from accidental spills, and suggests widespread wastewater contamination of stormwater. One of the benefits of the USCS approach is its support in tracing the sources of widespread urban contaminants (Figure 5-3), whereby identifying groups of co-occurring contaminants with similar uses (e.g., in turfgrass care) can inform managers of the contribution of relevant land uses within the sewershed. This insight can guide efforts to control emissions and support mitigation efforts, such as engineering solutions or behavioral changes that would prevent entry into the stormwater ponds (Figure 5-3).

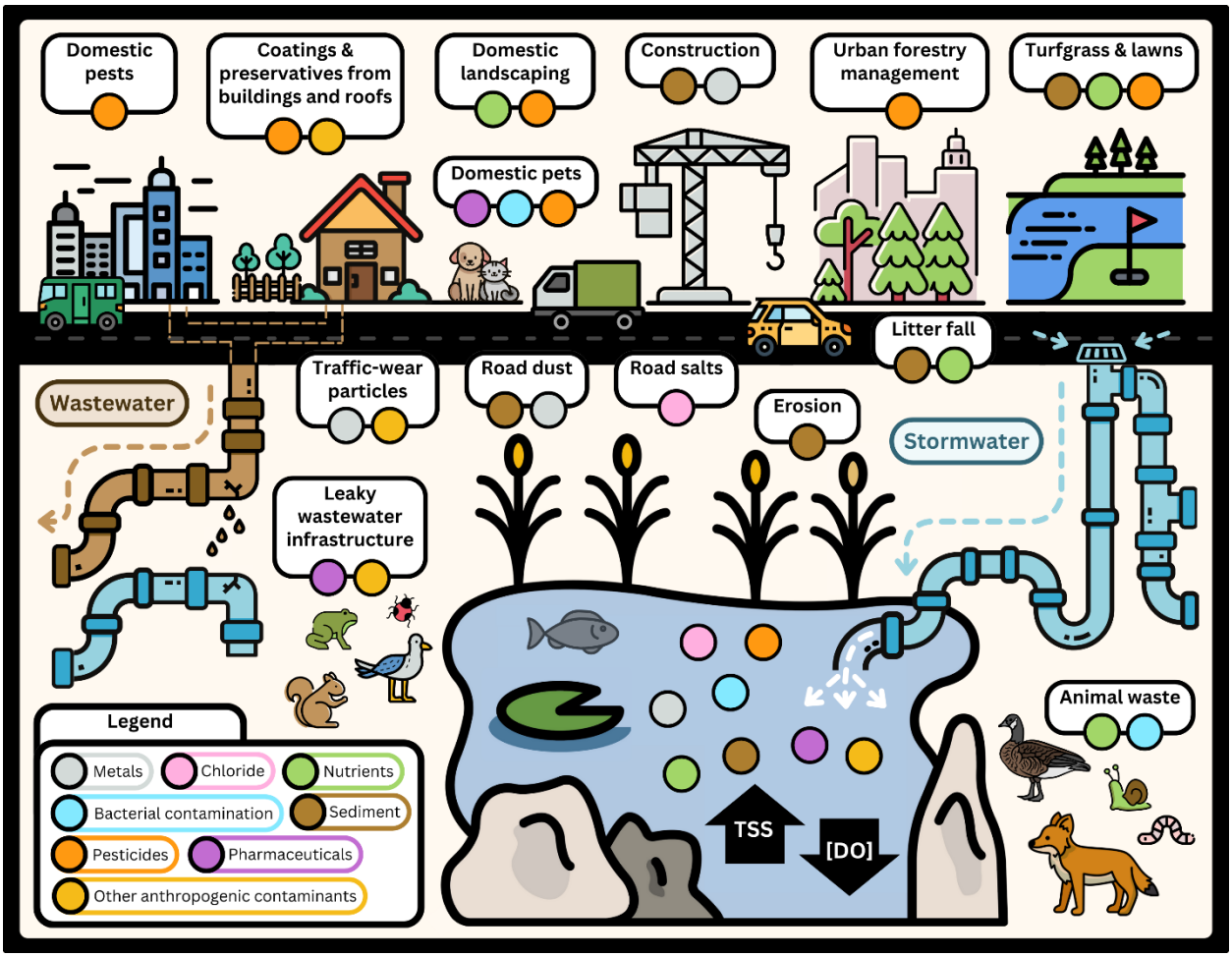


Figure 5-3. The urban stormwater contaminant signature (USCS) describes a common characterization of stormwater in urban areas. Here, the potential sources of common contaminant components of the USCS are shown.

5.4.2.1 Comparisons with other studies

Several contaminants with 100% detection frequencies were also reported as common contaminants of stormwater in other studies. For example, caffeine was widely detected in stormwater samples collected by Masoner et al. (2019; 96% detection frequency) in the United States and Tran et al. (2019; 100%) in Singapore. DEET, an ingredient in most insect repellents, also had near-ubiquitous detections in both studies (Masoner et al., 98% detection frequency; Tran et al., 100% detection frequency), while carbendazim was also very common (94% detection frequency) in American stormwaters (Masoner et al. 2019). A recent survey of urban streams in the Greater Toronto Area in Ontario found 6PPD-quinone, a

tire-related chemical of emerging ecological concern, in 80% of stream samples (Helm et al. 2024), two of which receive effluent from the SWP sites in our study. The UV stabilizer 4-methyl-1H-benzotriazole has a number of industrial applications, ranging from antifreeze in cars and aircrafts, to firefighting substances (Davis et al. 1977), and had high presence in Masoner et al.'s samples (92% detection frequency).

Other compounds that were sufficiently common (detected in > 80% of ponds sampled) to be incorporated into our USCS were also comparable to other studies. For example, carbamazepine, an anticonvulsant, and lidocaine, a local anesthetic and antiarrhythmic drug, were detected in 95% of our samples, and carbamazepine was detected in all samples collected by Tran et al. (2019) and lidocaine detected in 69% of samples by Masoner et al. (2019). Clothianidin and imidacloprid had detection frequencies of 90% and 86%, respectively, and are also common in stormwater in other countries with permitted uses (e.g., 86% detection frequency of imidacloprid by Masoner et al. 2019), but have been banned in the European Union due to being highly toxic to non-target invertebrates (EU Health and Food Safety, n.d.).

These remarkable similarities in contaminant profiles show that, although the exact blend of components in our USCS may not be universal, there is notable alignment with data from other stormwater surveys. The process of USCS development could be duplicated elsewhere to determine a USCS for different urban areas should there be more systematic differences. For example, locations in South America or Europe with differences in chemical permittance and applications would warrant an adaptation to the process of USCS development.

5.4.3 Considering Modes of Action (MOAs) in mixtures

Evidently, SWPs contain multifarious mixtures of organic and inorganic contaminants spanning a wide range of chemical classes and MOAs. While some regulatory and assessment frameworks, such as whole effluent toxicity tests, account for mixture effects, most risk assessments still rely on a chemical-by-chemical approach, which may underestimate risk when compounds co-occur at low concentrations yet interact additively or synergistically (Gomez-Eyles et al. 2009; Martin et al. 2021).

Two models commonly used to predict mixture toxicity, concentration addition and independent action, are based on assumptions of similar or dissimilar MOAs, respectively. Both require extensive toxicological data, which are often unavailable for emerging contaminants or alternative exposure routes such as dietary intake via biofilms, as discussed in Chapter 4 (see also Ijzerman et al. 2023). Additionally,

promising tools have been developed such as effects-based assays (e.g., Jager et al. 2010) and cell line assays (e.g., Hoover et al. 2019) for evaluating mixture toxicity.

Given the lack of effect data for 11 of our 46 most frequently detected organic compounds and the absence of biofilm-based thresholds for metals, in addition to the concentration data obtained from o-DGT analysis, we opted to qualitatively explore the toxicity potential of the proposed USCS based on the MOAs of the most frequently detected organics (Table 5-2). While detection does not equate to risk, certain groupings, such as synthetic auxin herbicides (e.g., 2,4-D, MCPA, mecoprop, triclopyr) and succinate dehydrogenase inhibitor fungicides (e.g., fluopyram, fluxapyroxad, penflufen), suggest potential for cumulative effects within shared MOA groups.

Overall, we identified over 13 distinct MOAs among the most frequently detected compounds (Table 5-2), alongside several substances with unknown or poorly characterized MOAs, especially among pharmaceuticals and building-derived chemicals. This chemical diversity complicates risk predictions using additive models alone and underscores the need for approaches that can accommodate mixtures with overlapping, unique, and unknown effects.

Some components in the USCS are also known or suspected synergists. For instance, piperonyl butoxide (90% detection frequency) enhances insecticide potency (Cross et al. 2017), while azole fungicides such as tebuconazole (100% detection frequency) and flupyradifurone (90% detection frequency) can amplify toxicity of co-occurring lipophilic insecticides (USEPA 2014; Cedergreen 2014). Such interactions suggest that even low concentrations of individual compounds may contribute to enhanced mixture toxicity.

Beyond chemical interactions, other factors such as salinity, microbial activity, and nutrient content, can modify toxicity. For instance, salinity may either intensify or mitigate toxicity depending on the mixture (Hall and Anderson 1995; Forget et al. 1999; Velasco et al. 2019), and bacteria in sediments can enhance the bioavailability of hydrophobic contaminants (Widenfalk 2005). Interactions between fecal bacteria and chemical mixtures remain poorly understood, but given that fecal indicators often signal wastewater intrusion (Sercu et al. 2011), further investigation is warranted.

Taken together, the chemical diversity, potential for interaction, and influence of environmental modifiers complicate risk predictions in urban stormwater. While our study did not quantify mixture toxicity, we emphasize that any framework aiming to assess urban stormwater impacts on both aquatic life within stormwater ponds and in receiving waters must acknowledge the limits of single-compound

assessments and consider cumulative and context-dependent effects to better reflect real-world exposures. The diversity and uncertainty of potential toxicities within the USCS further evidences the value of monitoring this group of contaminants.

5.4.3.1 Mixture toxicity: Future research needs

Our findings emphasize the need to expand ecotoxicological testing to better address mixture effects, a call that has been repeated by researchers for years (Altenburger et al. 2013; Kortenkamp and Faust 2018; Kortenkamp et al. 2019; Raby et al. 2019; Drakvik et al. 2020; Salvito et al. 2020; Mueller et al. 2023). The diverse array of substances detected in the SWPs suggests that exposures in urban environments reflect complex mixtures rather than isolated compounds. Although most detected compounds in our SWP water samples fall below individual guideline thresholds (Izma et al. 2024a [Chapter 3]), their combined impacts remain largely unknown. Current mixture risk approaches often require toxicological data for individual substances, which are lacking for many emerging or understudied compounds (Fairbairn et al. 2018); for example, several key USCS components such as the pharmaceuticals mirtazapine and paroxetine, and urban-use pollutants like 6PPD-quinone, TCEP, and 4-methyl-1H-benzotriazole, are currently lacking published ecological thresholds. Establishing predicted no-effect concentrations (PNECs) for these compounds would enable more accurate risk quotient calculations. In the meantime, there are a number of approaches to mixture assessments incorporated by the research community to address the lack of thresholds and complexity of mixture exposures (Brack et al. 2016), such as cell-line assays across MOAs (e.g., CALUX assays; Escher et al. 2013). We recommend future urban stormwater mixture studies prioritize compound groupings with shared MOAs (e.g., synthetic auxins, succinate dehydrogenase inhibitors) or known interactive effects (e.g., piperonyl butoxide with insecticides; azoles with pyrethroids or metals), as discussed above.

5.4.3.2 Monitoring and risk management implications

Given the high number of contaminants in urban runoff, there is a critical need for streamlined monitoring frameworks. Although we were unable to monitor all relevant contaminants (other micropollutants such as polycyclic aromatic hydrocarbons and phthalates are also widespread in urban stormwaters; Gasperi et al. 2022), the USCS offers a practical starting point for focused surveillance, enabling programs to prioritize frequently detected and environmentally relevant compounds. This approach supports improved spatial and temporal resolution by reducing the number of analytes screened, while still capturing representative exposure profiles. This approach aligns with international efforts to identify priority

mixtures and support mixture-based risk policies (e.g., Eriksson et al. 2007; Knapp et al. 2021; Rorije et al. 2022; Mueller et al. 2023). In light of the complex nature of monitoring and assessing mixture effects, precautionary risk management strategies are warranted - particularly those that consider cumulative exposures and trigger mitigation when thresholds are exceeded.

5.5 Conclusion

SWPs provide a unique window into urban contaminant mixtures due to their role in concentrating runoff from diverse sources. Our multi-matrix approach revealed frequent co-occurrence of a wide array of contaminants, many with overlapping or interacting MOAs, underscoring the limitations of single-compound risk assessments in these systems. To help streamline monitoring and guide future research, we propose the USCS: a targeted suite of frequently detected, environmentally relevant contaminants. The USCS can serve as a practical foundation for mixture-focused risk assessment, helping to prioritize bioassays and guideline development for under-regulated substances. As cities continue to densify, refining how we monitor and evaluate cumulative contaminant exposure will be essential for effective urban water management.

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Chapter 6: Biofilms, bugs, and the built environment: Exploring local and landscape drivers of diatom and macroinvertebrate assemblages in urban stormwater ponds

6.1 Overview

Stormwater management ponds (SWPs) are engineered systems designed primarily for flood control and sediment capture in urban landscapes. Despite their intended function, these ponds are often colonized by aquatic biota and can contribute to urban biodiversity. We investigated the ecological condition of 21 SWPs in a highly urbanized city in southern Ontario, Canada, by relating water quality, pesticide contamination, physical habitat features, and surrounding land use to the composition of periphytic diatom and aquatic macroinvertebrate assemblages. Assemblages were dominated by pollution-tolerant taxa. Non-metric multidimensional scaling (NMDS) ordinations indicated that water quality parameters were good predictors of variation in community composition for both assemblages, while pesticide contamination was associated with diatom relative abundances and local vegetation cover was associated with macroinvertebrate relative abundances. Landscape variables within a 300 m buffer surrounding the SWPs were not associated with community composition or taxonomic richness for either assemblage, suggesting that site-level conditions exert stronger ecological influence. These findings highlight the importance of using multiple biological assemblages to capture different aspects of ecological condition and the value of integrating biological monitoring into stormwater infrastructure planning. Enhancing emergent and riparian vegetation, reducing pollutant inputs, and managing contaminant pathways may improve biodiversity potential in urban SWPs.

6.2 Introduction

Urbanization is rapidly increasing worldwide, with more than two-thirds of the global population expected to live in cities by 2050 (United Nations, 2018). The resulting development, notably the expansion of impervious surfaces to support car-centric urban centers, alters natural hydrological systems (Walsh et al., 2005) and threatens biodiversity (Elmqvist et al., 2013; Piano et al., 2020; Szulkin et al., 2020), especially in conjunction with the increasing effects of climate change (Urban et al., 2024). In Canada, suburban sprawl, where the rate of developed surface areas increases faster than the population, has resulted in the rapid replacement of natural wetlands with stormwater management controls, such as

stormwater management ponds (SWPs), to account for the loss of natural runoff capture systems (Rooney et al., 2014; Birch et al., 2022).

Stormwater management ponds (SWPs) are engineered, end-of-pipe receptacles for storm runoff primarily constructed for flood mitigation purposes. In addition, SWPs are designed and managed to reduce sediment and nutrient loadings to downstream habitats (OMECP, 2003). Attempts to naturalize SWP designs have had limited success because the ecologically-beneficial biophysical features of natural wetlands are often at odds with stormwater management goals (Rooney et al., 2014). For example, SWPs have steep bank slopes to maximize runoff holding capacity following a storm event, whereas more gradual shoreline slopes promote higher emergent vegetation cover and more suitable habitat for biota (Rooney et al., 2014).

In urban areas, SWPs experience thermal pollution (Sabouri et al., 2013) and salinization (Van Meter et al., 2011), receive an assortment of contaminants, such as nutrients (Yang & Lusk, 2018), pesticides (Izma et al., 2025 [Chapter 2]), and heavy metals (Karlsson et al., 2010), and are periodically dredged to maintain their water storage capacity and to dispose of contaminated sediments. These inhospitable environmental conditions, coupled with decreasing urban green spaces and available habitats, can turn SWPs into ecological traps, where organisms are attracted to them as potential habitat, but are unable to sustain healthy populations (Robertson & Hutto et al., 2006; Ferzoco & McCauley, 2024).

Yet, SWPs in North America have been colonized by a variety of aquatic and terrestrial species, including birds (Ward et al., 2010; Rooney et al., 2014), fish (McIsaac 2022), amphibians (Gallagher et al., 2014; McIsaac 2022), aquatic plants (Rooney et al., 2014; Perron & Pick, 2020a), and invertebrates (Hassall & Anderson, 2015; Sinclair et al., 2021; Johnson et al., 2013). Although some studies have shown lower biodiversity in SWPs with the dominance of pollution-tolerant species (e.g., Rochfort et al., 2000; Sun et al., 2018), several others have found the biodiversity of benthic assemblages to be indistinguishable with that of natural ponds (e.g., Le Viol et al., 2009; Hassall & Anderson, 2015; Stephansen et al., 2016). Evidently, despite the intention of SWPs to serve as infrastructure rather than as natural heritage features, these spaces may inadvertently comprise habitat that is elsewhere unavailable to urban species . In this sense, SWPs can be considered *novel ecosystems*: ecological systems that have been significantly altered by human activity, lack historical analogues, and support persistent but non-traditional species assemblages (Hobbs et al., 2006; Murphy, 2013; Collier & Devitt, 2016). Their

hydrological regime, contamination profiles, and structural design differ fundamentally from natural wetlands, yet they maintain self-organizing biological communities, including invasive and opportunistic species such as goldfish and *Phragmites australis* (Rooney et al., 2015; McIsaac, 2022). Restoration to a pre-disturbance state is not applicable in such settings, reinforcing the importance of understanding and managing these systems as functional, albeit altered, components of urban biodiversity networks. With the stark decline of freshwater biodiversity both globally (WWF 2016; Tickner et al., 2020) and in Canada (Desforges et al., 2022), understanding the biological communities occupying these SWPs, and the stressors they face, is fundamental to urban biodiversity conservation efforts (Ferzoco & McCauley, 2024).

Macroinvertebrate and periphytic diatom assemblages are routinely incorporated into ecological assessments of aquatic systems due to their well-known responses to environmental conditions (e.g., Barbour, 1999; Belore et al., 2002). Although aquatic macroinvertebrates are one of the most studied taxonomic groups in SWPs (McKercher et al., 2024), few studies have explored the influence of contamination by organic chemicals on community structure, and fewer still have focused on this group within SWPs in Canada's urban landscapes. Periphytic diatoms are foundational to aquatic food webs (Wetzel, 2005), yet are seldom surveyed in SWPs (Minelgaite et al., 2020), despite being sensitive indicators of various water quality parameters (Kelly et al., 1998) and evidence that contaminants accumulate in periphyton (Izma et al., 2024 [Chapter 3]).

Our study contributes to understanding ecological diversity in urban SWPs in Ontario and provides insight into the stressors and drivers affecting aquatic assemblages in these understudied ecosystems. Specifically, our objectives were to 1) characterize the diversity of periphytic diatom and aquatic macroinvertebrate assemblages in urban SWPs; 2) determine attributes of the surrounding landscape and pond water quality and their relationships with the relative abundances of diatom and invertebrate taxa; and 3) propose hypotheses, based on information acquired from this exploration, to guide future research on these novel ecosystems.

6.3 Methods

6.3.1 Study area

Brampton, Ontario, is among the fastest-growing municipalities in Canada, experiencing rapid urban expansion (Statistics Canada, 2021; City of Brampton, 2022). This growth has resulted in 7,888 hectares,

or 45.4%, of the city being covered by impervious surfaces (TRCA, n.d.). Situated within the West Humber River and Credit River watersheds, Brampton contains over 190 stormwater ponds (SWPs) that ultimately discharge into Lake Ontario (City of Brampton, n.d.).

We selected 21 SWPs (Figure 6-1), which drain into four major watercourses: Etobicoke Creek, Mimico Creek, the Credit River, and the Humber River. SWPs were selected to capture independent gradients in urban intensity and habitat connectivity. Urban intensity was represented by the percentage of impervious cover within a 300 m buffer around each pond (ranging from 10.9% to 55.2%). Habitat connectivity was indicated by the percentage of open water cover (both lentic and lotic) within the same 300-m buffers (ranging from 0% to 7.6%; McIsaac, 2022). All selected ponds are classified as wet ponds, engineered to hold water year-round, and ranged in size from 1,051 to 3,686 m² (mean = 2,272 m²; standard deviation = 851 m²). Each pond was at least 10 years old and had not been dredged within the past decade. All selected SWPs were located a minimum of 800 m apart from each other to ensure the 300 m buffers did not overlap.

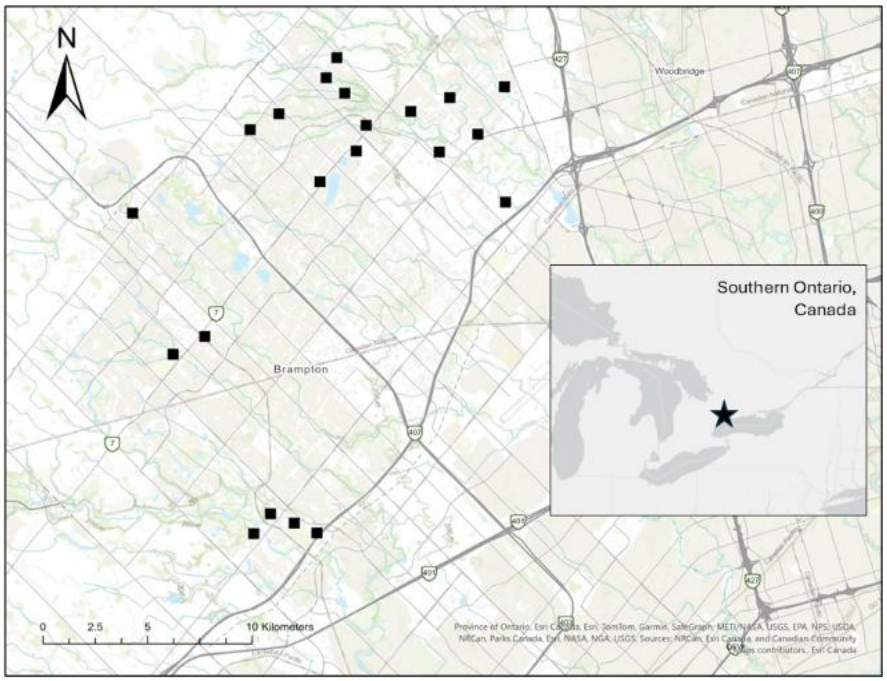


Figure 6-1. Map of stormwater pond (SWP) sites (black squares) in Brampton, Ontario, Canada.

6.3.2 Landscape and local measures

We used ortho-rectified aerial imagery (15 cm resolution, First Base Solutions 2020) using QGIS v3.10 (QGIS Development Team 2022) to measure the areas of surrounding canopy cover, impervious cover, roads, and water (all m^2) in a 300 m buffer zone around each SWP. These buffer zones are measured starting from the perimeter of the SWP. We calculated the area of impervious cover by summing the areas of roads, sidewalks, roofs, and other impervious surfaces such as parking lots and driveways.

Local variables included pond size, as well as the total spatial extent (in m^2) of vegetation cover (within the ponds and within 15 m of the pond perimeter) and the extent of robust emergent vegetation (e.g., cattails), narrowleaf emergent vegetation (e.g. grasses and sedges), shrubs (woody vegetation < 2 m in height), and dead trees as vegetation cover classes. We delineated the perimeter of the SWPs using an SX Blue II GNSS receiver with real time sub-meter accuracy (Geneq Inc., Montreal, Quebec) and vegetation-based indicators following the Ontario Wetland Evaluation System southern manual v 3.2 (OWES, 2013) and the Marsh Monitoring Program habitat description form (MMP Amphibians, 2009). Field surveys to identify plant species along the pond margin were conducted during the summertime. We interpreted the pond boundary as the transition zone where the presence of wetland indicator species declined and upland species became dominant (i.e., 50% cover by upland plant species). We digitized the collected perimeter points in QGIS v3.10 (QGIS Development Team 2022).

6.3.3 Water and biofilm sampling

6.3.3.1 Water quality surveys

Over a period of nine weeks (May 25 to July 25, 2022), we conducted weekly water quality surveys at each SWP site. We measured water temperature (°C), conductivity (mS cm^{-1}), and dissolved oxygen (DO; mg L^{-1}) at three equidistant points in the open water, at a depth of ~20 cm. We used a Hach HQ1140 Portable Conductivity/TDS Meter (Hach Sales and Services L.P., London, Canada) to measure temperature and conductivity, and a YSI ProSolo ODO Optical Dissolved Oxygen Meter (Xylem Inc., Miami, FL) to measure DO.

For each weekly water sampling event, we combined three 50 mL grab samples taken at equidistant points around the ponds at ~20 cm, using a high-density polyethylene Nalgene bottle. Composite samples from each pond were transported on ice to the lab for analysis of pH, total suspended solids (TSS; mg L^{-1}), and chloride ion concentration (mg L^{-1}). We determined TSS gravimetrically, and

we used an Orion 9156BNWP Combination pH Electrode and Orion Star A211 Bentchtop pH Meter (Thermo Fisher Scientific, Waltham, MA) to measure pH and a Chloride Checker® HC (Hannah Instruments, Woonsocket, RI) to colorimetrically determine chloride ion concentration.

Additional composite grab samples were collected twice during the survey period (once on June 21, 2022, once on July 11, 2022) for the fluorometric determination of chlorophyll-*a* (ug L^{-1}) and pheophytin (ug L^{-1}) concentrations with a Turner Designs TD 10AU fluorometer (Turner Designs Inc., Sunnyvale, California).

During the survey period we also submitted additional grab samples from each site (collected at the same three equidistant points as those collected for TSS and chloride assessment) for analysis of bioavailable nutrients at ALS Laboratories in Waterloo, Ontario. This analysis included the fluorometric determination of ammonia (NH_3), ion chromatographic determination of nitrate (NO_3) and nitrite (NO_2), and calorimetric analysis of orthophosphate (PO_4). We submitted grab samples to the Agriculture and Food Laboratory (AFL) in Guelph, Ontario, following a significant precipitation event on June 7, 2022, for the determination of total coliform bacteria and *Escherichia coli* by plate-count analysis.

6.3.3.2 Pesticide sampling

To characterize pesticide contamination in SWP water over the survey period, we collected a time-integrated composite sample from nine 150 mL subsamples, collected at ~20 cm depth each week. The composite sample, totaling 1.35 L, was stored at -20 °C prior to submission for pesticide analysis to the Agricultural and Food Laboratory (AFL; ISO/IEC 17025 accredited) in Guelph, Ontario.

We cultured biofilms on artificial substrates in each pond to assess pesticide contamination and biofilm community composition, detailed in Chapters 2 and 3. Briefly, we deployed samplers composed of ten acrylic plates (20.2 cm by 44.4 cm in area) in each pond, suspended at ~10 cm depth. We harvested the biofilm after 54 days of deployment by scraping both sides of each plate into a composite sampling vessel. An aliquot of 300 – 500 g (wet weight) of biofilm was removed from each composite sample and freeze-dried using a FreeZone I Labconco benchtop freeze dryer (Labconco Corporation, Kansas City, MI) prior to submission to AFL for pesticide analysis.

Water and biofilm samples were screened for 542 current-use and legacy pesticides by gas chromatography-tandem mass spectrometry (GC-MS/MS) and liquid chromatography/electrospray ionization-tandem mass spectrometry (LC/ESI-MS/MS). Details of this analysis have been published in

Izma et al., 2025 and are described in more detail in Chapter 2. Analyte lists, including their method detection and quantification limits, are provided in Appendix E. We were unable to harvest adequate biofilm mass from two sites to meet the submission requirements for the full screening of pesticides (these sites were only analyzed for polar pesticides and phenoxy herbicides). All other samples contained adequate biomass for the full analysis.

6.3.4 Periphytic diatom analysis

Biofilm samples from each pond were subsampled for diatom analysis and preserved with Lugol's iodine (~1% v/v). We removed organic matter from the samples by acid digestion in 5 mL of 100% (v/v) nitric acid for 15 hours, after which we added 1 mL of hydrogen peroxide 30% (v/v) to each sample and placed them in tubes in a 60 °C hot water bath for 1 hour. We allowed the samples to cool down to room temperature before centrifuging at 5500 rpm for 10 minutes. After discarding the acid supernatant, we added deionized water to rinse the retained pellets. We repeated this step of centrifusion and rinsing until the supernatant was no longer acidic (pH > 6). We mounted the diatom frustules onto microscope slides using Naphrax® (refractive index: 1.74; Brunel Microscopes Ltd., Wiltshire, UK). We used a Revolve Microscope (Discover Echo, San Diego, CA) at 1000x magnification to enumerate and identify diatoms to the genus level, following Lavoie et al. (2008) and Wehr et al. (2015).

We used four biological metrics to help characterize the diatom assemblages. These were taxonomic richness (the number of genera per sample), the percentage of diatoms from the genera *Achnanthidium* (% *Achnanthidium*), the percentage of motile diatoms (% motile), and the percentage of the most abundant taxon (% dominant). Members of the genus *Achnanthidium*, particularly the cosmopolitan species *Achnanthidium minitussimum*, have been used to predict levels of disturbance (Stevenson et al., 1999). Since this species is a pioneer species (often the first to attach and establish after a disturbance), its abundance is proportional to the time since the last disturbance event (Stevenson et al., 1999). The percentage of motile diatoms represents an index for siltation, as motile diatoms are able to move away from silty conditions (Bahls et al., 1992; Dickman et al., 2005; Weilhoefer & Pan, 2022). We calculated this index with the relative abundances of moderately-motile to very motile genera, including *Caloneis, Craticula, Ctenophora, Cymbella, Encyonema, Encyonopsis, Entomoneis, Eolimna, Epithemia, Grunowia, Halamphora, Hippodonta, Navicymbula, Navicula*, and *Nitzschia*.

6.3.5 Aquatic macroinvertebrate analysis

We sampled and identified aquatic macroinvertebrates from the 21 SWPs from August 29 to September 2, 2022, following the wetland version of the Ontario Benthic Biomonitoring Network (OBBN) Protocol Manual (Jones et al., 2007): a widely adopted standard for environmental monitoring of aquatic systems in Ontario. We chose to sample in the late summer to capture the time period where anthropogenic stressors are estimated to be more pronounced (Jones et al., 2007).

We used a 500 µm mesh D-net to perform the "jab and sweep" method, where the net is inserted ~5 cm into the substrate and swept forward to collect disturbed material at three equidistant points around each pond less than ~50 cm in dpeth. This method is recommended for mucky substrate types such as those found in wetlands or ponds. Following each jab and sweep, we washed the sample from the net into a bucket with water to ensure all material was collected. We repeated the jab, sweep and rinse a minimum of two times at each sample point. From the resulting composite sample, we transferred 200 mL subsamples onto a white tray where we picked out all visible live specimens and placed them into a clean, wide-mouth plastic container containing 250 mL of 90% ethanol. We continued to take subsamples until we collected at least 100 invertebrates, after which we finished processing the entire subsample. In samples with low abundances of invertebrates, we performed additional jab and sweep sampling to create additional composites and subsampled those until we collected a minimum of 80 specimens. We transported preserved samples from each replicate (n = 3) at each pond (total = 63 samples) to the lab for identification.

We counted and identified aquatic macroinvertebrates to the family level using Voshell (2002), with the exceptions of Amphipoda, Hirudinea, Hydrachnidia, Nematoda, and Oligochaeta, which were identified to their associated taxonomic level. This taxonomic resolution follows the Ontario Ministry of the Environment Rapid Bioassessment Protocol Taxonomic Level, found in Appendix 1 of the OBBN Protocol Manual (Jones et al., 2007). We processed each replicate (n = 3) for each site separately, after which we averaged total abundance counts across replicates and calculated an average relative abundance for each taxon at each site.

We calculated five biological metrics to describe the aquatic macroinvertebrate assemblages. Taxonomic richness represented the total number of taxa at each SWP site. We modified the commonly used metric of percent Ephemeroptera, Plecoptera, and Trichoptera (%EPT) to exclude members of the family Baetidae. Previous research has shown that members of the Baetidae family exhibit a wide range

of environmental tolerances and are the least sensitive family to organic pollution (with a saprobic index of 2.6) in Ephemeroptera (Menetrey et al., 2008). We also calculated the percentage of worms (%worms), including members from Nematoda, Oligochaeta, and Planariidae, the percentage of families in the order Diptera (%Diptera), and the percentage of the most abundant taxon (%dominant).

6.3.6 Statistical analysis

6.3.6.1 Principal components analysis

We grouped environmental variables hierarchically based on scale and data type (Table 6-1). At the largest scale, we defined landscape drivers (n = 4 variables) as land covers within the 300 m buffer surrounding each pond, which were measured using satellite data. Next, we grouped vegetation metrics and pond area as local drivers (n = 6 variables), which were measured in the field. For the purpose of statistical analysis, we converted total vegetation cover to the proportion of vegetation cover relative to pond area. We defined water quality based on standard water chemistry variables (n = 13 variables). This was distinct from pesticide contamination, which we split into two groups: pesticide concentrations in water samples (n = 13 variables) and pesticide concentrations in biofilm samples (n = 6 variables). To reduced the effects of rarely detected analytes, only pesticides from the suite of 542 analytes which were detected in a minimum of three out of the 21 SWPs were included in these analyses. We decided to separate pesticide detections in water samples from other metrics of water chemistry to simplify the interpretation of the analysis. Other chemicals that were rarely detected (i.e., fewer than three of 21 sites, or 14% of sites, for example NO_2 and NO_3) were also excluded from this analysis to limit matrix sparsity and better characterize general patterns in occurrence and concentration.

Table 6-1. Groupings of environmental variables, and their abbreviations, measured at 21 stormwater pond (SWP) sites.

Group	Variables included	Abbreviation
Landscape Drivers	Impervious cover	Impervious
	Road cover	Road
	Canopy cover	Canopy
	Water cover	Water
Local Drivers	Pond size	Size
	Proportional vegetation cover	Vegetation
	Narrowleaf emergent vegetation cover	NLE

Group	Variables included	Abbreviation
	Robust emergent vegetation cover	RE
	Shrub cover	Shrub
	Dead trees	DT
Water Quality	Surface water temperature	Temperature
	pH	pH
	Dissolved oxygen concentration	DO
	Conductivity	Conductivity
	Chloride ion concentration	Chloride
	Total suspended solids	TSS
	Chlorophyll-*a* concentration	Chlorophyll-*a*
	Pheophytin concentration	Pheophytin
	Chlorophyll-*a* to pheophytin ratio	CP
	Ammonia concentration	NH_3
	Orthophosphate concentration	PO_4
	Coliform bacteria	Coliform
	E. coli	*E. coli*
Pesticide Concentrations in Water	2,4-Dichlorophenoxyacetic acid	2,4-D
	2-(2,4-Dichlorophenoxy) propanoic acid	2,4-DP
	2,4,5-Trichlorophenoxyacetic acid	2,4,5-T
	Bentazon	Bentazon
	Chlorate	Chlorate
	Clothianidin	Clothianidin
	Dicamba	Dicamba
	Flonicamid	Flonicamid
	Flupyradifurone	Flupyradifurone
	Imidacloprid	Imidacloprid
	2-Methyl-4-chlorophenoxyacetic acid	MCPA
	Mecoprop	MCPP
	Triclopyr	Triclopyr
Pesticide Concentrations in Biofilm	2,4-Dichlorophenoxyacetic acid	2,4-D
	Azoxystrobin	Azoxystrobin
	Diuron	Diuron
	2-Hydroxyethylphosphonic acid	HEPA
	Nicotine	Nicotine
	Tebuconazole	Tebuconazole

All statistical analyses were performed using R Statistical Software (version 4.2.2.) in RStudio (version 2024.04.0; Rstudio Team 2021). We performed a principal components analysis (PCA) on each group (N = 5) of environmental variables to reduce groups of correlated variables into synthetic orthogonal variables for use in further analyses. Prior to this analysis, we prepared our dataset by replacing values from water quality or pesticide measurements which were below detection limit with one

half of the detection limit, and values which were below the quantification limit but above the detection limit with the value of the detection limit.

Following this preparation, we divided our dataset into five correlation matrices, one for each of the groups described in Table 6-1, and performed the PCA on each matrix resulting in a total of five PCA ordinations. We decided how many PC axes were significant based on a minimum required variance explained of 20% per component. We used biplots to interpret the loadings of environmental variables on each significant axis to synthesize a concise characterization of each axis.

6.3.6.2 Community composition of periphytic diatoms and aquatic macroinvertebrates

We assessed periphytic diatom and aquatic macroinvertebrate assemblage composition using non-metric multidimensional scaling (NMDS) ordinations on relative abundance data. Prior to these ordinations, rare taxa (observed at fewer than 3/21 SWP sites) were removed. We created Bray-Curtis dissimilarity matrices for each assemblage using the 'metaMDS' function in the *vegan* package in R (Oksanen et al., 2020). We decided on the optimal number of dimensions for each ordination by visualizing stress against dimensionality with scree plots, considering a stress value below 0.1 a fair fit.

To visualize relationships between environmental variables and variations in community compositions, we used the 'envfit' function in *vegan* (Oksanen et al., 2020) to plot the PCA site scores from each significant PC axis from the five PCA ordinations, which represented environmental predictor variables as characterized in Sections 6.3.4 and 6.3.5. We only included predictor variables that were reasonably correlated ($p < 0.20$, $r^2 > 0.10$) with at least one axis of the NMDS ordinations. We also used the 'envfit' function in *vegan* (Oksanen et al., 2020) to plot biological metric vectors which were reasonably correlated ($p < 0.20$, $r^2 > 0.10$) with at least one axis of the NMDS ordinations. We used the 'ggplot' function in *ggplot2* to visualize the ordinations (Wickham, 2021).

6.3.6.3 Regression analysis

We performed regression analyses with the significant PCA axes site scores and all biological metrics to help further explore variations in community composition of diatoms and macroinvertebrates. We used the 'ggplot' function in *ggplot2* (Wickham, 2021) to visualize significant ($p < 0.05$) relationships.

6.4 Results

6.4.1 Environmental variables

6.4.1.1 Landscape drivers

The first two PCA axes cumulatively explained 73.4% of the total variance in the four landscape drivers (Figure 6-2). We interpreted Axis 1 (eigenvalue = 2.079, explained 52.0% variance) as a gradient from a low to high built-up land cover in the 300 m buffer surrounding the SWP sites. PC1 axis scores increased with increasing impervious and road covers and decreased with tree canopy and open water cover. Impervious and road cover both had eigenvector values >0.5 and canopy and water cover in a 300 m buffer had eigenvector values of < -0.3 (Table E-1). We labeled Axis 1 *Developed Cover* (Table 6-2). Axis 2 (eigenvalue = 0.858, explained 21.5% variance) represented a range of the amount of tree canopy in the landscape, with canopy cover in a 300 m buffer having an eigenvector value of 0.890 (Table E-1). We labeled Axis 2 *Canopy Cover* (Table 6-2).

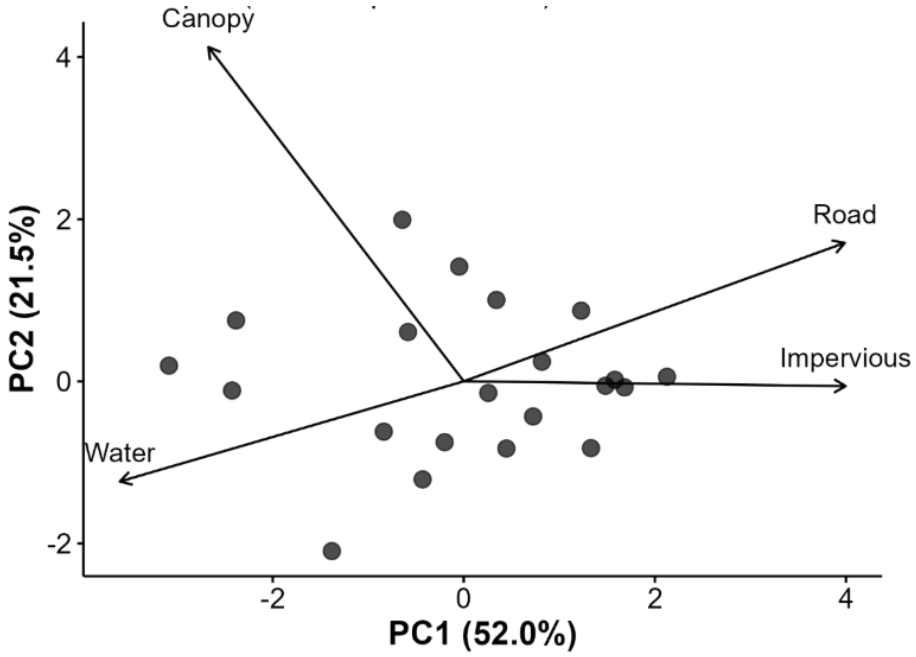


Figure 6-2. Biplot of principal components analysis (PCA) results based on a correlation matrix with four landscape variables (vectors) from 21 SWP sites (circles). Water = water cover in 300 m buffers; Canopy = canopy cover in 300 m buffers; Road = road cover in 300 m buffers; Impervious

= impervious cover in 300 m buffers. Ridged reflections on Axis 1 was done to improve interpretability. Vector length is 5 * r (correlation coefficient) for each PC axis.

6.4.1.2 Local drivers

Together, Axis 1 and Axis 2 explained 76.4% of the total variance in the six local driver variables (Figure 6-3). Axis 1 (eigenvalue = 2.634, explained 43.9% variance) represents a gradient of increasing pond size, shrub cover, dead tree cover, and narrowleaf emergent vegetation cover. We interpret Axis 1 as representing a gradient in pond structure and shoreline slope, from artificial to more naturalized. The eigenvector values for the cover of dead trees, narrowleaf emergent vegetation, and shrubs were all > 0.3 (Table E-1). We labeled Axis 1 *Naturalized Basin* (Table 6-2). We interpreted Axis 2 (eigenvalue = 1.951, explained 32.5% variance) as a metric of total area of reeds and cattails, since most robust emergent vegetation were identified as either *Phragmites australis* or *Typha* spp. Most of the surveyed ponds were not naturalized (Figure 6-2) and the most abundant vegetation cover type was robust emergent vegetation, therefore the proportion of total vegetation cover is likely largely representative of the proportion of robust emergent vegetation. Both variables had eigenvector values > 0.5 (Table E-1). We labeled Axis 2 *Reed and Cattail Cover* (Table 6-2).

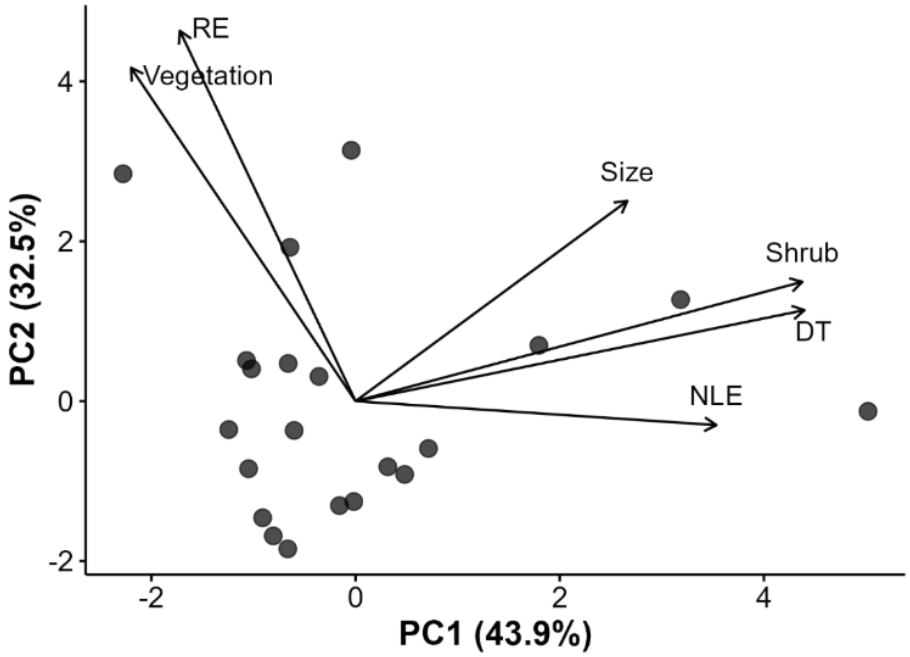


Figure 6-3. Biplot of principal components analysis (PCA) results based on a correlation matrix of six local driver variables (vectors) from 21 SWP sites (circles). Vegetation = percent vegetation cover; RE = area of robust emergent vegetation; Size = SWP size; Shrub = area of shrubs; DT = area of dead trees; NLE = area of narrowleaf emergent vegetation. Vector length is 5 * r (correlation coefficient) for each PC axis.

6.4.1.3 Within-pond water quality

The first and second axes cumulatively explained 55.2% of the total variance in water quality parameters (Figure 6-4). Axis 1 (eigenvalue = 4.122, explained 31.7% variance) scores decreased with increasing surface water temperature, dissolved oxygen (DO), and pH (eigenvector values < -0.2). Concentrations of PO_4, coliform bacteria, and *E. coli* had eigenvector values > 0.3 (Table E-1), representing an increase in nutrient pollution with increasing Axis 1 scores. Higher nutrient conditions also coincided with higher algae abundance, represented by chlorophyll-*a* and pheophytin concentrations in the water, both with eigenvector values > 0.2. Although chlorophyll-*a* and pheophytin concentrations had slightly higher loadings (0.3) on Axis 2, we decided to include these variables in the interpretation of Axis 1 to better describe the influence of nutrient pollution. We consequently labeled Axis 1 *Nutrient Pollution* (Table 6-2). Axis 2 (eigenvalue = 3.059, explained 23.5% variance) described a gradient from low to high conductivity, chloride concentration, and total suspended solids (TSS) (eigenvectors > 0.3; Table E-1), reflecting common attributes of road runoff. Thus, we labeled Axis 2 *Road Runoff* (Table 6-2).

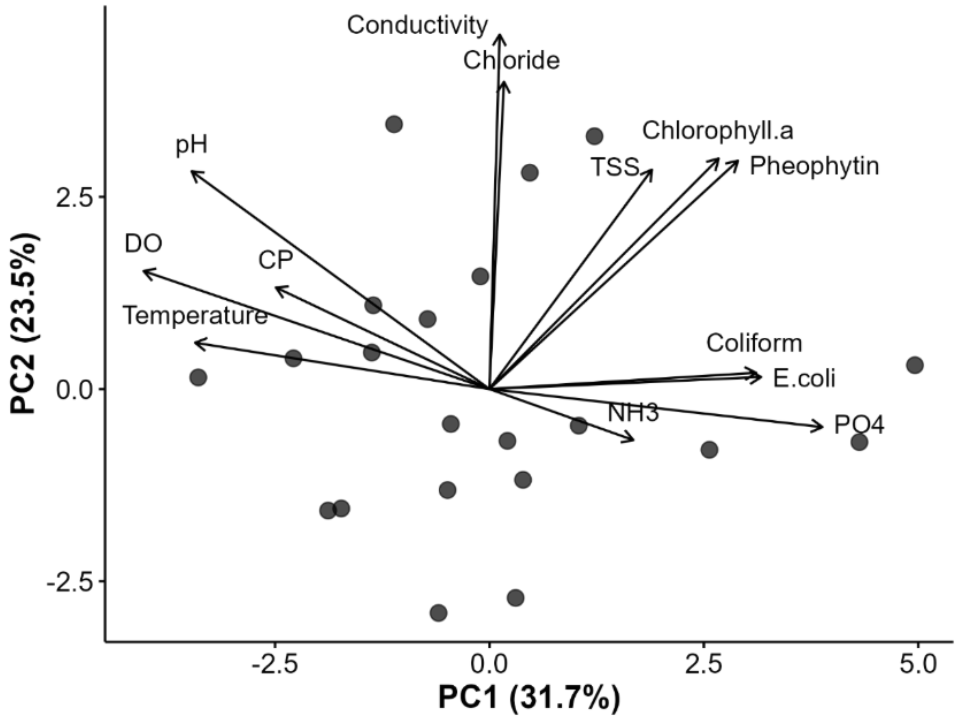


Figure 6-4. Biplot of principal components analysis (PCA) results based on a correlation matrix of 13 water quality variables (vectors) from 21 SWP sites (circles). Vector length is 5 * r (correlation coefficient) for each PC axis.

6.4.1.4 Pesticide concentrations in water samples

Only the first axis (eigenvalue = 3.440, explained 26.5% of variance) met our criteria of explaining > 20% of the total variance in pesticide concentrations in water samples (Figure 6-5). Axis 1 scores increased with synthetic auxin herbicides used to control broadleaf weeds. The eigenvector values for 2,4-D, MCPA, MCPP, dicamba, and triclopyr were all > 0.3 (Table E-1). We labeled this axis *Synthetic Auxins* (Table 6-2). Axis 2 (eigenvalue = 2.109, explained 16.2% variance) was not included in further analysis.

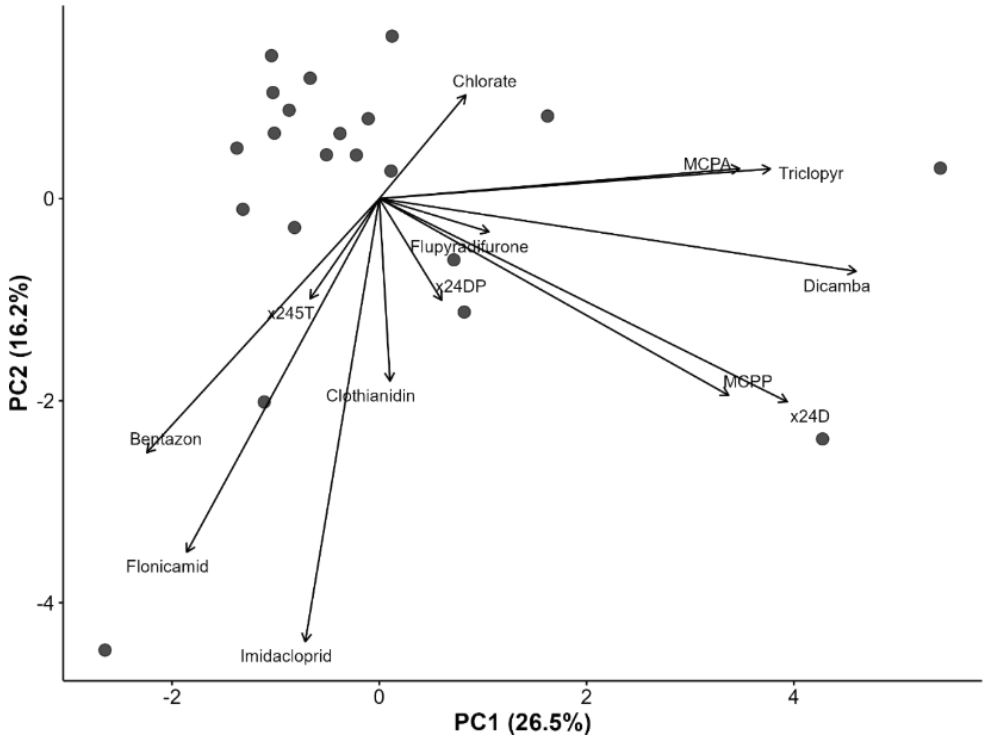


Figure 6-5. Biplot of principal components analysis (PCA) results based on a correlation matrix of concentrations of pesticides (vectors) detected in stormwater samples collected from 21 SWP sites (circles). Axis 2 was not included in further analysis. x245T = 2,4,5-T; x24DP = 2,4-DP; x24D = 2,4-D. Vector length is 5 * r (correlation coefficient) for each PC axis.

6.4.1.5 Pesticide concentrations in biofilm samples

The first two axes cumulatively explained 52.1% of the total variance in concentrations of pesticides detected in biofilm samples (Figure 6-6). Axis 1 (eigenvalue = 1.596, explained 26.6% variance) scores increased with the concentration of azoxystrobin and HEPA (eigenvector values both > 0.5), as well as 2,4-D (eigenvector = 0.2278; Table E-1). Scores decreased with the concentration of tebuconazole and nicotine, both with eigenvector values < -0.2. Azoxystrobin, HEPA (a metabolite of glyphosate), and 2,4-D have (or are sourced from) common applications on lawn and turf, whereas tebuconazole is a broad-spectrum fungicide used against diseases like rusts, powdery mildew, and leaf spots. Nicotine, likely is either leached from cigarette butts, a common type of urban litter, or enters the stormwater system from human wastewater. We labeled Axis 1 as *Non-Point Source Pesticides*. Axis 2 (eigenvalue = 1.527, explained 25.5% variance) scores increased with 2,4-D concentration (eigenvector = 0.2440). The

remaining pesticides had absolute eigenvector values < 0.2 (Table E-1). We labeled Axis 2 *Increasing 2,4-D*.

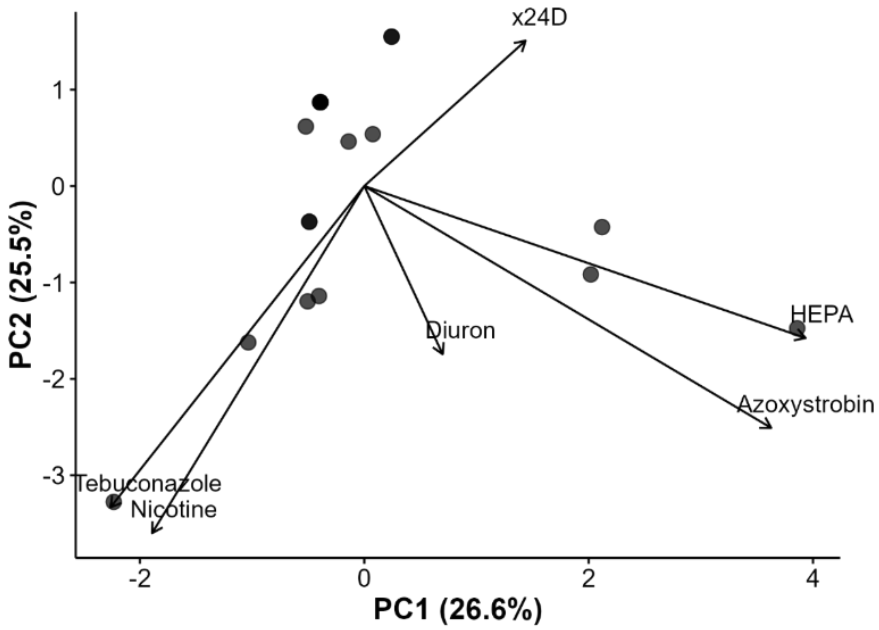


Figure 6-6. Biplot of principal components analysis (PCA) results based on a correlation matrix of concentrations of pesticides (vectors) detected in biofilm samples collected from 21 SWP sites (circles). x24D = 2,4-D. Vector length is 5 * r (correlation coefficient) for each PC axis.

Table 6-2. Summary of significant principal components analysis (PCA) axes. Axes were deemed significant if they explained 20% or more of the variance.

PCA	Axis	% variance explained	Description of gradient	Label
Landscape Drivers	Axis 1	52.0	Range from more naturalized to more built-up cover in the proximate area around the ponds.	Developed Cover
Landscape Drivers	Axis 2	21.5	Range from lower to higher proportion of canopy cover.	Canopy Cover
Local Drivers	Axis 1	43.9	Range from low to high pond size, cover of shrubs, dead trees, and narrowleaf emergent vegetation	Naturalized Basin

Local Drivers	Axis 2	32.5	Range from low to high robust emergent vegetation and proportional vegetation cover.	Reed and Cattail Cover
Water Quality	Axis 1	31.7	Range from low to high eutrophic conditions with higher nutrients, bacteria, chlorophyll-*a*, and pheophytin concentrations. Also associated with lower dissolved oxygen and temperatures, and higher acidity.	Nutrient Pollution
Water Quality	Axis 2	23.5	Range from low to high conductivity, chloride, and TSS.	Road Runoff
Pesticides in Water Samples	Axis 1	26.5	Range from low to high concentrations of synthetic auxin herbicides	Synthetic Auxins
Pesticides in Biofilm Samples	Axis 1	26.6	Increasing HEPA, azoxystrobin, and 2,4-D concentrations; decreasing nicotine and tebuconazole concentrations.	Non-Point Source Pesticides
Pesticides in Biofilm Samples	Axis 2	25.5	Increasing 2,4-D concentrations; azoxystrobin, diuron, HEPA, nicotine, and tebuconazole decreasing in concentration.	Increasing 2,4-D

6.4.2 Biological assemblages

6.4.2.1 Periphytic diatoms

We found a total of 16 families and 34 genera of periphytic diatoms across all 21 biofilm samples collected from the SWP sites. The most common genera were *Achnanthidium*, *Nitzschia*, and *Thalassiosira* which were present in all samples, followed by *Gomphonema*, *Navicula*, *Cocconeis*, and *Stephanocyclus* present in 95, 81, 71, and 71% of samples, respectively. The number of genera per site (i.e., taxonomic richness) ranged from 7 to 19 (Table E-2), averaging 13 (SD ± 4). Percent *Achnanthidium* ranged from 4 to 87%, averaging 38% (SD ± 28%). The percent of motile genera ranged from 2 to 75%, averaging 36% (SD ± 26%). The percent of dominant taxa ranged from 28 to 87%, averaging 53% (SD ± 18%).

6.4.2.2 Aquatic macroinvertebrates

We identified 45 taxa across our 63 samples from 21 SWP sites, including 40 identified at the family-level and the remaining five at the order, infra-order, or subclass level. The most abundant taxa were represented by the families Baetidae and Chironomidae, both occurring at 100% of sites, followed by Coengrionidae, occurring at 95% of sites, and Notonectidae, occurring at 76% of sites. Corixidae,

Libellulidae, and Oligochaeta were each present at 71% of sites. The total number of taxa per site (i.e., taxonomic richness) ranged from 7 to 26, averaging 14 (SD ± 5; Table E-3). The percentage of EPT (excluding members of the family Baetidae) ranged from 0 to 15%, averaging 2% (SD ± 4%). The percent of worms ranged from 0 to 32%, averaging 6% (SD ± 8%), while the percent Diptera ranged from 2 to 72%, averaging 19% (SD ± 19%) across all sites. Finally, the percentage of the most dominant taxon at each site ranged from 15 to 72% averaging 45% (SD ± 16%).

6.4.3 Periphytic diatom and aquatic macroinvertebrate community composition

6.4.3.1 Periphytic diatom community composition

The optimal NMDS solution for the Bray-Curtis dissimilarity matrix based on the relative abundances of periphytic diatom genera had two dimensions (stress = 0.098; Figure E-1), with NMDS1 approximating 73.1% of the variance and NMDS2 approximating 27.0% of the variance (Figure 6-7, Table E-5). *Nutrient Pollution* ($p = 0.010$), *Synthetic Auxins* ($p = 0.011$), and *Road Runoff* ($p = 0.13$) were the three PC axes from Table 6-2 that were reasonably correlated ($p < 0.2$, $r^2 > 0.010$; Table E-6) with the diatom dissimilarity matrix, and are presented as vectors in Figure 6-7. All four biological metrics calculated from the diatom assemblages were significantly correlated with the ordination scores of sites ($p < 0.05$; Table E-7).

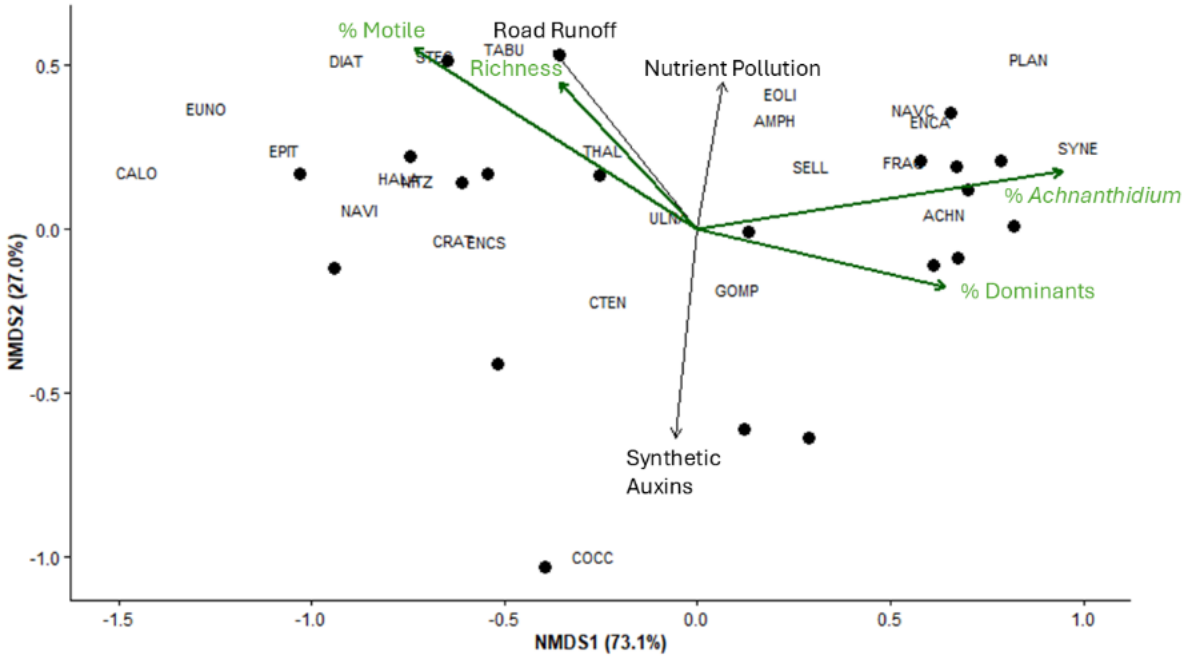


Figure 6-7. Non-metric multidimensional scaling (NMDS) ordination of periphytic diatom community composition in 21 SWP sites (circles). Environmental vectors (black arrows) represent selected principal component axis scores (Table 6-2). Only environmental vectors which were reasonably related to NMDS axes ($p < 0.20$, $r^2 > 0.01$) are included. Thick green arrows represent biological metrics which were significant with the ordination ($p < 0.05$). Refer to Table E-4 for a description of taxon codes.

NMDS1 was negatively correlated with *Road Runoff*. Sites with lower scores on NMDS1 therefore were characterized by higher concentrations of chloride, higher conductivity, and higher TSS levels, and had higher relative abundances of *Tabularia*, *Stephanocyclus*, and *Thalassiosira*, and lower relative abundances of *Gomphonema* (Figure 6-7). The percentage of motile taxa and total taxonomic richness was positively aligned with *Road Runoff*, and the percentage of *Achnanthidium* and percentage of dominant taxa were negatively aligned with *Road Runoff*. The percent of motile diatoms at a site was significantly and positively correlated with the PC axis scores from *Road Runoff* (coefficient = 8.164, p = 0.01; Table E-8, Figure 6-8A), and the percent *Achnanthidium* was significantly and negatively correlated with the PC axis scores from *Road Runoff* (coefficient = -7.270, p = 0.036; Table E-8, Figure 6-8B). Although both relationships were significant, their relatively low R^2 values (<0.3) indicates lower support

for the predictability of these relationships. Taxonomic richness ($p = 0.26$) and percent dominant taxa ($p = 0.188$) were not significantly correlated with *Road Runoff* (Table E-8).

NMDS2 was positively correlated with both *Road Runoff* and *Nutrient Pollution* (Figure 6-7). Sites with higher scores on NMDS2 therefore were characterized by higher concentrations of NH_3, PO_4, coliform bacteria, *E. coli*, chlorophyll-*a,* pheophytin, and TSS, and lower concentrations of DO, lower pH, and lower water temperatures, in addition to characteristics associated with *Road Runoff*. These sites tended to have higher relative abundances of *Tabularia*, *Diatoma*, and *Planothidium*, and lower relative abundances of *Cocconeis*. NMDS2 was negatively correlated with *Synthetic Auxins*, therefore sites with higher scores on this axis were characterized by higher concentrations of the herbicides 2,4-D, MCPA, MCPP, dicamba, and triclopyr in the water. These sites also had higher relative abundances of *Cocconeis* and lower relative abundances of *Tabularia*, *Diatoma*, and *Planothidium*. The percentage of motile taxa and total taxonomic richness were positively correlated with *Road Runoff* and *Nutrient Pollution* in this ordination but only the percent motile taxa and *Road Runoff* were significantly correlated (Table E-8).

The vector representing increasing *Nutrient Pollution* coincided with decreasing *Synthetic Auxins.* We did not observe any significant correlations between *Synthetic Auxins* or *Nutrient Pollution* axes scores with the biological metrics tested (Table E-8). The three environmental vectors (*Nutrient Pollution*, *Synthetic Auxins*, and *Road Runoff*) were correlated primarily with NMDS2; however, most of the variance (73.1%) in diatom relative abundances among sites was explained on NMDS1.

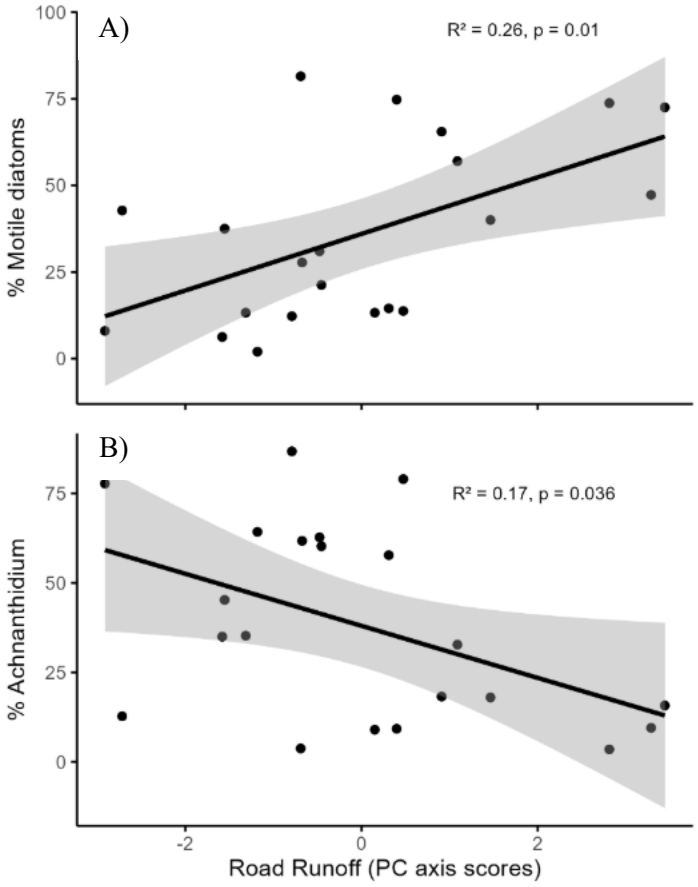


Figure 6-8. Simple linear regressions showing the relationship between increasing *Road Runoff* and A) Percent motile diatoms; and B) Percent *Achnanthidium*.

6.4.3.2 Aquatic macroinvertebrate community composition

The optimal NMDS solution for the Bray-Curtis dissimilarity matrix based on the relative abundances of aquatic macroinvertebrates had three dimensions (stress = 0.089; Figure E-2). NMDS1, NMDS2, and NMDS3 approximated 42.8, 35.3, and 22.0% of the variance, respectively (Table E-5). Environmental vectors, represented by PC axis scores, which were reasonably correlated ($p < 0.20$, $r^2 > 0.01$) were fitted onto the ordination (Table E-9). These were *Naturalized Basin* ($p = 0.025$), *Road Runoff* ($p = 0.051$), and *Nutrient Pollution* ($p = 0.090$). All biological metrics calculated from the macroinvertebrate assemblages, except for % dominants, were reasonably correlated with the ordinations ($p < 0.20$; Table E-10).

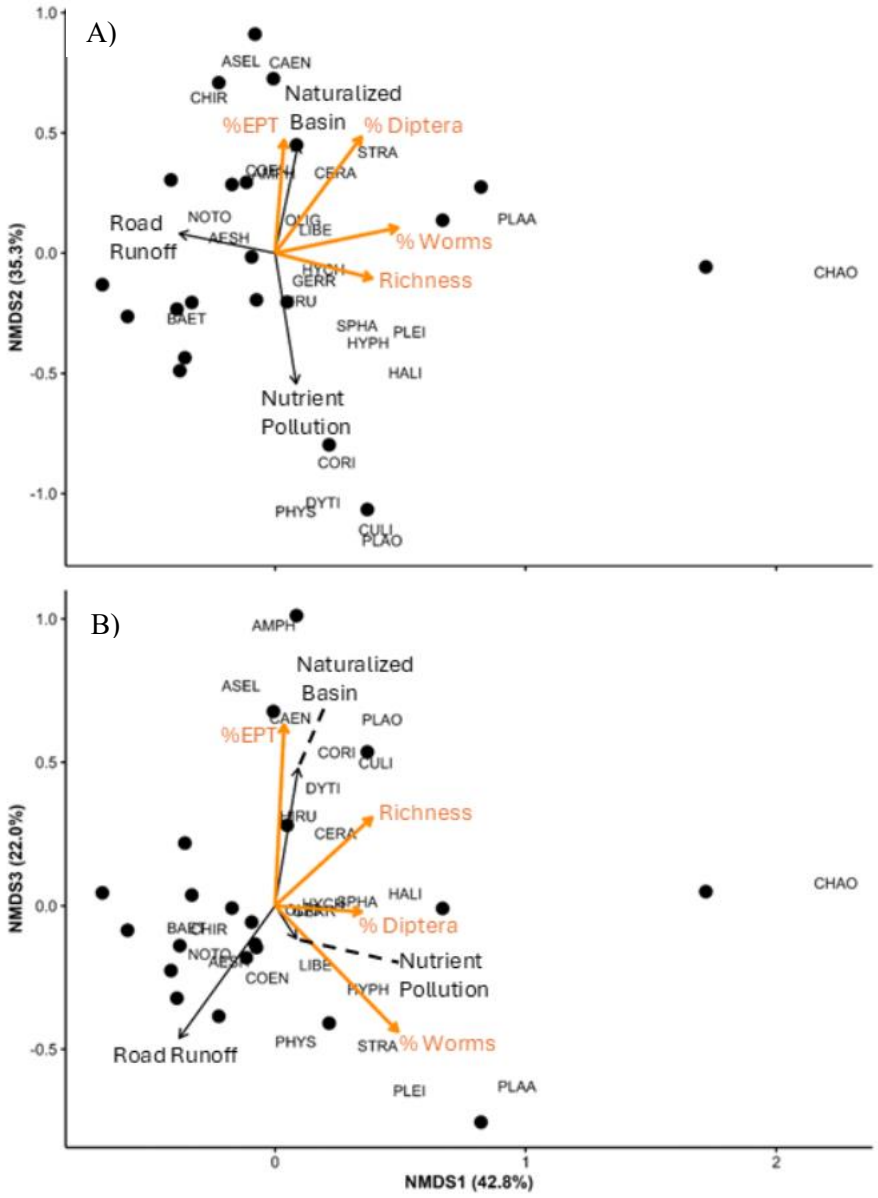


Figure 6-9. Non-metric multidimensional scaling (NMDS) ordination of aquatic macroinvertebrate community composition in 21 SWP sites (circles). Panel A shows NMDS1 and NMDS2; Panel B shows NMDS1 and NMDS3. Environmental vectors (black arrows) represent selected principal component axes scores (Table 6-2). Only environmental vectors which were reasonably related to NMDS axes ($p < 0.20$, $r^2 > 0.01$) are included. Orange arrows represent

biological metrics which were significantly correlated with the ordination ($p < 0.05$). Refer to Table E-4 for description of taxon codes.

NMDS1 (Figure 6-9A and B) was negatively correlated with *Road Runoff*. Sites with lower scores on NMDS1 therefore were characterized by higher concentrations of chloride, higher conductivity, and higher TSS levels. They also tended to have higher relative abundances of Notonectidae, Aeshnidae, Chironomidae, and Baetidae, and lower relative abundances of Chaoboridae and Planariidae. The percentage of worms and taxonomic richness were negatively correlated with *Road Runoff*, however only taxonomic richness was significantly and negatively correlated with the PC axis scores from *Road Runoff* (coefficient = 0.342, p = 0.003; Table E-11; Figure 6-10A).

NMDS2 (Figure 6-9A) and NMDS3 (Figure 6-9B) were both positively correlated with *Naturalized Basin* features. Sites with higher score on NMDS2 and NMDS3 therefore were characterized by higher cover of shrubs, dead trees, and narrowleaf emergent vegetation, and tended to have higher relative abundances of Caenidae, Asellidae, and Amphipoda, and lower relative abundances of Physidae. Percent EPT was positively correlated to *Naturalized Basin* on both NMDS2 and NMDS3, and the percentage of Diptera was positively aligned with *Naturalized Basin* on NMDS2 whereas total taxonomic richness was positively aligned on NMDS3. NMDS2 and NMDS3 were both negatively correlated with *Nutrient Pollution*, with NMDS2 more strongly correlated with this vector than NMDS3. Sites with lower scores on both these axes therefore had higher concentrations of NH_3, PO_4, coliform bacteria, *E. coli*, chlorophyll-*a,* pheophytin, and TSS, and lower concentrations of DO, lower pH, and lower water temperatures. On NMDS2, these sites had higher relative abundances of Physidae, Dytiscidae, Corixidae, Culicidae, and Planorbidae, while on NMDS3, these sites had higher relative abundances of Pleidae, Planariidae, Physidae, and Stratiomyidae. Of the biological metrics, only percent EPT was significantly and positively correlated with the PC axis scores from *Naturalized Basin* (coefficient = 0.202, p = 0.023; Table E-11, Figure 6-10B). We did not observe significant correlations between *Nutrient Pollution* axis scores and the biological metrics (Table E-11). The *Nutrient Pollution* and *Naturalized Basin* PC axis scores were inversely related, but not significantly (p = 0.055; Figure E-3).

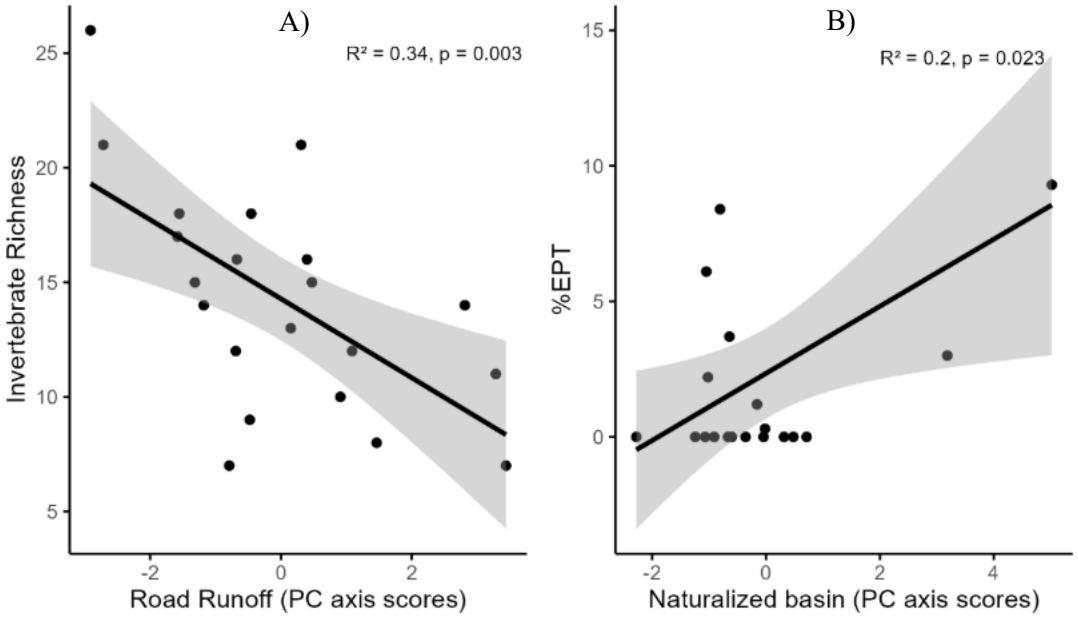


Figure 6-10. Simple linear regressions showing the relationships between A) increasing *Road Runoff* and taxonomic richness of invertebrates; and B) increasing *Naturalized Basin* and %EPT.

6.5 Discussion

Stormwater management ponds (SWPs) are fundamentally infrastructure, constructed to meet the needs of flood management in developed areas lacking natural infiltration cycles (Lusk et al., 2025). These constructed ponds are not intended to support urban wildlife. Consequently, the conditions within the ponds are often suboptimal for sustaining diverse and healthy biological communities (Hale et al., 2019). Nonetheless, SWPs are frequently colonized by various urban-adapted species, including taxa of conservation interest, thereby inadvertently contributing to urban biodiversity (Holtmann et al., 2019a; Holtmann et al., 2019b; McIsaac, 2022).

In our study, we explored two biological assemblages, periphytic diatoms and aquatic macroinvertebrates, both commonly studied indicators of ecosystem health, yet understudied in conjunction with SWP contamination and landscape features. In our exploratory approach, our objective was to characterize the composition of these assemblages and study the relationships between variance in these communities and external environmental factors to develop hypotheses that could be pursued by

future researchers (Table 6-3). As a brief overview, we found that diatom communities were predominantly composed of pollution-tolerant genera such as *Achnanthidium*, *Nitzschia*, *Gomphonema*, and *Cocconeis*. Similarly, macroinvertebrate assemblages were dominated by families like Baetidae, Chironomidae, and Coengrionidae, which exhibit broad environmental tolerances (Menetrey et al., 2008; Carew et al., 2007; Vilenica et al., 2021). Non-metric multidimensional scaling (NMDS) ordinations indicated that water quality parameters were associated with variation in community composition for both assemblages, while pesticide contamination was more predictive of diatom relative abundances and local vegetation cover was associated with macroinvertebrate relative abundances. Conversely, landscape variables within a 300 m buffer surrounding the SWPs did not significantly predict community composition or taxonomic richness for either assemblage. We provide a more detailed discussion of these results below.

6.5.1 Water quality and herbicide contamination are associated with diatom community composition

The diatom community composition was more associated with anthropogenic chemical stressors than the landscape and habitat characteristics of the SWPs. Our analysis identified that gradients associated with *Nutrient Pollution*, *Road Runoff*, and *Synthetic Auxins* related to the variability in periphytic diatom assemblages. However, these factors accounted for a limited proportion of the variance, suggesting additional unmeasured stressors with stronger relationships to community structure. Urban aquatic systems are subject to a multitude of stressors, including heavy metals, hydrocarbons, and emerging contaminants (Masoner et al., 2019), which may have substantial, yet in our case unquantified, impacts on diatom communities. Together, these measured and unmeasured stressors may exert complex mixture effects on exposed biota, complicating attempts to confidently discern key modulators of assemblage composition. Previous investigations into environmental drivers of diatom assemblage variability in stormwater-affected systems have also found a lack of explanatory relationships, likely due to the established tolerance to frequent disturbances (e.g., Gillet et al., 2017; Minelgaite et al., 2020), whereby sensitive taxa are already excluded from these polluted and stressful conditions.

Assessment of water quality parameters revealed an unexpected alignment of temperature, dissolved oxygen (DO), and pH measurements. Typically, in natural aquatic systems, DO and pH exhibit an inverse relationship with temperature. In the case of DO, this is due to the decreased solubility of oxygen at higher temperatures (USGS, 2018). However, our sampling occurred during daylight hours

(10:00–16:00), coinciding with peak photosynthetic activity, which can elevate DO even during peak diurnal temperatures (Tarr et al., 2005; EPA, 2023). Additionally, we observed an inverse relationship between nutrient concentrations and DO, suggesting eutrophic conditions associated with higher nutrient levels resulted in reduced DO levels, or conversely, oligotrophic conditions associated with lower nutrient levels supported higher DO levels. Trophic classification is typically based on total phosphorus (TP) concentrations (e.g., CCME, 2004; where TP > 20 µg L^{-1} is classified as eutrophic and TP <10 µg L^{-1} is oligotrophic). As our nutrient analysis only included inorganic phosphorus in the form of orthophosphate (PO_4), which ranged from < MDL (3.0 µg L^{-1}) to 70.9 µg L^{-1}, we can only suggest that the three sites with PO_4 concentrations above 20 µg L^{-1} were likely eutrophic, while those with PO_4 concentrations below 5 µg L^{-1} (n = 17), experienced more oligotrophic conditions at the time of sampling.

The elevated pH values observed are typical of SWPs in Ontario (e.g., Chiandet & Xenopoulos, 2016; Hassal & Anderson, 2015). These conditions may be attributed to the peak photosynthetic activity at the time of sampling (as the removal of carbon dioxide from the water elevates pH levels) or to the weathering of construction materials and pavements, such as limestone-based aggregates commonly used in southern Ontario (Holt et al., 2011), which release carbonates into the water, thereby increasing alkalinity (Wright et al., 2011). As the temperature of stormwater increases when running over warm surfaces like pavements, in a sewershed with higher coverage of impervious cover, we could therefore expect an increase in both thermal pollution and pH. The presence of limestone riprap and concrete structures in SWPs could also contribute to these elevated pH levels, although further research is necessary to test these hypotheses (Table 6-3; Hypotheses 1 and 2).

The relationship between *Nutrient Pollution* and diatom community composition was somewhat perplexing. For instance, members of the genus *Cocconeis*, such as *Cocconeis placentula*, are known to dominate in eutrophic and turbid conditions (Lavoie et al., 2008), however, this genus was most abundant in SWPs characterized by lower nutrient concentrations. *Cocconeis* was also more abundant in sites with higher *Synthetic Auxins*, indicated by elevated levels of herbicides used to treat broadleaf vegetation. These herbicides are often applied on turf, which can be found in parklands, sports fields, areas lining transportation corridors, municipal lands, school fields, and golf courses, and includes the mowed areas around SWPs. The opposing alignment of the vectors representing *Nutrient Pollution* and *Synthetic Auxins* suggests that excess nutrient inputs may not be primarily sourced from fertilizer use on these landscaped areas, and that there are likely differing pathways for nutrient and herbicidal contamination to SWPs. Moreover, *Cocconeis* species are often associated with aquatic vegetation (Olding 2000) or

riparian vegetation (Comeau 2016), implying that runoff associated with *Synthetic Auxins* may serve as a proxy for the presence of manicured or ornamental vegetation around SWPs or within their sewersheds. This relationship is an important area for future study as it may advise on the sources of these contaminants to SWPs (Table 6-3; Hypothesis 3).

The positive correlation between motile diatom taxa and *Road Runoff* indicates that SWPs receiving higher inputs of total suspended solids (TSS) and chloride, and with higher conductivity, favour diatoms capable of moving away from silty conditions or relocating to more favourable microhabitats (Jones et al., 2017; Stenger-Kovacs et al., 2023). Genera such as *Stephanocyclus* and *Tabularia*, which can inhabit both benthic and planktonic environments, were also prevalent in these conditions. Additionally, salt-tolerant taxa, such as *Diatoma,* were abundant in SWPs characterized by *Road Runoff*, consistent with findings from urban ponds in Toronto, Ontario (Olding, 2000). Future research could survey a wider range of conditions, with more varying rates of deposition and chloride concentrations, to test more directly the effect of these parameters on the prevalence of motile and salt-tolerant diatom taxa (Table 6-3; Hypothesis 4).

Pollution-tolerant taxa were well represented in the biofilm from our SWPs. For example, members of the genus *Nitzschia*, such as *Nitzschia palea*, members of *Gomphonema*, such as *Gomphonema parvulum*, and members of the genus *Achnanthidium*, such as *Achnanthidium minitussimum*, are widespread in contaminated urban surface waters (e.g., Olding 2000; Comeau 2016; Minelgaite et al., 2020). However, despite its dominance, the proportion of diatoms from the genus *Achnanthidium* was significantly and negatively correlated with *Road Runoff*. This opposes the expectation that pollution tolerance increases with percent impervious cover in a catchment (Muscio 2002), and could suggest that the type of disturbance matters*: Achnanthidium* are known to be tolerant of substrate and hydrological disturbances (Biggs et al., 1999), yet the chronic, chemically stressful conditions characteristic of runoff from roads may exceed their tolerances to silty and salty conditions. Interestingly, SWPs seemed to be either dominated by *Nitzschia* or *Achnanthidium*, but rarely both, suggesting differing niche preferences or environmental filtering of the two genera. Differing tolerances to stressors are seen in some species within these two genera (e.g., *Nitzschia palea* and *Achnanthidium* spp., have different sensitivities to herbicides; Esteves et al., 2017) and reciprocal dominance has been observed in *Nitzschia* and *Achnanthidium* species in river systems of differing environmental conditions (Shen et al., 2018). However, whether these differences at the species-level can be generalized at the

genus-level warrants further investigation into the underlying ecological mechanisms driving this differential dominance (Table 6-3; Hypothesis 5).

From these results, it is difficult to make generalizations about whether diatom communities in biofilms are as useful for indicating water quality in SWPs as they are in other, more variable environments. Urban SWPs may represent a narrow portion of the pond condition spectrum, likely on the extreme end, and thus may simply have low diversity consisting of mainly tolerant taxa. This may provide us with less resolution to explore the variability of assemblages among SWPs. Another caveat of our approach is that we sampled diatoms from biofilms grown on artificial substrates in the middle of the ponds, and although this technique is commonly employed in biofilm assessments (Sabater et al., 2007), this method may not provide us a true reflection of the natural diatom assemblages present in the ponds (Miao et al., 2021). Using this standardized approach minimizes the effect of substrate differences (e.g., between biofilms on rocks or macrophytes if sampling on natural substrates) or heterogeneity in water depth on the cultivated biofilm communities, which enhances comparability among sites. However, there are trade-offs with this approach, including lost realism. For example, ecological selection on which taxa occur in assemblages will differ based on whether the biofilms are newly established (i.e., are colonizing) or have already established some growth (i.e., are accumulating; Tien et al, 2009). Future research should consider comparing diatom assemblages from both natural and artificial substrates and of various ages to determine the merit of using diatoms as representative indicators of water quality specifically in SWPs (Table 6-3; Hypothesis 6A and 6B).

Table 6-3. Hypotheses proposed from our exploration of local and landscape drivers of periphytic diatom and aquatic macroinvertebrate assemblages in urban stormwater ponds (SWPs).

Number	Hypothesis
1	SWPs with higher proportion of impervious cover in their sewersheds have higher water temperatures and alkalinity.
2	SWPs with more structural features made from limestone have higher alkalinity.
3	A higher proportion of manicured or ornamental vegetation surrounding SWPs is associated with higher levels of synthetic auxin herbicides.
4	Higher rates of deposition from roads, measured by higher TSS and chloride concentrations, drive higher proportions of motile and salt-tolerant diatom taxa in SWPs.
5	The reciprocal dominance observed between species of *Nitzschia* and *Achnanthidium* in stormwater-impacted systems is driven by differing environmental tolerances.
6	The response of diatom community composition in SWPs to water quality conditions is significantly different when sampled from biofilms grown on artificial substrates compared

	to those from natural substrates (A). The response of diatom community composition in SWPs to water quality conditions is influenced by the time since initial colonization (B).
7	Species- and genus-level identification of macroinvertebrate samples from SWPs provides richness and composition data more strongly related to environmental conditions than at the family- and order-level.
8	Sewershed size and morphology correlate to water quality metrics and (A) macroinvertebrate or (B) diatom community composition in SWPs.
9	Macroinvertebrate taxonomic richness in SWPs is more strongly influenced by substrate composition than by other local pond features.
10	Macroinvertebrate assemblage composition and richness are influenced by road-related contaminants in both the water column and sediments in SWPs.
11	Pond connectivity (i.e., the proportion of water within a 300 m buffer surrounding a SWP) is correlated to variability in macroinvertebrate community composition in SWPs.
12	Landscape features (e.g., impervious cover, road cover) within the sewershed of a SWP are more predictive of variability in diatom and macroinvertebrate assemblages than landscape features of the surrounding landscape (i.e., in a symmetrical buffer zone).

6.5.2 Macroinvertebrate community composition is associated with local pond features and water quality

Aquatic macroinvertebrate communities within the studied SWPs were dominated by taxa with high tolerance to urban-associated stressors, such as nutrient enrichment and degraded water quality. For example, families like Chironomidae and Oligochaeta are commonly abundant in such environments due to their resilience to pollution (Carew et al., 2007; Sinclair et al., 2021). The widespread presence of the family Baetidae, regardless of environmental conditions, supports the notion that members of this family are generalists with broad ecological tolerances (Barbour et al., 1999; Menetrey et al., 2008), are often not as sensitive as other families in Ephemeroptera (e.g., Alhejoj et al., 2014), and are thus best excluded from biological indices, such as %EPT (Carpenter et al., 2016).

Based on the PCA analysis, we characterized the local features of the SWPs as indicators of the naturalization of the pond design. Compared to constructed SWPs, naturalized ponds and constructed wetlands tend to be larger in size, have more dead woody debris and peripheral shrub cover, and more gradual slopes providing a more suitable habitat for narrowleaf emergent vegetation like sedges, rushes, and flowering plants (Chambers & Kalff, 1986; Hudon et al., 2004; Rooney et al., 2015). Artificial SWPs, on the other hand, tend to have steep slopes to maximize holding capacity, often with stone or concrete banks and turfgrass-style landscaping. The extent of naturalization was the strongest predictor of

macroinvertebrate community composition in our SWPs, likely because complex vegetation structures offer refuge and food resources, enhancing habitat suitability for various taxa (Hassall et al., 2011). For example, emergent macrophytes are crucial for the life cycles of Odonata, serving as sites for oviposition, emergence, and shelter (Johansson et al., 2019). Contrary to Sinclair et al. (2021), who reported dominance of Amphipoda and Chironomidae in structurally simple habitats, our findings indicated higher abundances of these taxa in more naturalized SWPs.

Caenidae, a moderately sensitive family of mayflies, and the cosmopolitan detritivores Asellidae and Amphipoda were more abundant in SWPs with naturalized basin features. These features contribute to a higher diversity of macrophytes, increasing the availability of organic matter and refugia to these taxa (Thornhill et al., 2017). However, environmental tolerances can be variable within families and orders and are most accurately described at the species- or genus-level (Jones, 2008). The presence of sensitive taxa, where that sensitivity is described at coarser taxonomic resolution, does not unequivocally indicate habitat quality, and may merely reflect the fulfillment of minimal habitat requirements of some, but not necessarily all, of the members in that taxonomic group. Gleason and Rooney (2017) found that disturbances from agricultural activities were not predicted by macroinvertebrate assemblage composition in prairie pothole wetlands in Alberta, Canada, when taxa were identified to the family level. This lack of relationship, despite the wetlands being located across a large range of agricultural cover, suggests that family-level assessment of macroinvertebrate community composition may not be an effective bioindicator tool for environmental conditions in small lentic ecosystems (Gleason & Rooney, 2017).

Additionally, SWPs generally only represent a small range in habitat conditions compared to the full range of possible environments occupied by aquatic macroinvertebrates. Most of the taxa identified in our samples were generalists with wide environmental tolerances, likely because the unnatural conditions characteristic of stormwater ponds filter out most of sensitive taxa in the sample pool. However, an assessment of SWP macroinvertebrate communities employing finer taxonomic resolution, such as with eDNA sampling and analysis, could improve our understanding of how pond conditions relate to taxonomic richness and composition without generalizing about environmental tolerances and sensitivities (Table 6-3; Hypothesis 7).

The outlying SWP site on the NMDS ordination of the macroinvertebrate assemblages (Figure 6-9; right side) is located within 200m of Professor Lake, a small urban lake, but is otherwise very isolated from natural areas. It is surrounded by berms on two long sides and ringed by shrubby vegetation. The

pond has primarily steep concrete banks, so there is little emergent vegetation, making the site more pond-like than wetland like. This is reflected in the predominance of Chaoboridae and Planariidae - two taxa that are both predators on zooplankton and other small invertebrates. These taxa thrive in nutrient rich environments and are generally light-adverse, often preferring deeper, darker waters (Kenk, 1980; Borkent, 1981; DeWalt et al., 2010; Sluys & Riutort, 2018). This more pond-like character may be from the influence of nearby Professor Lake, the contribution of litter fall from the ringing shrubs, the berms that isolate the pond from direct runoff, and the steeply sloped sides that limit the growth of rooted aquatic vegetation. This is one of the few privately-owned and managed ponds that we surveyed, which might also explain the low evidence of road runoff influence, as it is likely not connected to the catch basin network of the surrounding roads, despite the high road density. The effect of sewershed size and morphology on the resulting water quality and macroinvertebrate community composition of the SWP is an interesting area for future study and could inform future development designs (Table 6-3; Hypothesis 8A). These pond attributes could also be explored in relation to the diatom community composition, given the correlation between water quality metrics and the diatom community (Table 6-3; Hypothesis 8B).

The percentage of EPT taxa (excluding Baetidae) was positively correlated with the degree of naturalization, and overall %EPT values were similar to those reported in other studies of stormwater impacted assemblages (e.g., Carpenter et al., 2016; Goldyn et al., 2018; Fanelli et a., 2019). Taxonomic richness did not exhibit a significant relationship with naturalization, despite evidence suggesting that features like gentle shore slopes and diverse vegetation enhance invertebrate richness (Becerra Jurado et al., 2010). More, larger ponds are thought to have increased abilities to buffer disturbances, for example by diluting incoming contaminant loads (Sun et al., 2018). The vector representing *Naturalized Basin* in the NMDS ordinations (Figure 6-9) was inversely related to *Nutrient Pollution* on NMDS2 (Figure 6-9A) and slightly inversely related with *Road Runoff* on NMDS3 (Figure 6-9B), suggesting that the riparian buffer and emergent vegetation may be effective at taking up nutrients, and possibly even intercepting TSS. The lack of relationship between macroinvertebrate taxonomic richness and pond naturalization may be attributed to the influence of unmeasured factors, such as substrate composition, which is known to be an important determinant of macroinvertebrate community structure (Mishra & Nautiyal, 2016). We would recommend testing this relationship in a SWP context (Table 6-3; Hypothesis 9). SWPs have occasionally been documented to exhibit higher macroinvertebrate richness than natural ponds, albeit with differing community compositions (Le Viol et al., 2009; Becerra Jurado et al., 2010; Hill et al., 2017; Meland et al., 2020). These differences may be more discernible at finer taxonomic resolutions

(Meland et al., 2020), underscoring the importance of detailed taxonomic identification in ecological assessments. Finally, the degree of isolation of SWPs from other habitats may restrict colonization by new species to those with better modes of dispersion.

The community composition of macroinvertebrates was also substantially associated with the quality of the pond water. SWPs with elevated TSS, chloride, and conductivity, harbored greater relative abundances of strong swimmers like Baetidae, Notonectidae, and Aeshnidae, paralleling the increased prevalence of motile diatoms under similar conditions. Chloride concentrations exceeded the Canadian Water Quality Guidelines for long-term exposure (120 mg L^{-1}; CCME, 2011) in 17 of the 21 SWPs. Elevated levels chloride have detrimental effects on freshwater macroinvertebrates (Soucek & Kennedy, 2005; Elphick et al., 2011) and can also damage pond vegetation resulting in less habitat and food (Blasius & Merritt, 2002). Elevated conductivity has been shown to influence macroinvertebrate communities (e.g., Perron and Pick, 2020b), with some studies reporting positive correlations with richness (e.g., Barman & Gupta, 2015), likely due to the proliferation of tolerant taxa like Coenagrionidae and Libellulidae (Capinera, 2008; Meland et al., 2020). Conductivity levels in our SWP sites are likely to be largely driven by the concentration of chloride, rather than other anions such as sulfate that may be more responsible for conductivity in natural systems, and which may have lower toxicities (e.g., Soucek & Dickenson, 2015). Our findings indicated a negative relationship between macroinvertebrate richness and *Road Runoff*, suggesting that extreme conditions may surpass the tolerance thresholds of even resilient taxa. Higher abundances of Baetidae were found at sites characterized by increased *Road Runoff*, which is supported by other studies which have found that Baetids are more tolerant of elevated salt than other taxa of mayflies (Pond et al., 2010; Timpano et al., 2018; Meland et al. 2020). An important limitation of our study was that we did not measure contaminants in the pond sediments, and it is possible that the indicators of Road Runoff in the water (e.g., chloride concentration and TSS) increase in tandem with deliveries of sediment-associated contaminants like heavy metals, which may also contribute to the effects on assemblage variability. This is another critical avenue for future work (Table 6-3; Hypothesis 10)

Nutrient Pollution, associated with decreasing DO and pH levels, favored taxa capable of atmospheric respiration, such as Physidae, Planorbidae, Dytiscidae, and Culicidae. These taxa are less affected by low DO conditions (Sun et al., 2019). Conversely, Amphipoda, which require higher DO levels, were more abundant in SWPs with better oxygenation, aligning with the findings of Sinclair et al.

(2021). However, unlike their study, we did not observe a dominance of Amphipoda in low pH conditions, and Odonata and Oligochaeta appeared less sensitive to pH variations in our SWPs.

The effects of vegetation cover and water quality on macroinvertebrate communities are likely integrated (Becerra Jurado et al., 2010; Sinclair et al., 2021). Riparian and emergent vegetation not only provide structural habitat and food resources but also influence water chemistry through processes like leaf litter decomposition, which can alter pH and nutrient dynamics (Stoler & Relyea, 2011). Thus, the interplay between habitat complexity and water quality is critical in shaping macroinvertebrate assemblages in SWPs.

Some invertebrates who disperse aerially need other waterbodies nearby to be successful (Briers & Biggs, 2005), and previous studies have found that urban pond connectivity was important for invertebrate richness (Sun et al., 2018), density (Holtmann et al., 2018), and community composition (Becerra Jurado et al., 2010). Thus, we expected pond connectivity (measured as the presence of other water bodies within the 300 m buffers) to have some association with the variation in macroinvertebrate composition. However, our SWP sites did not necessarily represent an evenly dispersed gradient of connectivity, thus further research is needed to investigate this relationship (Table 6-3; Hypothesis 11). The correlation between biological assemblages and habitat connectivity is important to urban biodiversity conservation and management, as recent evidence suggests thoughtful placement of SWP locations can contribute to landscape connectivity in cities (Birch et al., 2023).

6.5.3 Stormwater ponds do not reflect their locality

The relationship between the quality of stormwater and the extent of altered, or urbanized, land cover in a watershed has been well studied (e.g., Alley & Veenhuis, 1983; Liu et al., 2012; Arnold & Gibbons, 1996; Brabec et al., 2002), with increased impervious surface cover and road density linked to degraded water quality and altered hydrology (Walsh et al., 2005). However, landscape variables, such as road and impervious cover, showed no correlation to the richness and community structure of either the diatom or the invertebrate assemblages in our study. There are a few potential explanations for this finding. First, our approach to characterizing landscape drivers for predicting effects on biological assemblages took a buffer approach, in which variables were calculated based on a 300 m buffer surrounding each pond. However, much of the water received by SWPs is delivered by sewer pipes from artificial catchment areas that do not necessarily follow the natural topography of the land (Lim et al., 2017). The boundaries of these catchment areas, called sewersheds, are not easily delineated, due to their highly modified

construction and the cryptic influence of underground pipes, culverts, and catch basins. Although ecological connectivity could be influenced by impervious cover in a symmetrical buffer around a SMP, water quality is more likely influenced by the proportion of impervious cover within the sewershed. A symmetrical buffer around each pond may not best represent the range and relative contribution of different land cover types and land uses to the water quality in the ponds. The diatom and macroinvertebrate communities in ponds in our study do not seem to relate to variation in these proximal buffer zones; thus, we predict that the sewershed is more influential on the habitat quality of the pond, than the surrounding landscape. This is an area of inquiry requiring further exploration (Table 6-3; Hypothesis 12).

Secondly, the design and maintenance of individual SWPs may exert a stronger influence on local biological assemblages than the surrounding land use. Features such as sediment forebays, vegetated buffers, or outlet configurations can mediate the effects of catchment-level urbanization by altering flow regimes, residence time, and contaminant retention (OME 2003; Hatt et al., 2009; LeFevre et a., 2015; Walsh et al., 2012).

Our results align with recent research suggesting that pond biotic communities can be strongly associated with local-scale factors (Sinclair et al., 2021), occasionally more than by landscape context (Mackintosh et al., 2015; Mathers et al., 2024), particularly in urban settings where anthropogenic modifications have decoupled land-water interactions. This underscores the importance of site-level management practices, such as enhancing vegetation cover, minimizing concrete infrastructure, and reducing direct pollutant inputs, in shaping the ecological function of SWPs.

6.5.4 Implications for stormwater pond management and monitoring

Stormwater management ponds, though primarily designed for flood control and sediment capture, are increasingly recognized for their unintended but important role in supporting urban aquatic biodiversity (Ferzoco & McCauley, 2024; McKercher et al., 2024). Our findings emphasize that SWPs can provide habitat for diverse biological assemblages, but that their ecological quality is variable and assemblages are sensitive to local environmental conditions, particularly water quality and habitat structure.

The SWPs in our study which were more naturalized and had indications of lower nutrient pollution had higher %EPT (excluding the Baetidae family), and those associated with lower levels of road-related contamination had higher taxonomic richness, suggesting that management of these parameters could improve the robustness of the macroinvertebrate communities within urban ponds. To

enhance the ecological function of SWPs, management strategies should prioritize naturalization of shorelines and basins. Incorporating emergent vegetation, maintaining gradual slopes, and minimizing turfgrass or hardscaping around pond margins can improve habitat heterogeneity and provide refuge for aquatic organisms (Oertli & Parris, 2019; Sinclair et al., 2021). These design elements not only benefit biodiversity but may also improve water quality through enhanced nutrient uptake and sediment stabilization.

Given the prevalence of herbicides, chloride, and other urban pollutants in SWP water (Masoner et al., 2019) and biofilms (Izma et al., 2024a [Chapter 3]), regular monitoring of these compounds is essential. Our results suggest that biological assemblages are receptive to these contaminants, showing shifts in dominant taxa and reductions in sensitive groups. However, the presence of pollution-tolerant species does not preclude the potential for restoration; rather, it highlights the resilience of urban biota and the opportunity for gradual ecological improvement through better management.

Our study reinforces the value of integrating multiple bioindicator groups to assess ecological condition. While diatoms were more responsive to water chemistry (including contaminants associated with nutrient and road pollution), macroinvertebrates were more influenced by the habitat structure in each pond. Together, these assemblages offer complementary insights into the biophysical integrity of SWPs and can inform adaptive management strategies aimed at reconciling stormwater infrastructure with urban biodiversity goals. There are, however, opportunities for improvement for these approaches. For example, in the OBBN protocol, there are limited biological metrics that have been validated in wetlands and many of the suggested metrics may not be applicable for lentic waters (e.g., %EPT is a widely used metric, but Plecoptera is rarely found in still or slow-moving waters; Kashian and Burton, 2000). Small lentic habitats might benefit from additional metric development to improve the applicability of standardized biomonitoring tools like OBBN.

6.6 Conclusion

Our study explored the ecological characteristics of SWPs by examining patterns in periphytic diatoms in biofilms grown on artificial substrates and aquatic macroinvertebrate assemblages alongside water quality, pesticide contamination, and habitat and landscape features. Our findings highlight that SWPs, despite being artificial systems embedded in urban landscapes, can support a range of biological communities whose composition varies with site-specific environmental conditions.

Water chemistry, particularly concentrations of chloride, nutrients, and herbicides, was most strongly associated with variation in diatom assemblages, while macroinvertebrate richness was more closely linked to physical habitat features such as vegetation cover and pond morphology. These differing patterns reflect the differences in sampling conditions; for example, macroinvertebrates were sampled from microhabitats around the pond edges where vegetation and basin features have more influence, whereas diatoms were cultivated on artificial substrates suspended in the upper water column where they were more exposed to the water and less to the vegetation and sediment. The differing patterns also underscore the value of examining multiple biological communities, as each may reflect distinct aspects of the local environment.

Contrary to common assumptions, landscape-scale urbanization metrics such as impervious cover and road density did not show strong relationships with biological community composition. This suggests that conditions within the pond, both chemical and structural, may have a more direct association with aquatic assemblages than broader land use measures. We also predict that urbanization metrics based on land use within the sewersheds of SWPs are more associated with water quality and biological community composition than metrics calculated from the proximate land use surrounding the SWPs.

While this study does not identify diagnostic biological responses, the patterns observed emphasize the potential ecological value of stormwater ponds and the relationship between site-level design and maintenance choices with biological communities. Maintaining vegetated margins, limiting hardscaping, and reducing pollutant inputs may support more diverse and resilient assemblages. As cities continue to urbanize, efforts to understand and enhance the ecological function of SWPs will be critical to supporting urban aquatic biodiversity and improving the multifunctionality of these engineered systems.

6.7 References

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Chapter 7
Conclusion

Urban stormwater management ponds (SWPs) are increasingly recognized as important components of the urban landscape, both for their role in runoff mitigation (Jefferson et al., 2017) and for the biodiversity they support (Oertli & Parris, 2019; Ferzoco & McCauley, 2024). However, these systems are also recipients of diverse and complex contaminant mixtures originating from urban runoff, including a wide range of pesticides, industrial chemicals, pharmaceuticals, metals, and other pollutants (Masoner et al., 2019; Flanagan et al., 2021; Karlsson, 2021). My thesis explores the nature and potential ecological consequences of contaminant accumulation in stormwater ponds using a suite of chemical, biological, and toxicological tools. In this conclusion, I first give an overview of the research context of my work and outline my main research objectives. Next, I outline five important points of discussion that arose throughout the course of my thesis work. I then provide a brief synopsis of each data chapter, followed by a review of limitations and recommendations for further research priorities.

7.1 Overview of research context and objectives

My field research was conducted at 21 SWPs in Brampton, Ontario, a rapidly urbanizing city in the Greater Toronto Area built on the traditional territory of the Anishinaabe, Haudenosaunee, and Huron-Wendat peoples. Brampton's population growth and expanding suburban and industrial development have greatly increased impervious surface cover, altering natural hydrological processes and prompting the construction of over 190 SWPs to manage runoff and protect water quality. The 21 ponds I studied were originally selected by my lab mate, Danny McIsaac (Figure 7-1), for his Master's research to represent gradients in urbanization, canopy cover, and surrounding open water (McIsaac, 2022). Building on his foundation, I employed a multidisciplinary approach using chemical and biological tools to further assess contamination, water quality, and ecological condition across these sites. The samples of water, biofilm, and passive samplers I collected during one field season were analyzed for pesticides and other urban-use contaminants, and these results formed the backbone of my thesis. In each chapter, I conduct distinct explorations of these data to create a

cohesive and in-depth evaluation of the pesticide contamination and ecological condition of the ponds.



Figure 7-1. My field partner Danny McIsaac, pictured in both photos above, studied the anuran and fish communities of the same 21 SWP for his Master's research in the years prior to my field work. [Photos by G. Izma, 2022].

The core objectives of my thesis (summarized in Figure 7-2) were:

1. To characterize the pesticide contamination profile in urban SWPs using three complementary approaches (water sampling, biofilms grown on artificial substrates, and o-DGT passive samplers) and compare their sensitivities and applications to make recommendations for future pesticide monitoring programs;
2. To quantify the accumulation of pesticides into biofilms in SWPs and investigate parameters influencing this process;
3. To test the effects of consuming contaminated biofilms from SWPs on the growth and survival of two invertebrate grazers;
4. To characterize a broad range of contaminants present in urban SWPs and identify a suite of ubiquitous contaminants across ponds; and

5. To explore the relationships between local and landscape factors with periphytic diatom and benthic macroinvertebrate assemblages in SWPs.

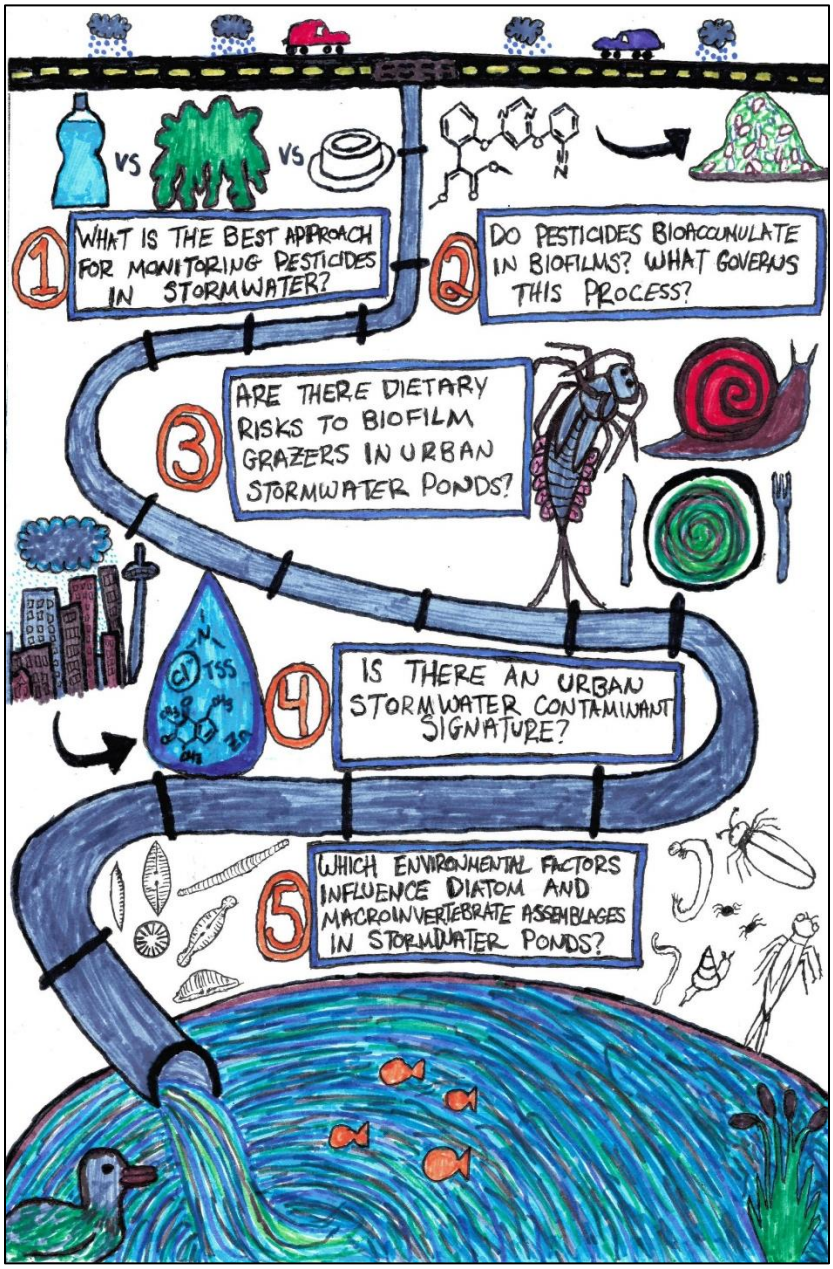


Figure 7-2. A summary of my main thesis questions, by chapter. [Drawing by G. Izma, 2025].

7.2 Points of discussion

In this section, I will briefly discuss five philosophical and scientific queries that arose during my time working on this thesis. I do not intend to provide decisive conclusions, but I hope that this discussion will contribute to the larger conversation on these important issues in the field.

7.2.1 The use of reference sites for SWPs

It is typical for biomonitoring studies to characterize a reference site for comparison with test sites. This reference site is intended to represent an acceptable condition, one minimally impacted by stressors of concern (e.g., human activity), where the biological communities are considered robust and healthy (Bailey et al., 2004). Finding such a site is often difficult, given there are few places left on this planet that have evaded the destruction of human development and activity. Considering SWPs are man-made, engineered infrastructure, which have become colonized by biota unintentionally, the question of identifying a reference site, or even simply defining an acceptable ecological condition, for such a novel ecosystem is controversial (Tixier et al., 2011; Kattan et al., 2016; Miller & Bestelmeyer, 2016). Good reasons exist for using a natural wetland or an engineered wetland as reference points – the former represents the ecosystem that SWPs are often replacing, and the latter represents an attempt to reconstruct the ecosystem services of a natural wetland in an urban context. However, neither are truly analogous to SWPs, and this begs the question – what is the target biological state for SWPs? When selecting a reference site, we are also inadvertently selecting a target state, or acceptable condition, for which our test sites should be managed. But if the primary functions of SWPs are not for habitat provision or supporting biodiversity, and if these ecological functions are often at odds with the original, engineered intentions of the facility (e.g., a steep, bathtub-like basin shape is optimal for maximum water storage capacity, but limits growth of emergent vegetation), then is selecting a reference site for SWPs truly appropriate? This question has followed me throughout my studies - and a major limitation of my work is that I still do not have an answer! In the beginning of my project, I planned to include two reference sites: one at Long Point Provincial Park in Lake Erie, and one at the Experimental Lakes Area near Kenora, Ontario. Samples from both reference sites were unfortunately unusable – perhaps a message from the universe telling me to think twice before including data from these wildly different ecosystems for comparisons with urban SWPs. I did use a reference site for the bioassay dietary tests – however biofilm from this clean river site was included mainly to control for any effects from feeding the test subjects biofilm that had

been frozen and rethawed (mayflies are known to be picky eaters). However, a relatively unimpacted headwater stream is a poor analogue for any lentic system and would not constitute a reference in the sense of Bailey et al. (2004).

The question of identifying a reference site for urban habitats like SWPs can lead to a never-ending rabbit hole of questions: What functions should urban blue-green spaces serve, and for whom? Should we allow the replacement of natural wetlands for SWPs for the purpose of development? What does a healthy urban ecosystem look like? Is a healthy urban habitat a realistic goal for SWPs? And so on…. Although this is a well-discussed topic of considerable interest (e.g., Hobbs et al., 2014; McNellie et al., 2020; Ferzoco & McCauley, 2024), it is not one my thesis helped me to reach a conclusion about.

7.2.2 Balancing realism and control

A common challenge in ecotoxicological research is finding the balance between realism and control, where laboratory exposure experiments tend not to reflect realistic environmental conditions, and biomonitoring in the field often lacks a form of control (Beketov & Liess, 2012). In my work, I strived to strike a balance between these two approaches by incorporating both field-based and lab-based methods. For example, the treatment diets in the bioassay experiments were field-collected biofilm – more analogous to the test subjects' natural diets than if I were to spike their normal in-lab culturing food with various concentrations of pesticides. However, what I gained in realism with this approach, I lost in control (i.e., knowing the exact contents of the treatment diets). I was therefore restricted in the way in which I could interpret the results, because there may have been nutritional deficiencies or additional contaminants present in the wild-cultured biofilm that I did not measure and therefore could not account for. Despite this caveat, my position remains that integrating both biomonitoring and toxicology approaches into risk assessments is the best way to manage trade-offs in realism and control.

7.2.3 The impossibility of achieving true comprehensiveness

Looking back on the past four years of research, there are, of course, many things I wish I had done differently and many things I would have done, had I had access to unlimited time, money, and resources. The most obvious addition I would make is to have conducted a truly comprehensive analysis of contaminants present in all environmental compartments in the SWPs – including in

sediment. Chemical analysis is expensive: for example, the cost of pesticide analysis was approximately $500 (paid in 2022) per sample per matrix (water and biofilm) submitted to the Agriculture and Food Lab (AFL) in Guelph, Ontario. For 21 ponds (plus two "reference sites"), our budget only permitted us to pay for the analysis of pesticides in water and biofilm, metals in biofilm, and fecal bacteria and nutrients in water samples. By measuring many other parameters alongside these analyses (e.g., water quality surveys, macroinvertebrate and diatom analyses, using the biofilm for toxicity assay treatments, etc.), I squeezed as much data as I possibly could out of my field season. We were also beyond privileged to partner with Agriculture and Agri-Food Canada (AAFC), which enabled me to investigate other urban-use contaminants in o-DGT samplers at no cost – an opportunity I am deeply grateful for. We estimated that this in-kind contribution was worth over $100,000. However, we know that SWPs collect a large assortment of contaminants far beyond what was analyzed for in any of the matrices, and we certainly missed characterizing these mixture exposures of potential toxicological concern. All studies must contend with limited resources and time that constrain possible analyses, and I had to make choices about what to include and what not to include. In hindsight, there were a few things I wish I had measured (such as industrial contaminants like PAHs and PFAS), and there were also a few things that were not as helpful to measure (such as metal concentrations in the biofilm – quantifying these in water or sediment samples would have been more informative as there are actual reported ecological thresholds with which to compare them to). Discovering that there is likely widespread wastewater contamination of my SWP sites opens up a new array of possible contaminants for monitoring, such as legal and illicit drugs or other types of bacteria of public health concern. True comprehensiveness may be a pipe dream, but I believe that forming research partnerships and working collaboratively can greatly expand the breadth of analyses a project can encompass and can result in a more in-depth and holistic understanding of contamination than is possible individually. I hope that the next student who wants to study this topic will find these suggestions useful when deciding on their research approach.

7.2.4 Detection limits in multiple matrices

A detection limit is a value that represents the lowest concentration of a substance that can be reliably distinguished from zero (or from background noise) by a particular analytical method. In other words, the detection limit is the smallest amount of a chemical that can be confidently *detected* in a sample, but not necessarily *quantified* accurately, and therefore gives us only "presence or absence" data. The method detection limit (MDL) is a statistical estimate of the detection limit based on repeated

measurements of a low concentration sample (USEPA, 2016). The MDL reflects the sensitivity of the analytical instruments or methods used (e.g., gas chromatography-mass spectroscopy (GC-MS) or liquid chromatography-tandem mass spectroscopy (LC-MS/MS)) and can vary depending on the matrix being analyzed (in our case: water, biofilm, or o-DGT extracts) and the target analyte. Results below the MDL are reported as "non-detects", even though the chemical might still be present at levels below this threshold.

The three matrices I used to monitor contaminants in SWPs each had their own set of detection limits. The extracts from the o-DGTs had the lowest MDLs, ranging from 0.0000002–0.0007 μg L^{-1}. The MDLs for water ranged from 0.0006 – 0.2 μg L^{-1} while MDLs for biofilm ranged from 3 - 50 μg kg^{-1}. It is important to consider matrix-specific MDLs because a "non-detect" in one matrix does not necessarily mean that compound is absent, rather, it may simply be present at a concentration below a relatively higher MDL. One reason for the differences in MDLs among the matrices is matrix interference, where different background materials, such as organic matter or particulates, can interfere with compound detection. Organic and inorganic material in the biofilm samples can mask or suppress the signal of the target analyte, leading to a higher MDL (Bonnineau et al., 2020). For example, biologically derived oils may "hold on" to hydrophobic contaminants, or biological materials may be extracted along with contaminants, creating "noise" in the sample. The thin films, on the other hand, are more homogenous and chemically-known compared to the complex composition of microorganisms and extracellular polymeric substances that comprise the biofilm samples. Additionally, complex matrices are likely to have more variability between replicates, which can mean the calculated MDL from replicate analyses is higher to reflect the reduced precision (EPA, 2016).

Extraction efficiency can also cause substantial variability in MDLs, where differences in sample preparation can influence the measured concentrations. In the o-DGTs, the thin films are a standardized thickness, and after collection are fully extracted, dried down to a pure crystal, and then rewetted with a consistent volume of solvent. Compounds measured in biofilms are in units of dry mass and are therefore a function of the water content of the biofilms themselves, which can vary from 90 – 99%. Drying down the biofilm samples introduces this variability into the MDL values. For example, the MDL for atrazine in biofilm is 20 μg kg^{-1} dry weight. Let us suppose there are two fresh biofilm samples containing the exact same concentration of atrazine, 0.5 μg kg^{-1} wet weight, but biofilm A is 90% water by mass and biofilm B is 99% water by mass. These concentrations would

translate to 5 µg kg^{-1} dry weight (< MDL) for biofilm A and 50 µg kg^{-1} dry weight (> MDL) for biofilm B, meaning atrazine would only be reported as a detection in biofilm B, despite both samples containing the same original concentration. Prior to the analysis of water samples, unlike passive sampler thin films or biofilms, contaminants accumulated in the samples are not concentrated in solution (i.e., the samples are not dried down and reconstituted with solvent). Consequently, the probability of detection is not increased in the pre-analysis phase, as it is with the other matrix types where the possible quantity of contaminant going into the analytical machine is maximized.

The method of drying prior to analysis is similarly influential to the detection limits and measured concentrations in field samples. For example, in a recent study, my colleague Dr. Ijzerman and I compared our pre-analysis processing of biofilm, where Dr. Ijzerman used centrifugation to dry down the biofilms prior to extraction and analysis by AFL, whereas I used freeze-drying, as was done in previous work in our research lab (Rooney et al., 2020). Dr. Ijzerman found that differences in these methods can significantly affect the number of compounds detected (Ijzerman et al., 2025).

The resulting variability in MDLs among the three sampling matrices has an important effect on the detection profiles of the SWPs and reinforces the notion that multi-matrix sampling to capture different environmental compartments is crucial to illustrating a more holistic picture of contamination. Equally important is the development of research-grade analytical methods. For example, when I compared pesticide detections in water samples with o-DGT samples in a hypothetical scenario where the MDLs for both matrices were the same (i.e., at the MDL levels for water analysis), I found that detections between the two matrices were similar. Other researchers have similarly found that MDLs can have a strong influence on how monitoring data is interpreted (e.g., de Solla et al., 2012). Understanding how different matrices and their different MDLs can affect chemical analysis is an important consideration for interpreting the results.

7.2.5 Lack of structure in SWPs

The racks I constructed to cultivate biofilms in the SWPs looked a bit like floating trash (Figure 7-3) and were sometimes harassed by wildlife (Figure 7-4), but also provided often otherwise-sparse structure for emerging invertebrates (Figure 7-5) and other basking creatures (Figure 7-6). Natural wetlands tend to have more downed woody debris and shallower slopes with emergent vegetation (Hudon et al., 2005; Rooney et al., 2015), both of which provide substrates for microorganisms, invertebrates, and other animals to grow, forage, emerge, or bask on. The use that diverse fauna made

of my floating racks speaks to the limited extent of substrate present in the SWPs. I also observed fish feeding on the biofilm growing on the acrylic plates, geese pecking at emerging invertebrates on the pool noodles, and freshwater bryozoans (Figure 7-7) making use of the surfaces provided by the racks. Based on my observations, I believe it is likely that SWPs contain more nutrients than they have structure, that the lack of structure could be a limiting factor for many organisms, and that even small structural additions could create hotspots for productivity and biodiversity. This could have implications for SWP management, where increasing the amount of structure within the ponds, such as with floating wetlands (McAndrew & Ahn, 2017), could provide important habitat improvements, without being at odds with the primary objectives of maximizing water holding capacity and settling suspended solids.



Figure 7-3. The biofilm sampling racks, pictured above, looked a bit like floating garbage, especially by the end of the season, but they successfully enabled biofilm sampling across a variety of pond conditions (and they were good on a budget!). [Photos by D. McIsaac, 2022].



Figure 7-4. Sometimes geese pecked at the pool noodles holding the biofilm sampling rack – likely snacking on the emerging invertebrates that used the noodles for structure. I never managed to catch it on camera, but here is evidence of a group of them pecking at an anchor. [Photo by G. Izma, 2022].

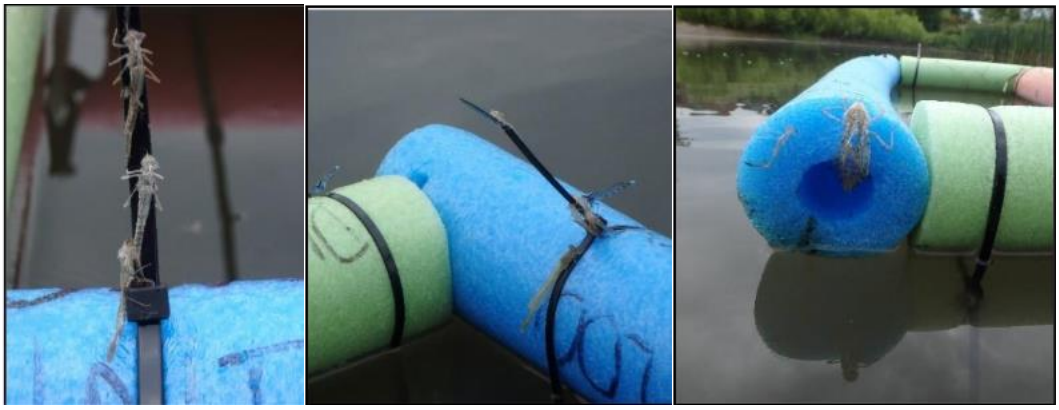


Figure 7-5. Exuviae left behind by emergent insects. The left and central panel show exuviae of zygopterans, whereas the right panel shows an exuviae of an anisopteran. [Photos by G. Izma, 2022].



Figure 7-6. A painted turtle basks in the sunlight, resting on top of one of my biofilm sampler racks. [Photo by G. Izma, 2022].



Figure 7-7. Freshwater bryozoan colonies (also known as "moss animals") growing on the biofilm sampler rack. [Photo by G. Izma, 2022].

7.3 Summary of the thesis data chapters

7.3.1 Chapter 2 – "Three complementary sampling approaches provide comprehensive characterization of pesticide contamination in urban stormwater"

In the field, I used three complementary chemical monitoring methods to characterize pesticide contamination in the 21 SWPs: time-integrated water sampling, biofilms grown on artificial substrates, and organic-diffusive gradients in thin films (o-DGT) passive samplers (Figure 7-8). Two sets of analyses were performed at two different laboratories (water and biofilms at AFL in Guelph; o-DGTs at AAFC in London) with varying analytical methods and sensitivities, so to make comparisons among the three matrices, I only looked at the results of the 282 pesticide analytes they had in common. To quantify time-weighted concentrations in o-DGTs, I modeled the diffusion coefficient of each pesticide analyte to calculate their sampling rates and time-weighted average (TWA) concentrations in the water (C_w). I found 82 different pesticides across all three sampling types, with the highest detection frequency in o-DGT samplers. This confirmed their utility in capturing time-integrated exposures to complex pesticide mixtures, however between-duplicate analysis of concentration data estimated from these samplers instilled a lack of confidence in their ability to provide reliable data for comparisons with ecological thresholds for assessing toxicological risks. While concentrations in water and biofilm were generally low and often below known toxicological thresholds, the wide range of compounds identified, spanning herbicides, insecticides, and fungicides, demonstrated the chronic, multi-compound exposures that urban biota are likely experiencing. I was able to use the results of this study to make recommendations for future pesticide monitoring programs, such as the type of approach best suited for a program given its objectives. I also highlighted the need for research-grade analytical limitations for chemical analysis, as this was an important driver in detection sensitivity among the three matrices I tested.



Figure 7-8. I could deploy two duplicate o-DGT samplers in this holder, which was suspended in the water column from the biofilm sampling rack. [Photo by G. Izma, 2022].

7.3.2 Chapter 3 – "Urban-use pesticides in stormwater ponds and their accumulation in biofilms"

Building on this work, I explored the value of biofilm sampling specifically as a biologically relevant monitoring tool (Figure 7-9), as well as the mechanisms influencing pesticide accumulation from the ambient water into biofilms. I used the complete results of the analysis of composite water and biofilm samples for 542 pesticides (due to both matrices being analyzed by the same laboratory) to calculate bioconcentration factors (BCFs). Then, I used the results of our extensive water quality surveys (Figure 7-10), conducted alongside the collection of water and biofilm samples across the 9-week survey period, to explore the influence of these measurements, in addition to the physicochemical properties of the pesticides (e.g., Log K_{ow}) and biofilm quality (e.g., AFDW), on the magnitude of accumulation. I found 32 pesticide compounds across the SWP sites, with water samples capturing a broader suite of pesticides compared to biofilms. Notably, over half of the pesticides detected in biofilms were not detected in water samples, implying that this traditional approach to pesticide assessment fails to capture all exposure routes, as even when pesticides are below detection limits in the water, biota could still be exposed to ecologically impactful levels of contaminants through their diet. These results indicate that biofilm serves as both an integrator of contamination and a potential route of exposure that is missed by conventional water monitoring. The accumulation of pesticides in biofilm was highly variable and not well predicted by traditional

physicochemical parameters and water quality, suggesting the need for further study into the mechanisms driving this accumulation.



Figure 7-9. A particularly productive biofilm crop, ready for harvest! [Photo by D. McIsaac, 2022].



Figure 7-10. Conducting water quality surveys in the SWPs. [Photo by D. McIsaac, 2022].

7.3.3 Chapter 4 – "Dietary exposure of stormwater contaminants in biofilm to two freshwater macroinvertebrates"

To assess whether this biofilm grown in SWPs poses a risk to aquatic organisms, I conducted two dietary toxicity experiments using biofilm collected from 15 of the SWPs. Dr. Ijzerman (Figure 7-11), from the School of Environmental Sciences at the University of Guelph, had previously developed a novel bioassay approach to assess dietary pesticide exposure through biofilms collected from agricultural streams (Ijzerman et al., 2023), which I adapted for use with SWP biofilms (Figure 7-12). I exposed two invertebrate test species to the SWP biofilm treatment diets: the mayfly nymph *Neocloeon triangulifer* in an acute bioassay and the freshwater snail *Planorbella pilsbryi* in a chronic bioassay (Figure 7-13). Both test species showed reduced survival and growth compared to controls, although pesticide concentrations in the diets were not consistently correlated with these outcomes. This suggests that other, unmeasured contaminants may contribute to observed toxic effects, and that chronic exposure, particularly for longer-lived organisms like snails, may increase the likelihood of toxicity. These findings provide evidence that contaminated biofilm represents a dietary exposure

pathway of ecological concern—one that is largely overlooked in traditional water-based risk assessments.









Figure 7-11. Dr. Moira Ijzerman is an incredibly dedicated and intelligent scientist, an enthusiastic and caring friend, and an all-around amazing human - I wouldn't be here without her! Top left: Moira with a stonefly. Top right: Moira and friend Yaryna sampling benthic invertebrates on the Grand River (Ontario), which I gladly tagged along for. Bottom left: Moira with a jar of adult snails – the "egg layers" of my snail trail. Bottom right: Moira holding used labels after we finished measuring mayfly nymph sizes on the microscope (a nymph is seen on the screen). [Photos by G. Izma, 2023].



Figure 7-12. A photo of Moira and me during the mayfly bioassay tests. We used jewelers' glasses to help see the tiny mayfly nymphs, which were newly hatched at the beginning of the trials. [Photo by N. Letwin, 2023].



Figure 7-13. Mayfly trial (left) [Photo by M. Ijzerman, 2023], and snail trial (right) [Photo by E. Neilly, 2023].

7.3.4 Chapter 5 – "An Urban Stormwater Contaminant Signature: Defining priority contaminants for urban stormwater research"

To better understand the broader contaminant landscape of urban SWPs, I used the full results of the analysis of 542 pesticides in water and biofilm samples and 491 urban contaminants in o-DGT

samplers to conduct a broader chemical survey across the same 21 ponds. I detected 200 organic compounds across sampling media, including pharmaceuticals, personal care products, vehicle-related chemicals, industrial compounds, and pesticides. I also documented widespread chloride pollution and fecal contamination, as well as elevated levels of traffic-related metals in biofilm. From these data, I developed the Urban Stormwater Contaminant Signature (USCS): A proposed list of frequently detected and environmentally relevant pollutants in urban stormwater ponds. This tool is intended to help inform more targeted and effective monitoring strategies while guiding future research into mixture toxicity and ecological risk.

7.3.5 Chapter 6 – "Biofilms, bugs, and the built environment: Exploring local and landscape drivers of diatom and macroinvertebrate assemblages in urban stormwater management ponds"

Finally, I examined how these chemical exposures, along with pond-level habitat characteristics and surrounding land use, related to the structure of biological communities within SWPs. Using periphytic diatoms (Figure 7-14) and benthic macroinvertebrates as biological tools, I found that both assemblages were largely dominated by pollution-tolerant taxa. Diatom communities were more strongly shaped by water quality parameters, particularly nutrients, chloride, and herbicides, while macroinvertebrate assemblages responded more to pond morphology and vegetation structure, suggesting that naturalization features can enhance ecological integrity. Interestingly, landscape-scale factors such as impervious cover and road density did not predict community composition, indicating that local conditions and potentially upstream sewershed characteristics may exert stronger influence on assemblage structure.



Figure 7-14. A few pictures of the diatoms I found in my biofilm samples. Clockwise from the top left: *Cocconeis*; *Stephanocyclus*; *Epithemia*; *Gomphonema*; *Navicula*; *Diatoma*; *Grunowia*; *Hippodonta*. [Phots by G. Izma, 2025].

7.4 Limitations of my research and future research priorities

7.4.1 Biofilm sampling for monitoring contaminants

When I first began my research, my initial focus was on developing biofilm sampling as a novel pesticide monitoring tool for use in urban aquatic systems. Dr. Ijzerman, who was also working on testing a biofilm sampling tool around the same time, used similar samplers, called "periphytometers", for monitoring pesticides in agricultural streams. We both came to similar conclusions when identifying the major limitations of this approach. First and foremost, this technique should be used in tandem with, not in replacement of, other sampling methods, such as grab water sampling or passive sampler deployment. An exception to this limitation would be if the goal of a monitoring effort is to target a specific pesticide or pesticides which are known to be largely absent in water, but accumulate in biofilms (e.g., glyphosate; Ijzerman et al., 2024). Biofilms can accumulate contaminants that are below the detection limits in the ambient water and thus can supplement water

sampling to give a more complete picture of the exposome (Rooney et al. 2020). However, on their own, the number of chemicals detected in biofilms is often low relative to other matrices. Additionally, there are a number of practical limitations when sampling biofilm: The large amount of dry mass required for chemical analysis can be difficult to obtain, requiring many artificial substrates and optimal growing conditions (i.e., nutrient availability, limited scouring; see Appendix F for experimental insight into optimizing biofilm sampling); the amount of labour required for harvesting biofilm from substrates is higher than for grab water sampling or using passive samplers; invertebrates can graze on the biofilm (Figure 7-15), which could impact the standing crop available for harvest; and keeping large pieces of equipment deployed for long periods of time raises the risks of vandalism and theft. I would recommend future study into the improvement and standardization of the biofilm sampling approach for the purpose of chemical monitoring, specifically with respect to the optimization of cultivated biomass in various environmental conditions and the improved efficiency of harvesting techniques. From an ecotoxicological point of view, biofilms grown on artificial substrates may not be representative of the typical diets of invertebrate grazers compared to biofilms growing on natural substrates. More insight is needed into the realism of using artificially-grown biofilms in bioassay in a laboratory setting.



Figure 7-15. Biofilm growing on the acrylic plates can act like an all-you-can-eat buffet for grazing invertebrates. [Photos by G. Izma, 2022].

7.4.2 Describing nutritional value

In a similar vein, I was restricted in my evaluation of the nutritional value of the biofilm diets in the bioassay tests. Ash-free dry weight and chlorophyll-*a* content are important metrics but represent a mere fraction of the number of parameters needed to truly evaluate the quality of food given to an organism. Fatty acid content, caloric density, and mineral content, to name a few, would have given me significantly more insight into the differences in quality among test diets. Identifying the microbial assemblages more comprehensively (e.g., via sequencing) and comparing this to known feeding preferences of the test subjects, would have also been useful. I would suggest that future dietary exposure tests include a more comprehensive characterization of the quality of the biofilm test

diets. This insight would also be valuable when exploring the effects of water quality parameters on biofilm nutritional quality *in situ*.

7.4.3 Concentration data from o-DGTs

In my first data chapter (Chapter 2), I modeled time-weighted average (TWA) concentrations of pesticides in the water from quantities measured in the o-DGT passive samplers. In Chapter 5, my intention was to expand on these results and model the TWA concentrations for all detected contaminant analytes (n = 200), which would have provided an excellent, information-rich data set which could also be used in Chapter 6 to better characterize the water quality of the SWPs. However, in comparing the analyte quantities between duplicate o-DGT samplers in Chapter 2, I found these measurements were not reliable enough to be used in future analyses. Concentration data is important to properly define exposure and risk; detection does not equate to risk, and likewise, a detection tells us little about the magnitude of exposure and whether or not ecological thresholds have been exceeded. Additionally, to model the TWA concentrations of contaminants, I first estimated the diffusion coefficients of each analyte using the Hayduk-Laudie diffusion model. However, empirically-derived diffusion coefficients for organic compounds are being reported by researchers in this field, particularly by Dr. Jonathan Challis at AAFC in Alberta (Challis et al., 2016). The calculated TWA concentrations I used in my work may have been limited by the use of estimated diffusion coefficients, and could be improved once more experimental values are generated and reported. The science of passive sampling and analysis is relatively new and is being improved by many researchers around the world. I would suggest that future use of o-DGT samplers for chemical monitoring continue to deploy samplers in duplicate or triplicate sets, and that where available, empirically derived diffusion coefficients are used when modelling TWA concentrations.

7.4.4 Taxonomic sufficiency

In my final data chapter (Chapter 6), I characterized the diatom assemblages in the biofilm samples grown in the SWPs. Diatoms are widely used in biomonitoring and are often described as being sensitive to pollution and good indicators of water quality (Vilmi et al., 2015; Masouras et al., 2021). However, I could not come to such a clear conclusion to describe the variability of the diatom assemblages in my biofilm samples and I propose that my particular approach, with a single sampling effort in such highly variable systems, is not the strongest method for bioassessment in SWPs. This discrepancy could be driven by another major limitation in my analyses, which was taxonomic

resolution: I identified diatoms to the genus-level, yet many bioassessment metrics are based on results from species-level identifications. I often made generalizations about the environmental preferences of a genera given information reported at the species-level (is this why it's called "genera-lizations"?), and this introduced some uncertainty into my interpretation of the results. Given species-level descriptions often differ among keys and are still evolving and being updated, I opted to stick with genus-level descriptions, despite their limitations. When harvesting my biofilm samples, I took an aliquot from each composite sample and froze it for the purpose of sequencing (16S and 18S) to better characterize the microbial community in the biofilms. Unfortunately, we did not have enough funding to complete this analysis, however these samples are still preserved and could be used by a future graduate researcher. My understanding of the biofilm assemblages, their uptake of chemicals, and their nutritional value to consumers, was limited by my approach to evaluate them (mainly through parameters such as ash-free dry weight and chlorophyll-*a* content, in addition to diatom analysis). I would encourage future research in this area to explore whether species-level data from sequenced samples improves the usefulness of biofilms as indicators of water quality, compared with the genus-level taxonomic identification approach.

7.4.5 OBBN

To characterize the aquatic macroinvertebrate communities in the SWPs, I followed the Ontario Benthos Biomonitoring Network (OBBN) protocol for sampling in a wetland (Jones et al., 2007). Out of the three protocols in the manual (streams, lakes, and wetlands), this was the most relevant method for SWPs and has been used by other SWP studies (e.g., Hassall & Anderson, 2015) as well as in agricultural ponds (Pavusa, 2023). The benefit of following OBBN methods is the access to a range of metrics from which we can derive information about habitat quality, despite the absence of appropriate reference condition values (as discussed in Section 7.2.1). These metrics have directionality (e.g., lower %EPT is worse than higher %EPT), and although the protocols were certainly not designed to survey SWPs specifically, using a standardized protocol gives us the ability to compare to other studies and to other ecosystems. The caveat of this is it is yet to be adapted for sampling in such a unique environment. At some sites, it took nearly six hours to collect the required 300 specimen; there simply was very little life present. At others, perhaps those near due to be dredged, the sediment build up was extreme, and even one "jab and sweep" produced mostly large amounts of sediment with few organisms. The challenge to obtain adequate specimen counts in SWPs to meet OBBN guidelines has also been documented in other surveys in the province (e.g., Hassall &

Anderson, 2015). This presents a unique opportunity for future researchers to improve this method for use in a wider range of aquatic systems, such as in urban environments where sediment build-up and low specimen counts may limit sampling efficiency. Another limitation to this approach is the coarse taxonomic resolution, where most specimens are identified to the family or order level, with a few groups left at the subclass or phylum level. Similarly to the diatom assemblages, finer taxonomic resolution would provide increased insight into variation in the macroinvertebrate assemblages and their relationships with external environmental factors, and I would encourage future studies to employ this approach.

7.4.6 Characterizing the sewershed

A significant limitation to my exploration of how landscape factors affect stormwater quality and biological assemblages in SWPs was the lack of information on the sewershed of each pond site. I did not find significant relationships between landscape parameters measured in 300 m buffers surrounding each pond, however most of the water entering the ponds is piped in via the underground storm sewer network, and the boundaries of this sewershed are artificially, not topographically, defined. It is likely that landscape parameters within the sewershed, such as the proportion of impervious cover or green spaces, are more correlated to the resultant water quality of the SWPs. Future work on this subject should attempt to delineate the sewersheds and digitize the catchment according to land cover attributes and explore whether this characterization better predicts the SWP conditions.

7.4.7 Lack of temporal representation

The data I used throughout my thesis originated from samples taken and surveys completed during one field season. These samples and surveys form the foundation of my thesis, meaning that my findings across chapters are directly linked and enable a thorough assessment and understanding of the data I collected. However, the strengths of centring my thesis around the collection of a core group of samples from one season come at the expense of temporal resolution. Due to the high costs of analyses, I was unable to repeat my sampling and surveys in subsequent years, and was thus unable to assess the variability in physical, chemical, and biological conditions among seasons and years in the SWPs. The variability in the conditions of these highly dynamic systems may have contributed to the effects I observed, and may have explains some of the results that appeared unusual, contradictory, or confusing. Surveying 21 sites across a large urban city with varying degrees of

urbanization allowed me to capture a degree of spatial representation, and the relatively homogenous contamination profiles across these sites suggest that other SWPs in Brampton likely experience similar conditions. This evidence could allow for future monitoring to focus on a smaller set of sites, with the spared resources allocated to improving the temporal representation of the program. Alternatively, the set of chemicals identified in the USCS enables a reduction in the number of analytes measures in samples, and thus the costs of analysis, and similarly, could allow for an exploration of contamination through the seasons and years.

7.5 Conclusion

My thesis demonstrates that SWPs are complex and dynamic urban ecosystems that experience chronic exposure to a complex mixture of contaminants. These contaminants, many of which are unmonitored or rarely included in routine assessments, can accumulate in biologically relevant matrices such as biofilms, posing ecotoxicological risks through dietary exposure pathways. Through integrative monitoring approaches and biological assessment, I highlight the importance of accounting for chronic, low-level, exposures to diverse chemical mixtures in urban water bodies. My work also underscores the need for more multi-matrix monitoring and ecologically grounded risk assessments to guide sustainable urban water management. Although challenging, I maintain that SWPs be recognized not only as functional infrastructure but also as ecosystems that require careful design and adaptive management to support their contribution to urban biodiversity conservation, whilst meeting flood protection and downstream habitat quality objectives.

Looking ahead, future research should prioritize identifying the specific mechanisms of biofilm-mediated contaminant uptake and transfer through aquatic food webs, particularly under chronic exposure scenarios. There is also a pressing need to better understand the ecotoxicological risks associated with chronic exposures to chemical mixtures modelled off the USCS. Additionally, evaluating how pond design features and naturalization strategies influence contaminant retention and habitat quality can inform best practices for improving the ecological performance of urban stormwater infrastructure. Finally, linking upstream land use and sewershed characteristics to in-pond contamination patterns may help guide more targeted mitigation strategies at the landscape scale.

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Appendix A:
Supplementary information for Chapter 2

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5. **Table A-1.2**. The names of pesticides detected in water, biofilm, and o-DGT samples.
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List of files available in the FigShare drive

The FigShare drive for this research can be found here: https://figshare.com/projects/Assessing_the_performance_of_three_complimentary_sampling_approaches_for_a_comprehensive_characterization_of_pesticide_contamination_in_urban_stormwater/226992 It contains the following items:

1. List of stormwater pond sites, their locations, and GPS coordinates.
2. Characteristics of pond sites (e.g., surface area, impervious cover).
3. Lists of target pesticide analytes and their MDLs/MQLs screened for in water and biofilm samples by AFL
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6. Raw results of pesticide analyses.

7. Calculations of sampling rates and TWAC in o-DGTs.

Description of biofilm sampler and o-DGT holder construction

We constructed a floating biofilm sampling rack for each stormwater pond site to be deployed for the purpose of standardizing the growth and harvesting of biofilms *in situ*. Each floating rack consisted of 10 acrylic plates suspended from a rectangular floating rack. Acrylic plates were cut from large acrylic sheets to 44.4 cm in length and 20.2 cm in height, and two small holes were drilled in the top of each plate for affixation to the rack. Each plate was gently scraped three times on each side with sandpaper to facilitate an ideal colonization surface. The floating rack consisted of a rope ladder with six rugs and pool noodles attached along the sides and rungs (see Figure 2-2). One acrylic plate was affixed with zip ties to each rung, and two were affixed to each side of the ladder, for a total of 10 plates. When deployed, the rack was attached to a marine rope and anchor to prevent drifting.

The o-DGT holders were constructed by cutting acrylic sheets into 15 cm by 15 cm squares and cutting out two circles 3.5 cm in diameter in the centre of the plates. This diameter fits the face of the o-DGTs but not the base, allowing the o-DGTs to sit in the holders. We secured the bases of the o-DGTs by affixing a small metal grate "door" with plastic screws to facilitate easy opening and closing of the holders. We drilled a small hole into the top of each holder to affix the holders to the floating racks with zip-ties.

Detailed methods of pesticide analysis

Analysis of pesticides in water and biofilm samples by AFL

The description below was adapted from text provided by Brian Atkinson at the Agriculture and Food Lab, Guelph, Ontario, and was previously published in the Supplementary Information section of Izma et al. (2024a).

Subsampling/Preprocessing

Biofilm samples are homogenized to a powder with dry ice and a small food processor to ensure test portion taken for analysis is representative of the entire sample. Stormwater samples are thawed and

shaken for one minute by hand before being subsampled for each test. No filtering of the water was used. Meaningful residue data can only be obtained when a representative sample is obtained.

Sample Analysis

All sample analyses were performed at the ISO/IEC 17025 accredited Laboratory Service Division, University of Guelph. The multi-residue pesticide determination (AFL Method ID: TOPS-142) was performed by liquid chromatography/electrospray ionization-tandem mass spectrometry (LC/ESI-MS/MS) and gas chromatography-tandem mass spectrometry (GC-MS/MS). Pesticides were extracted from water and biofilm using the method known as the quick, easy, cheap, effective, rugged, and safe (QuEChERS) method with dispersive solid phase extraction (SPE). A representative sample (20 mL for water and 3 g for biofilm) was extracted into acetic acid in acetonitrile in the presence of anhydrous sodium acetate and magnesium sulfate. The supernatant was centrifuged and then split, evaporated and diluted with methanol/ammonium acetate (for LC analysis) or hexane (for GC analysis). Sample extracts were analyzed in positive polarity mode using SCIEX5550 ESI-MS/MS with Agilent 1260 HPLC and on Agilent GC 7890 Quadrupole GC-MS/MS. This method detects for over 500 pesticide compounds.

The phenoxy pesticide screen (AFL Method ID: CHEM-069) was a general solid phase extraction (SPE) 2 step method. 500 mL of water is initially acidified with sulfuric acid, passed through the SPE, eluted with methanol, concentrated and analyzed in both positive and negative modes using a SCIEX 5500 LC-MS/MS with an Agilent HPLC. This method screens for 19 phenoxy acidic herbicides as well as for 9 neonicotinoid class pesticides. For the biofilm samples a 3 g subsample is extracted using a QuEChERS style method of acidified (with formic acid) acetonitrile in the presence of anhydrous magnesium sulfate and citrate buffers. The supernatant is centrifuged followed by dilution into a 50:50 MeOH/Water mixture filtered and analyzed in both positive and negative modes using a SCIEX 5500 LC-MS/MS with an Agilent HPLC.

The polar pesticide screen (AFL Method ID: CHEM-334) is a modified version of the EU's QuPPE method. A representative subsample (10 mL for water and 2 g for biofilm with 5 mL of Nanopure water to rehydrate) is extracted using acidified (with formic acid) methanol and then placed into a freezer for 30 minutes. The supernatant is then centrifuged and filtered and analyzed in positive and negative modes using a SCIEX 5500 LC-MS/MS with an Agilent HPLC.

Each pesticide in the analyzed matrices was quantified using a matrix-matched calibration curve with six concentrations. A quadratic regression with a 1/x weighting and a $r > 0.99$ was used to construct

the calibration curve for quantitation. Deuterated internal standards were added to samples to account for any matrix effect. An expanded uncertainty (U) that is statistically derived for each analyte was used for method validation.

Quality Controls

All methods use several quality control points that confirm the presence of a compound in the sample: retention time (RT), M+1 target mass, 2 fragment qualifier ions, and the ratio of the two fragment ions. For the identification and quantification of the compounds, each method utilizes deuterium labelled internal standards, matrix-matched blanks, spikes, and calibration curves. The analysis of each batch of samples included a reagent blank, reagent spiked, matrix blank, and three matrix spiked quality control samples. The reagent and matrix spiked quality control samples were conducted at various concentrations and all recoveries were between 80 and 120%. As part of our method validation, we use an expanded uncertainty (U) that is statistically derived for each analyte in our methods.

Analysis of pesticides in o-DGTs by AAFC

Samples were analyzed by LC-MS/MS using a Vanquish-Duo HPLC coupled to a Thermo Altis triple quadrupole mass spectrometer. 95 µL of sample was injected onto an initial preconcentration trap column (Hypersil Gold aQ; 20 mm × 2.1 mm 12 µm; Thermo Scientific). Following the injection, the trapped analytes were eluted off the online-SPE column and onto an analytical, Agilent Zorbax Eclipse Plus; (2.1 x 50mm, 1.8 µm); maintained at 35 °C with a flow rate of 300 µL min-1. Mobile phase A (H2O + 0.1% FA ; Optima LC-MS Grade) was held at 2% for 0.750 min. Mobile phase B (acetonitrile + 0.1% FA; Optima LC-MS Grade) was then increased to 15% over 1.05 min and to 24% over 5.6 min. Finally, mobile phase B was increased to 98% over 15.1 min and held for 2.4 min. The OptaMax NG H-ESI source was operated with capillary voltages of 3.5 kV in both positive and negative mode, ion transfer tube temperature of 325 °C and vaporizer temperature of 350 °C. The sheath, auxiliary and sweep gases were set to 35, 10 and 1 arbitrary units respectively. Analytical standards were purchased from Toronto Research Chemicals and Sigma-Aldrich. Target analytes, along with their LODs, LOQs, MRM transitions and retention times are available in the FigShare repository. LODs were determined by International Council for Harmonisation (ICH) guidelines, accessed 2019. Quantification was performed in Thermo TraceFinder 5.0.

Table A-1.1. Derived method detection limits (d-MDLs) as time-weighted average concentrations in µg L^{-1} in o-DGT samplers, for comparison with MDLs in water samples. For this purpose, only pesticides detected at field sites are included here.

Compound	o-DGT d-MDL (µg L^{-1})	Water MDL (µg L^{-1})	o-DGT d-MQL (µg L^{-1})	Water MQL (µg L^{-1})
2,4-D	0.000007	0.0006	0.00002	0.002
acetamiprid	0.000009	0.0006	0.00003	0.002
ametryn	0.000005	0.03	0.00002	0.1
atrazine	0.000001	0.07	0.000003	0.2
azoxystrobin	0.000003	0.09	0.00001	0.3
benalaxyl	0.000003	0.08	0.00001	0.3
bentazon	0.000003	0.0008	0.00001	0.002
bromacil	0.0007	0.06	0.002	0.2
bromoxynil	0.00001	0.001	0.00004	0.004
bromuconazole	0.00003	0.08	0.00009	0.2
bupirimate	0.000003	0.05	0.00001	0.2
carbaryl	0.00001	0.07	0.00004	0.2
carbendazim	0.0000002	0.1	0.000001	0.4
chlorantraniliprole	0.00002	0.1	0.00007	0.4
chlorpropham	0.00002	0.05	0.00006	0.2
clomazone	0.00001	0.05	0.00004	0.2
clothianidin	0.00001	0.002	0.00005	0.006
cyantraniliprole	0.00003	0.1	0.00009	0.3
diazinon	0.00004	0.03	0.0001	0.1
dichlorprop	0.00001	0.0007	0.00005	0.002
difenoconazole	0.000003	0.04	0.00001	0.1
dimethenamid	0.00002	0.2	0.00006	0.7
dimethomorph	0.00003	0.06	0.00009	0.2
dithiopyr	0.00006	0.001	0.0002	0.004
diuron	0.00001	0.2	0.00004	0.6
ethiofencarb	0.00002	0.08	0.00006	0.3
etoxazole	0.00001	0.04	0.00004	0.1
fenazaquin	0.00002	0.04	0.00007	0.1
fenobucarb	0.00003	0.1	0.0001	0.3
fenpropimorph	0.00001	0.05	0.00003	0.1
fluazifop-p-butyl	0.00005	0.04	0.0002	0.1
fludioxonil	0.00007	0.06	0.0002	0.2
flufenoxuron	0.00008	0.04	0.0003	0.1
fluopyram	0.00001	0.08	0.00004	0.2

Compound	o-DGT d-MDL (µg L^{-1})	Water MDL (µg L^{-1})	o-DGT d-MQL (µg L^{-1})	Water MQL (µg L^{-1})
flupyradifurone	0.000003	0.08	0.00001	0.2
flutriafol	0.00003	0.1	0.00009	0.3
hexaconazole	0.00002	0.07	0.00007	0.2
hexazinone	0.0000006	0.1	0.000002	0.5
imazethapyr	0.0000009	0.005	0.000003	0.01
imidacloprid	0.000008	0.0006	0.00003	0.002
indaziflam	0.000002	0.05	0.00001	0.1
indoxacarb	0.0001	0.08	0.0003	0.3
MCPA	0.00004	0.0007	0.0001	0.002
mecoprop	0.000003	0.0007	0.00001	0.002
metalaxyl	0.000007	0.04	0.00002	0.1
methiocarb	0.00001	0.1	0.00003	0.3
metolachlor	0.0000003	0.03	0.000001	0.1
metribuzin	0.000006	0.05	0.00002	0.2
myclobutanil	0.00002	0.05	0.00005	0.2
ofurace	0.00003	0.07	0.00009	0.2
paclobutrazol	0.000003	0.08	0.00001	0.3
picolinafen	0.00004	0.05	0.0001	0.2
picoxystrobin	0.000003	0.06	0.00001	0.2
piperonyl-butoxide	0.000003	0.1	0.00001	0.4
prometon	0.000005	0.05	0.00002	0.2
prometryn	0.000005	0.03	0.00002	0.1
propazine	0.0000002	0.04	0.000001	0.1
propiconazole	0.000003	0.08	0.00001	0.3
propoxur	0.000007	0.07	0.00002	0.2
pyraclostrobin	0.000003	0.04	0.00001	0.1
pyridate	0.00003	0.09	0.00009	0.3
pyrimethanil	0.000005	0.1	0.00002	0.4
pyriproxyfen	0.000003	0.06	0.00001	0.2
quizalofop-ethyl	0.00005	0.07	0.0002	0.2
simazine	0.000003	0.08	0.00001	0.3
Simetryn	0.000005	0.09	0.00002	0.3
spiromesifen	0.00003	0.1	0.0001	0.3
spiroxamine	0.000003	0.05	0.00001	0.1
sulfentrazone	0.00002	0.09	0.00006	0.3
tebuconazole	0.000003	0.1	0.00001	0.4
tebufenozide	0.00003	0.09	0.0001	0.3

Compound	o-DGT d-MDL (µg L^{-1})	Water MDL (µg L^{-1})	o-DGT d-MQL (µg L^{-1})	Water MQL (µg L^{-1})
tebuthiuron	0.000004	0.04	0.00001	0.1
terbuthylazine	0.000003	0.08	0.00001	0.3
terbutryn	0.000005	0.06	0.00002	0.2
tetraconazole	0.000003	0.05	0.00001	0.1
thiabendazole	0.000002	0.07	0.00001	0.2
thiacloprid	0.000004	0.001	0.00001	0.003
trifloxystrobin	0.000003	0.07	0.00001	0.2

Table A-1.2. List of pesticides detected in water, biofilm, and o-DGT samples.

Detected only in water	Detected only in biofilm	Detected only in o-DGTs	Detected in biofilm and o-DGTs	Detected in water and o-DGTs	Detected in all 3 matrices
flonicamid	thiophanate-methyl	fluopyram	azoxystrobin	clothianidin	chlorpropham
imazapyr		metalaxyl	thiabendazole	flupyradifurone	carbendazim
triclopyr		metolachlor	tebuconazole	imidacloprid	diuron
		propazine		bentazon	2,4-D
		propiconazole		dichlorprop	MCPA
		desethylatrazine		mecoprop	
		chlorantraniliprole			
		clomazone			
		simazine			
		prometon			
		tebufenozide			
		tebuthiuron			
		imazethapyr			
		paclobutrazol			
		dimethenamid			
		difenoconazole			
		ametryn			
		carbaryl			

	epoxiconazole
	myclobutanil
	hexazinone
	sulfentrazone
	cyantraniliprole
	ethiofencarb
	diazinon
	dimethomorph-II
	pyrimethanil
	picoxystrobin
	benalaxyl
	indaziflam
	terbuthylazine
	terbutryn
	flutriafol
	metribuzin
	prometryn
	spiroxamine
	bromoxynil
	dithiopyr
	flufenoxuron
	pyridate
	trifloxystrobin
	fludioxonil
	propoxur
	pyridaben
	picolinafen
	pyraclostrobin
	pyriproxyfen
	tetraconazole
	bromacil
	bromuconazole
	bupirimate

	etoxazole
	fenazaquin
	fluazifop-p-butyl
	hexaconazole
	indoxacarb
	quizalofop-ethyl
	Simetryn
	thiacloprid
	fenobucarb
	methiocarb
	ofurace
	acetamiprid
	atrazine

Table A-1.3: Detection frequencies (%) of pesticides in o-DGTs and water samples following adjustment of o-DGT MDLs to the same value as water MDLs. Pesticides listed in order of increasing detection frequency.

Pesticide	Detection Frequency (%)	
	Water	o-DGTs
2,4-D	100	76
MCPA	100	14
mecoprop	100	57
triclopyr	95	n.d.
imidacloprid	62	62
flupyradifurone	38	n.d.
clothianidin	33	n.d.
bentazon	24	n.d.
dichlorprop	19	5
flonicamid	19	n.d.
tebuconazole	n.d.	14
atrazine	n.d.	10
carbendazim	5	n.d.
chlorpropham	5	5
difenoconazole	n.d.	5
diuron	5	5
imazapyr	5	n.d.
metolachlor	n.d.	5
propiconazole	n.d.	5

Table A-1.4. Physico-chemical properties and ecotoxicology information of select pesticides detected in stormwater ponds. All information in this table is sourced from either NCBI's PubChem (Kim et al., 2023) or the University of Hertfordshire's Pesticide Properties Database (University of Hertfordshire, 2020). N/A = not appliable, pesticide does not dissociate.

Pesticide	CAS	Type	Possible urban use	MOA	Solubility in water (mg L^{-1}) at 20 C	Log K_{ow}	pKa	Log D_{ow}	K_{oc} (mL g^{-1})
2,4-D	94-75-7	Phenoxy herbicide	Lawn and turf	Synthetic auxin	24,300	-0.82	3.4	-5.49	39.3
Flonicamid	158062-67-0	Pyridine insecticide	Greenhouses	Chordotonal organ modulator	5300	0.1	11.6	-3.43	5.4
Flupyradifurone	951659-40-8	Organofluoride insecticide	Greenhouses	Blocks nAChR channel	3200	1.2	N/A	N/A	98.4
Imazapyr	81334-34-1	Imidazolinone herbicide	Lawn and turf, forestry, ponds and wetlands, industrial and residential areas	Inhibits plant amino acid synthesis	9740	0.11	1.9	-6.06	8.81
Imidacloprid	138261-41-3	Neonicotinoid insecticide	Lawn and turf, domestic pests	Blocks nAChR channel	610	0.57	11.12	-2.48	156
MCPA	94-74-6	Phenoxyacetic herbicide	Lawn and turf	Synthetic auxin	250,000	-0.81	3.73	-5.15	73.88
Mecoprop	7085-19-0	Phenoxypropionic herbicide	Lawn and turf, industrial sites, uncultivated areas	Synthetic auxin	250,000	-0.19	3.11	-5.15	47
Thiophanate-methyl	23564-05-8	Carbamate fungicide	Lawn and turf, veterinary, greenhouses	Inhibits mitosis and cell division	18.5	1.4	12.3	-2.83	330
Triclopyr	55335-06-3	Pyridine herbicide	Turf, industrial sites, uncultivated areas	Synthetic auxin	8100	-0.45	3.97	-4.55	27

Table A-1.5. Results of linear regressions between pesticide concentrations in water and o-DGT samples.

Pesticide	Regression equation	Estimated effect of coefficient	Standard error	T statistic	*p* value	Residuals		Adjusted r-squared	F-statistic	
						Standard error	Degrees of freedom		F-statistic value	Degrees of freedom
Flupyradifurone	y = 2300 + 260 (log10)x	264.49	48.66	5.44	4.45E-05	231.00	17	0.613	29.55	1 and 17
Imidacloprid	y = 3100 + 370 (log10)x	373.50	36.90	10.12	1.30E-08	201.10	17	0.849	102.40	1 and 17
Mecoprop	y = 46 + 7300x	7262.24	826.06	8.79	4.02E-08	363.90	19	0.792	77.29	1 and 19
2,4-D	y = 85 + 6300x	6316.11	1008.08	6.265	5.13E-06	913.00	19	0.657	39.26	1 and 19
MCPA	y = 9.9 + 2000x	2027.33	44.14	45.93	<2e-16	51.35	19	0.991	2110.00	1 and 19

Table A-1.6. Agreement in pesticide detections between paired o-DGT duplicates. Deployment section (S1 or S2) refers to either the first or second batch of duplicates deployed.

SWP site	Deployment section	# of detection agreements	# of detection disagreements	Agreement (%)
10	S2	21	6	77.8
10	S1	26	14	65.0
14	S2	20	5	80.0
14	S1	26	3	89.7
26	S1	30	3	90.9
46	S2	24	4	85.7
49	S1	30	10	75.0
51	S2	24	1	96.0
51	S1	23	3	88.5
56	S2	20	2	90.9
56	S1	20	9	69.0
58	S2	22	4	84.6
58	S1	28	6	82.4
78	S2	12	3	80.0
78	S1	12	5	70.6
85	S2	24	6	80.0
85	S1	30	8	78.9
96	S2	20	7	74.1
106	S2	23	9	71.9
106	S1	30	12	71.4
109	S2	34	8	81.0
109	S1	39	5	88.6
113	S2	21	2	91.3
113	S1	21	6	77.8
116	S2	24	3	88.9
130	S2	15	4	78.9
130	S1	21	8	72.4
153	S1	19	5	79.2
156	S2	22	2	91.7
156	S1	29	0	100.0
174	S2	25	2	92.6
174	S1	25	3	89.3
188	S1	27	10	73.0
191	S2	22	8	73.3
191	S1	29	13	69.0

	Average agreement (%):	81.4
	Standard deviation:	8.7

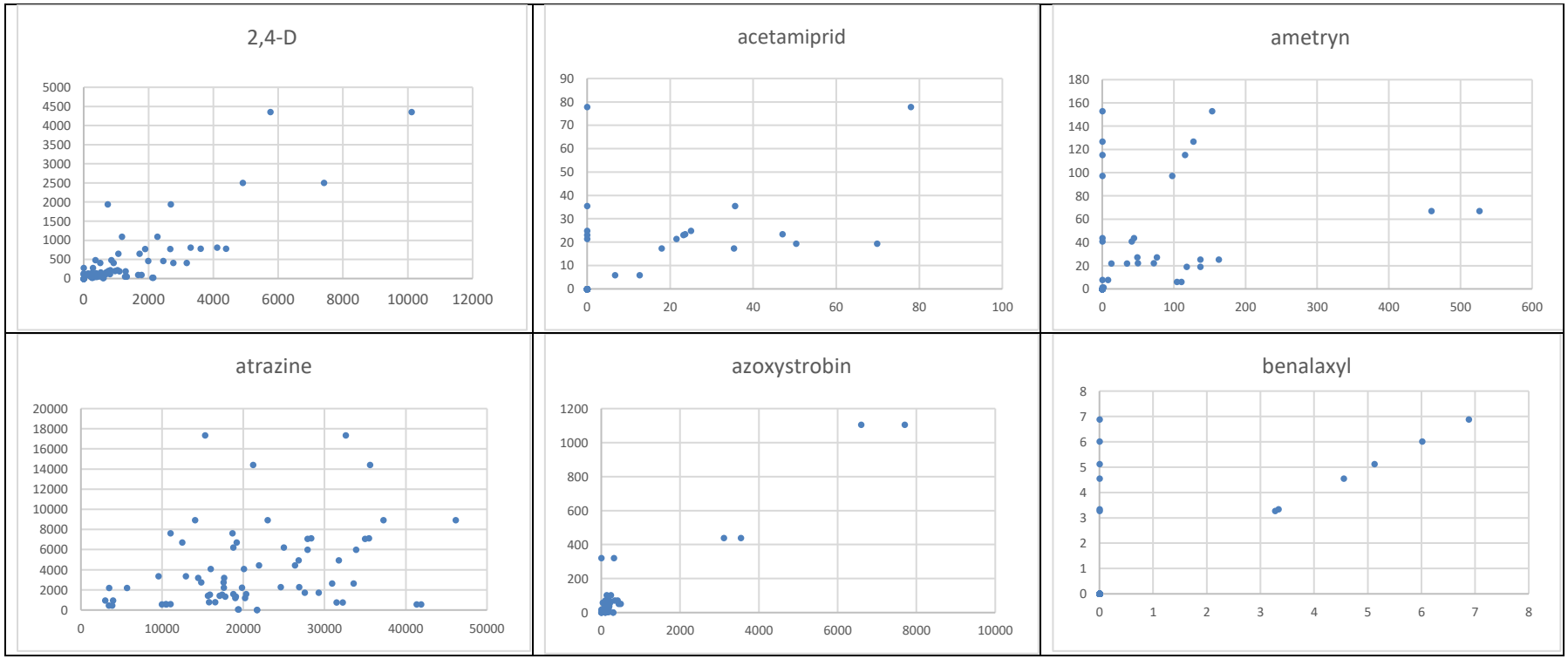
Table A-1.7. Mean differences (and standard deviation) between pesticide quantities measured in paired o-DGT duplicates, averaged across all stormwater pond sites. The coefficients of variance are calculated from the means and standard deviation.

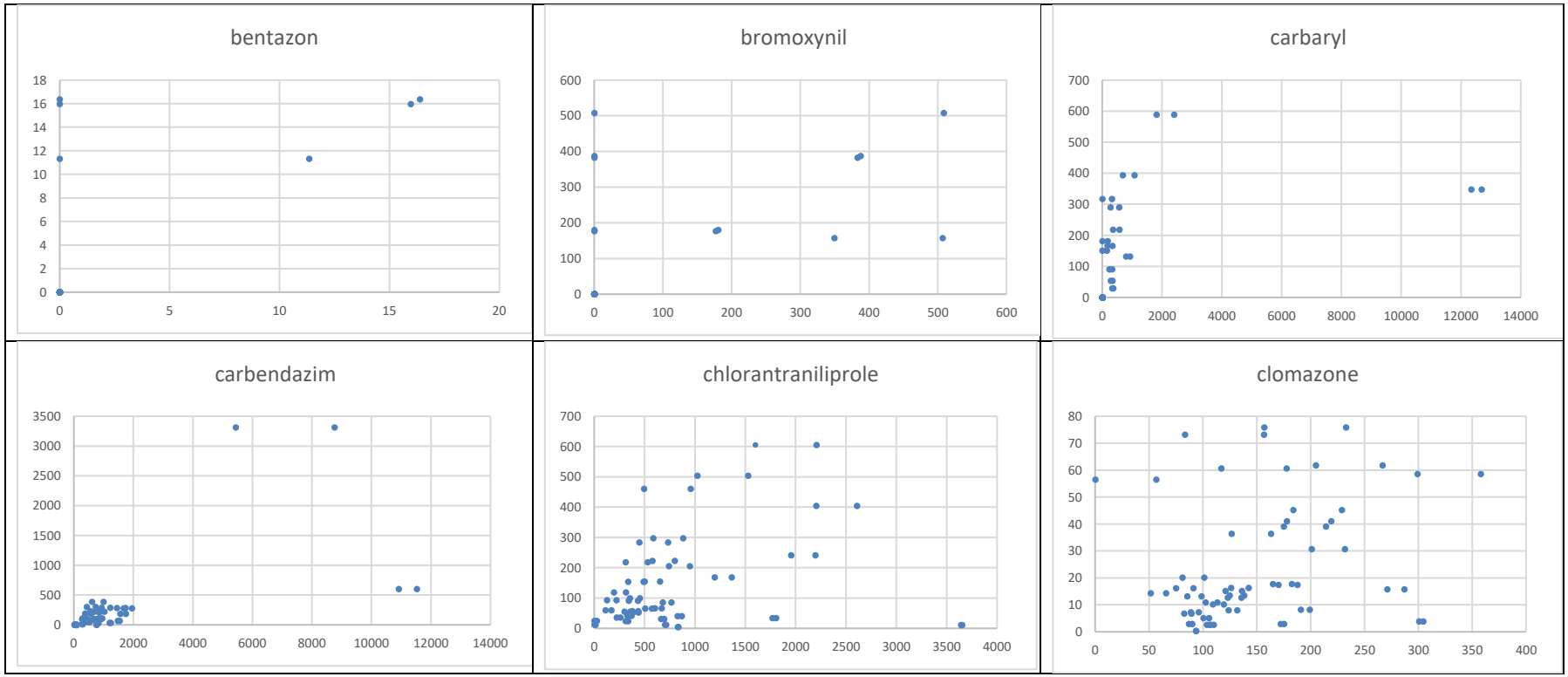
Pesticide	Mean difference between duplicates (pg/sampler)	Standard deviation	Coefficient of variance (%)
2-4,D	547.81	902.64	165
acetamiprid	27.70	20.36	74
ametryn	51.76	48.61	94
atrazine	3878.70	4007.86	103
azoxystrobin	86.31	203.01	235
benalaxyl	4.86	1.44	30
bentazon	14.56	2.80	19
bromacil	553.40	0.00	0
bromoxynil	298.98	146.87	49
bromuconazole	105.26	0.00	0
bupirimate	30.22	0.00	0
carbaryl	227.91	156.52	69
carbendazim	222.24	554.37	249
chlorantraniliprole	144.36	152.63	106
chlorpropham	27290.67	1910.27	7
clomazone	23.59	22.14	94
clothianidin	75.74	74.28	98
cyantraniliprole	62.91	63.41	101
diazinon	75.63	92.65	122
dichlorprop	8.44	0.00	0
difenoconazole	425.78	916.71	215
dimethenamid	39.85	34.85	87
dimethomorph	27.26	20.76	76
dithiopyr	25.43	24.91	98
diuron	3923.77	11565.99	295
epoxiconazole	53.46	39.95	75
ethiofencarb	128.95	73.04	57
etoxazole	28.37	0.00	0

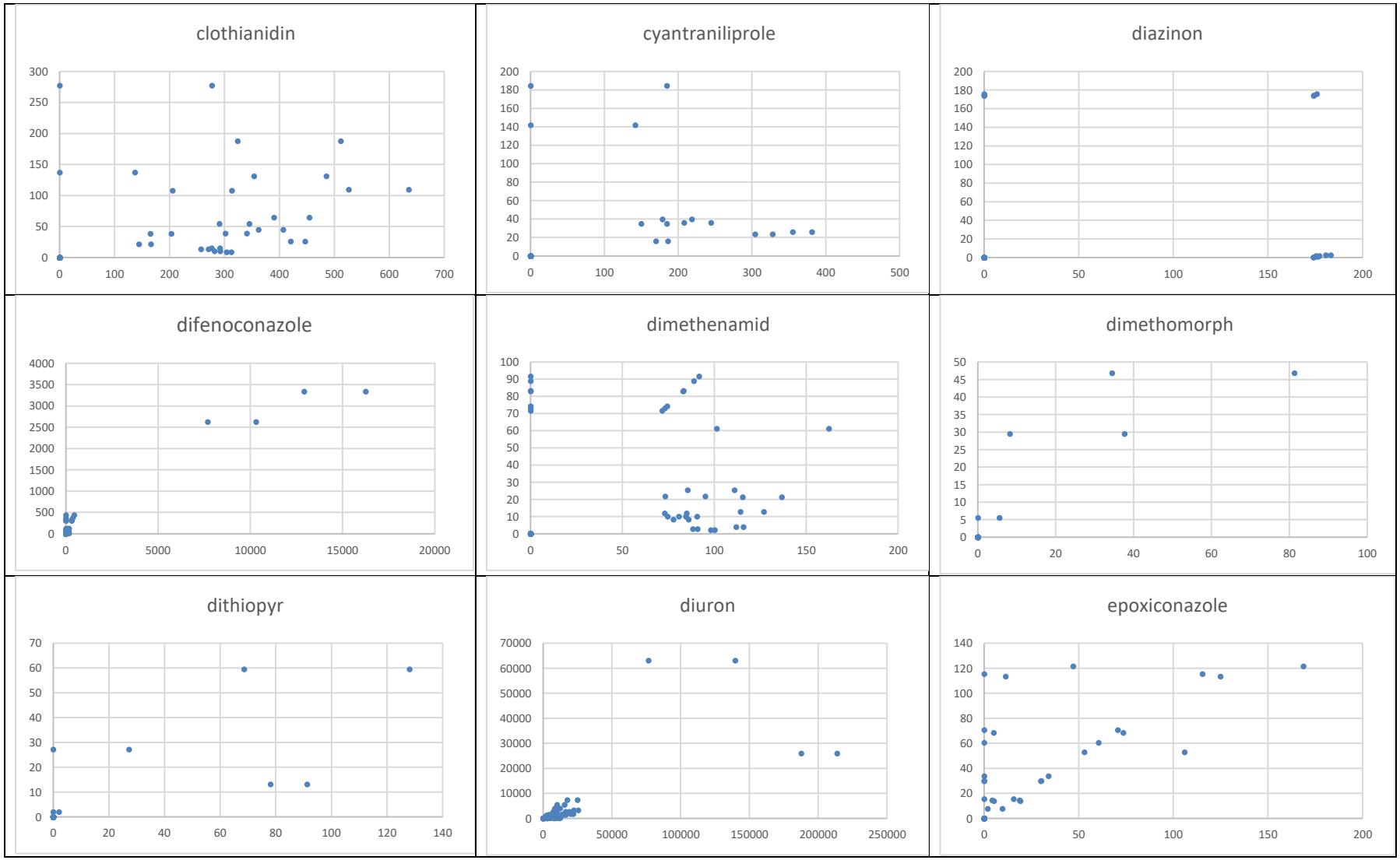
Pesticide	Mean difference between duplicates (pg/sampler)	Standard deviation	Coefficient of variance (%)
fenazaquin	110.37	0.00	0
fenobucarb	33.81	0.00	0
fenpropimorph	109.24	56.12	51
fluazifop-p-butyl	6.38	0.00	0
fludioxonil	561.07	643.56	115
flufenoxuron	872.22	214.76	25
fluopyram	125.30	106.11	85
flupyradifurone	328.83	879.84	268
flutriafol	311.42	95.19	31
hexaconazole	64.32	0.00	0
hexazinone	11.12	7.81	70
imazethapyr	3.96	3.77	95
imidacloprid	302.66	851.59	281
indaziflam	56.97	33.89	59
indoxacarb	25.62	0.00	0
MCPA	174.30	288.71	166
mecoprop	194.72	348.06	179
metalaxyl	40.29	50.32	125
methiocarb	41.03	0.00	0
metolachlor	574.09	621.00	108
metribuzin	524.82	359.61	69
myclobutanil	107.41	76.99	72
ofurace	305.10	0.00	0
paclobutrazol	68.07	54.74	80
picolinafen	321.48	106.85	33
picoxystrobin	16.04	0.00	0
prometon	10.78	9.40	87
prometryn	10.74	7.00	65
propazine	18.60	17.39	94
propiconazole	533.84	548.15	103
propoxur	83.11	57.84	70
pyraclostrobin	36.09	23.56	65
pyridaben	964.24	1312.91	136
pyridate	1445.96	1496.86	104
pyrimethanil	77.20	54.28	70
pyriproxyfen	53.00	62.30	118

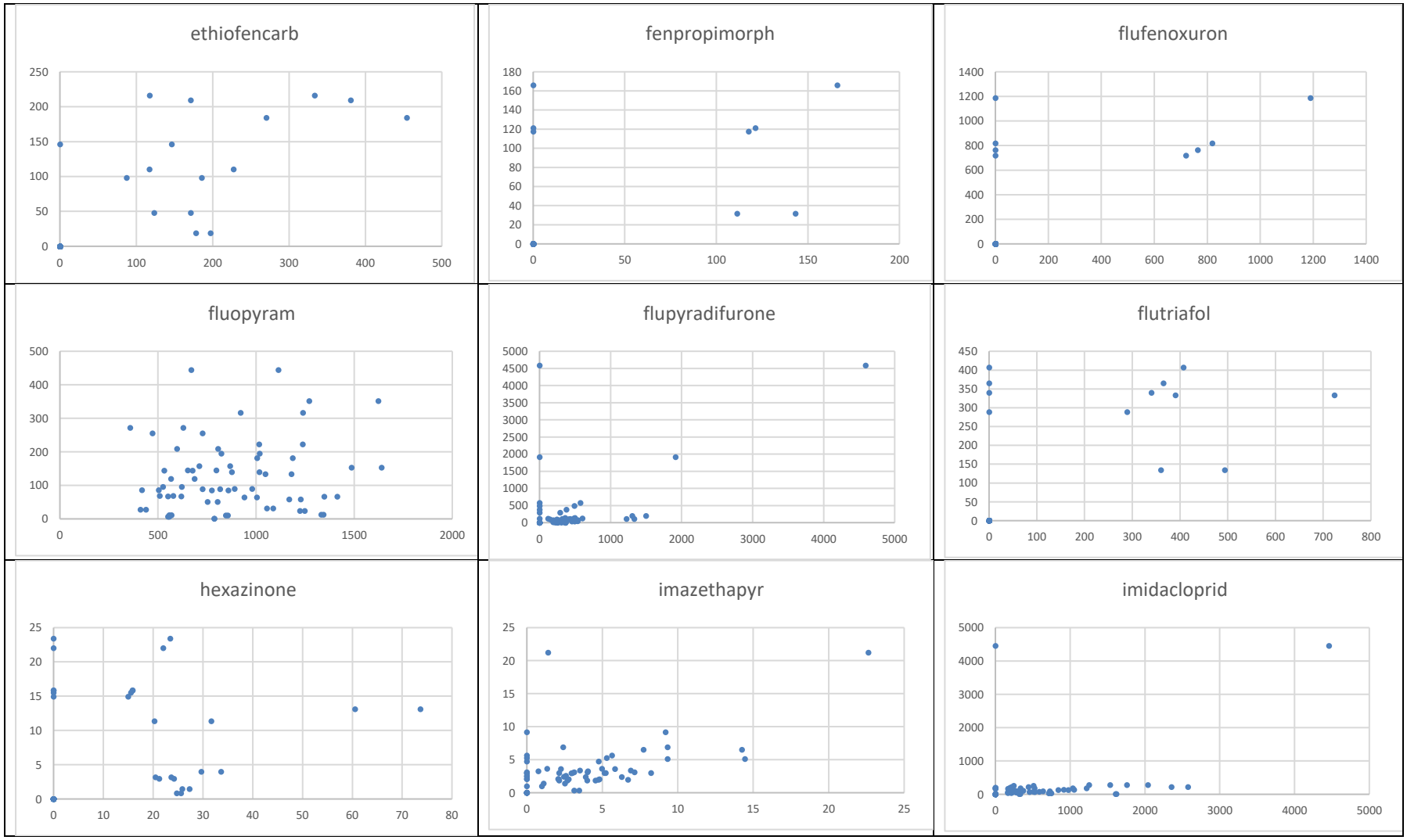
Pesticide	Mean difference between duplicates (pg/sampler)	Standard deviation	Coefficient of variance (%)
quizalofop-ethyl	467.20	0.00	0
simazine	96.45	65.26	68
Simetryn	13.07	13.09	100
spiromesifen	654.58	568.53	87
spiroxamine	7.65	1.92	25
sulfentrazone	104.90	85.07	81
tebuconazole	4511.64	10028.41	222
tebufenozide	152.61	126.47	83
tebuthiuron	10.28	22.21	216
terbuthylazine	69.17	80.19	116
terbutryn	7.66	7.56	99
tetraconazole	260.95	0.00	0
thiabendazole	145.73	404.36	277
thiacloprid	39.09	0.00	0
trifloxystrobin	3.16	2.64	83

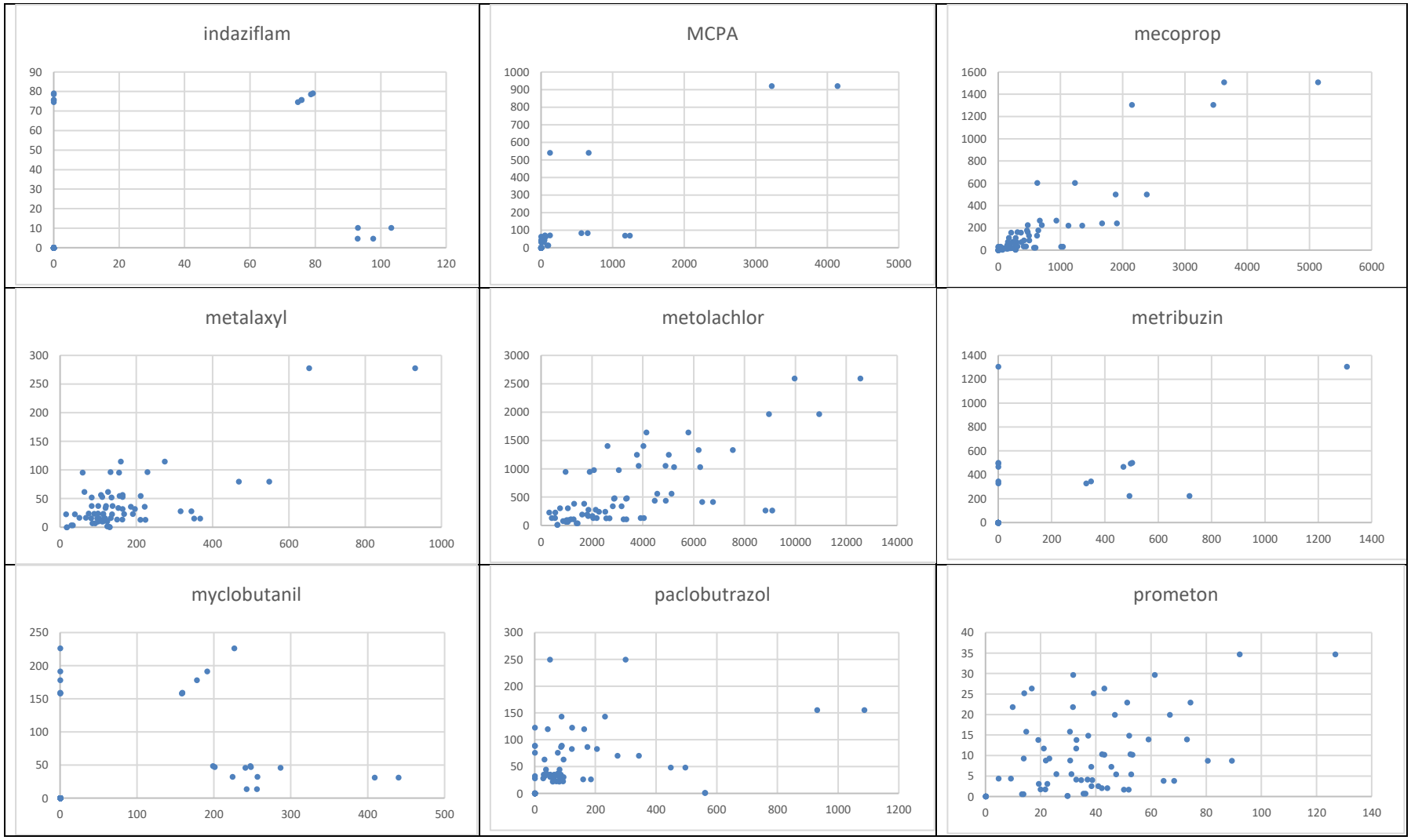
Table A-1.8. Plots of 55 pesticides showing the relationship between the mass measured in the samplers (**x-axis**; pg/sampler) and the difference in the masses measured in sampler pairs (**y-axis**; pg). Only pesticides detected at more than two sites are plotted.

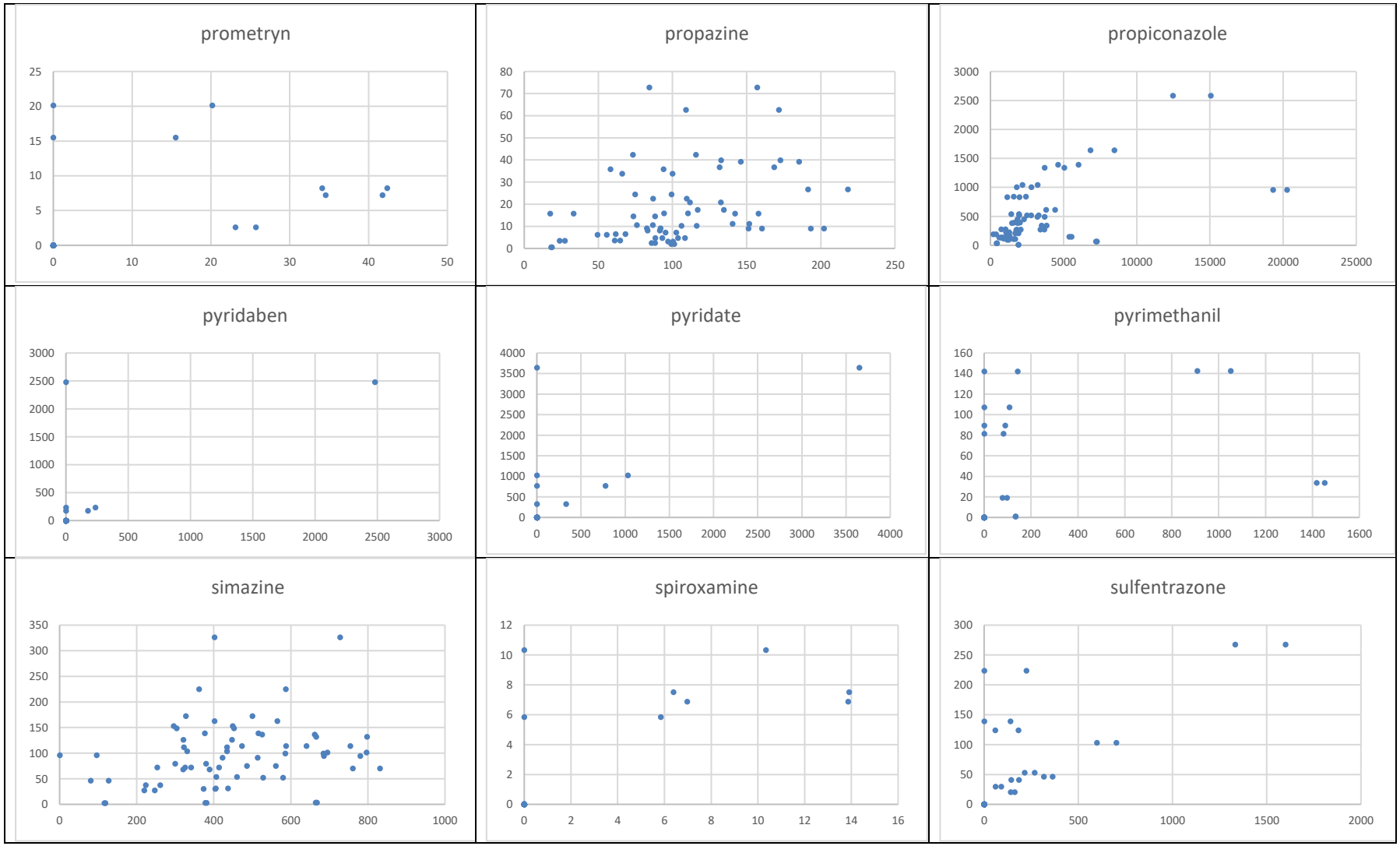


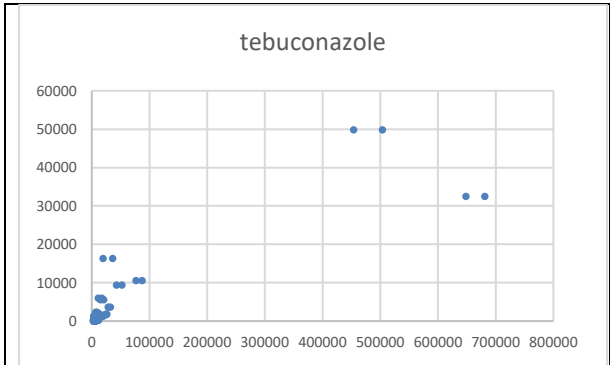

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16
14
12
10
8
6
4
2
0
0
5
10
15
20
bromoxynil
600
500
400
300
200
100
0
0
100
200
300
400
500
600
carbaryl
700
600
500
400
300
200
100
0
0
2000
4000
6000
8000
10000
12000
14000
carbendazim
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3000
2500
2000
1500
1000
500
0
0
2000
4000
6000
8000
10000
12000
14000
chlorantraniliprole
700
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500
400
300
200
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0
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1000
1500
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2500
3000
3500
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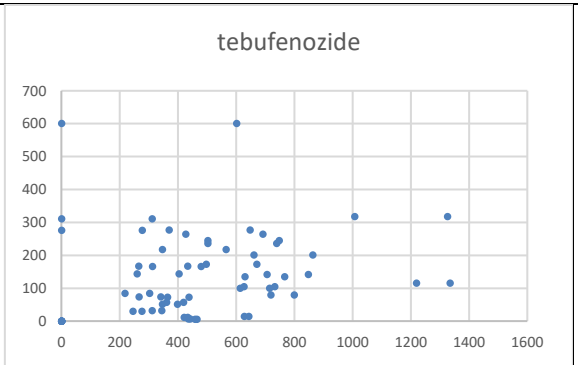

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dimethomorph
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diuron
epoxiconazole

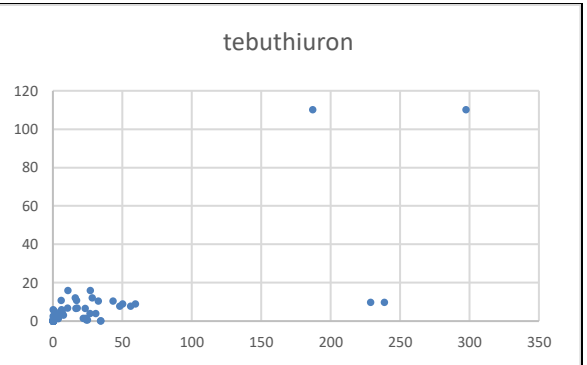

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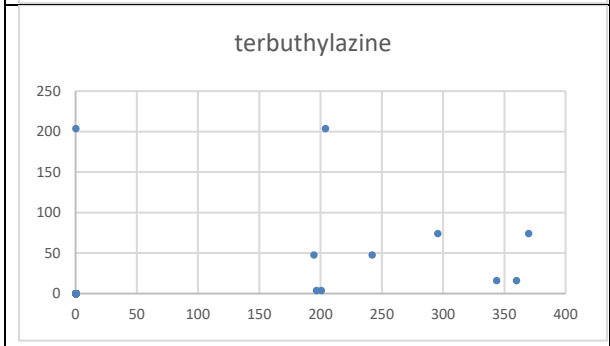

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paclobutrazol
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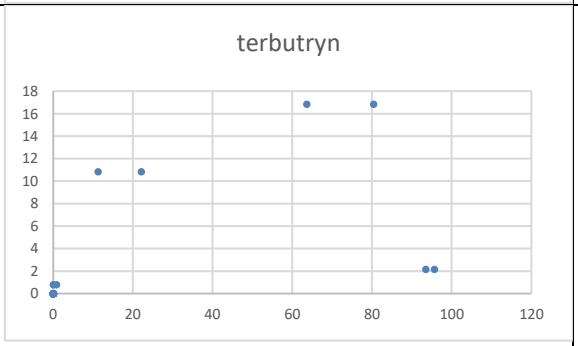

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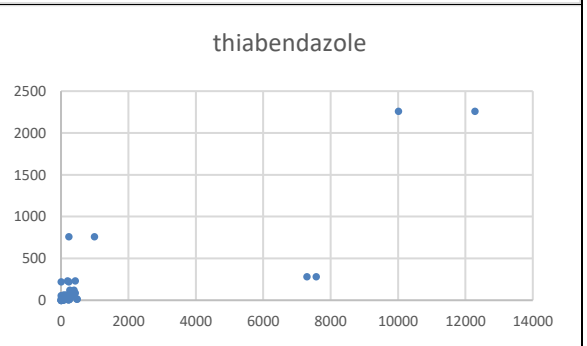

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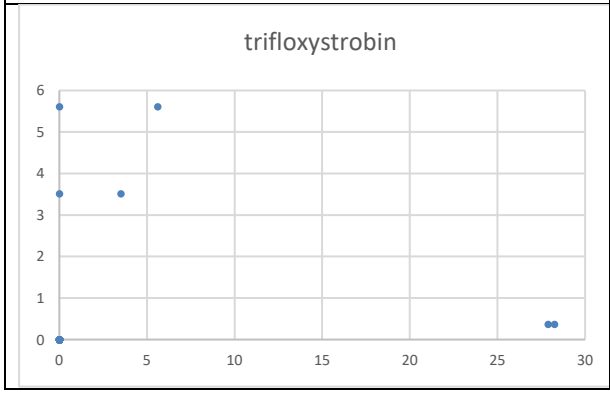

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400
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800
1000
1200
1400
1600


tebuthiuron
120
100
80
60
40
20
0
0
50
100
150
200
250
300
350


terbuthylazine
250
200
150
100
50
0
0
50
100
150
200
250
300
350
400


terbutryn
18
16
14
12
10
8
6
4
2
0
0
20
40
60
80
100
120


thiabendazole
2500
2000
1500
1000
500
0
0
2000
4000
6000
8000
10000
12000
14000


trifloxystrobin
6
5
4
3
2
1
0
0
5
10
15
20
25
30

Appendix A
Supplementary information for Chapter 3

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1. List of pesticide analytes and their MDLs/MQLs in water and biofilm samples
2. Methodology for pesticide analysis
3. Table B-1. Water Quality Index Scores
4. Figure B-1. Photos of biofilm samplers.
5. Table B-2. Pesticide detections in stormwater and in biofilm by pesticide type and use.
6. Pesticide detections and concentrations in water samples
7. Pesticide detections and concentrations in biofilm samples
8. Figure B-2. GLMs of pesticide detections in water and biofilm samples.
9. Figure B-3. GLMs of pesticide BCF_{DW}s and physicochemical properties
10. Figure B-4. GLM of pesticide BCF_{DW}s and water quality
11. Figure B-5. GLM of pesticide BCF_{DW}s and ash-free dry weight
12. Other information

List of pesticide analytes and their MDLs/MQLs in water samples:
Available on FigShare: https://figshare.com/s/549156f3317ddc839d69

Methodology for pesticide analysis:

Adapted from Brian Atkinson, Agriculture and Food Lab, Guelph, Ontario.

Subsampling/Preprocessing

Biofilm samples are homogenized to a powder with dry ice and a small food processor to ensure test portion taken for analysis is representative of the entire sample. Stormwater samples are thawed and shaken for one minute by hand before being subsampled for each test. No filtering of the water was used. Meaningful residue data can only be obtained when a representative sample is obtained.

Sample Analysis

All sample analyses were performed at the ISO/IEC 17025 accredited Laboratory Service Division, University of Guelph. The multi-residue pesticide determination (AFL Method ID: TOPS-142) was performed by liquid chromatography/electrospray ionization-tandem mass spectrometry (LC/ESI-MS/MS) and gas chromatography-tandem mass spectrometry (GC-MS/MS). Pesticides were extracted from water and biofilm using the method known as the quick, easy, cheap, effective, rugged, and safe (QuEChERS) method with dispersive solid phase extraction (SPE). A representative sample (20 mL for water and 3 g for biofilm) was extracted into acetic acid in acetonitrile in the presence of anhydrous sodium acetate and magnesium sulfate. The supernatant was centrifuged and then split, evaporated and diluted with methanol/ammonium acetate (for LC analysis) or hexane (for GC analysis). Sample extracts were analyzed in positive polarity mode using SCIEX5550 ESI-MS/MS with Agilent 1260 HPLC and on Agilent GC 7890 Quadrupole GC-MS/MS. This method detects for over 500 pesticide compounds.

The phenoxy pesticide screen (AFL Method ID: CHEM-069) was a general solid phase extraction (SPE) 2 step method. 500 mL of water is initially acidified with sulfuric acid, passed through the SPE, eluted with methanol, concentrated and analyzed in both positive and negative modes using a SCIEX 5500 LC-MS/MS with an Agilent HPLC. This method screens for 19 phenoxy acidic herbicides as well as for 9 neonicotinoid class pesticides. For the biofilm samples a 3 g subsample is extracted using a QuEChERS style method of acidified (with formic acid) acetonitrile in the presence of anhydrous magnesium sulfate and citrate buffers. The supernatant is centrifuged followed by dilution into a 50:50 MeOH/Water mixture filtered and analyzed in both positive and negative modes using a SCIEX 5500 LC-MS/MS with an Agilent HPLC.

The polar pesticide screen (AFL Method ID: CHEM-334) is a modified version of the EU's QuPPE method. A representative subsample (10 mL for water and 2 g for biofilm with 5 mL of Nanopure water to rehydrate) is extracted using acidified (with formic acid) methanol and then placed into a freezer for 30 minutes. The supernatant is then centrifuged and filtered and analyzed in positive and negative modes using a SCIEX 5500 LC-MS/MS with an Agilent HPLC.

Each pesticide in the analyzed matrices was quantified using a matrix-matched calibration curve with six concentrations. A quadratic regression with a 1/x weighting and a $r > 0.99$ was used to construct the calibration curve for quantitation. Deuterated internal standards were added to samples to account for any matrix effect. An expanded uncertainty (U) that is statistically derived for each analyte was used for method validation.

Quality Controls

All methods use several quality control points that confirm the presence of a compound in the sample: retention time (RT), M+1 target mass, 2 fragment qualifier ions, and the ratio of the two fragment ions. For the identification and quantification of the compounds, each method utilizes deuterium labelled internal standards, matrix-matched blanks, spikes, and calibration curves. The analysis of each batch of samples included a reagent blank, reagent spiked, matrix blank, and three matrix spiked quality control samples. The reagent and matrix spiked quality control samples were conducted at various concentrations and all recoveries were between 80 and 120%. As part of our method validation, we use an expanded uncertainty (U) that is statistically derived for each analyte in our methods.

Table B-1. Water Quality Index (WQI) scores for each stormwater pond site calculated using the CCME Water Quality Index Calculator version 1.2. Sites are listed in order of increasing WQI score.

Stormwater pond site	CCME WQI
130	33.5
109	35.6
116	44.7
26	46.2
153	47.2
191	47.5
106	49.4
51	50.7
174	50.8
10	56.8
49	56.9
46	59.2
58	60.1
188	61.2
56	64.6
14	67.1
85	71.5
96	72.2
113	74.0
156	76.1
78	94.6

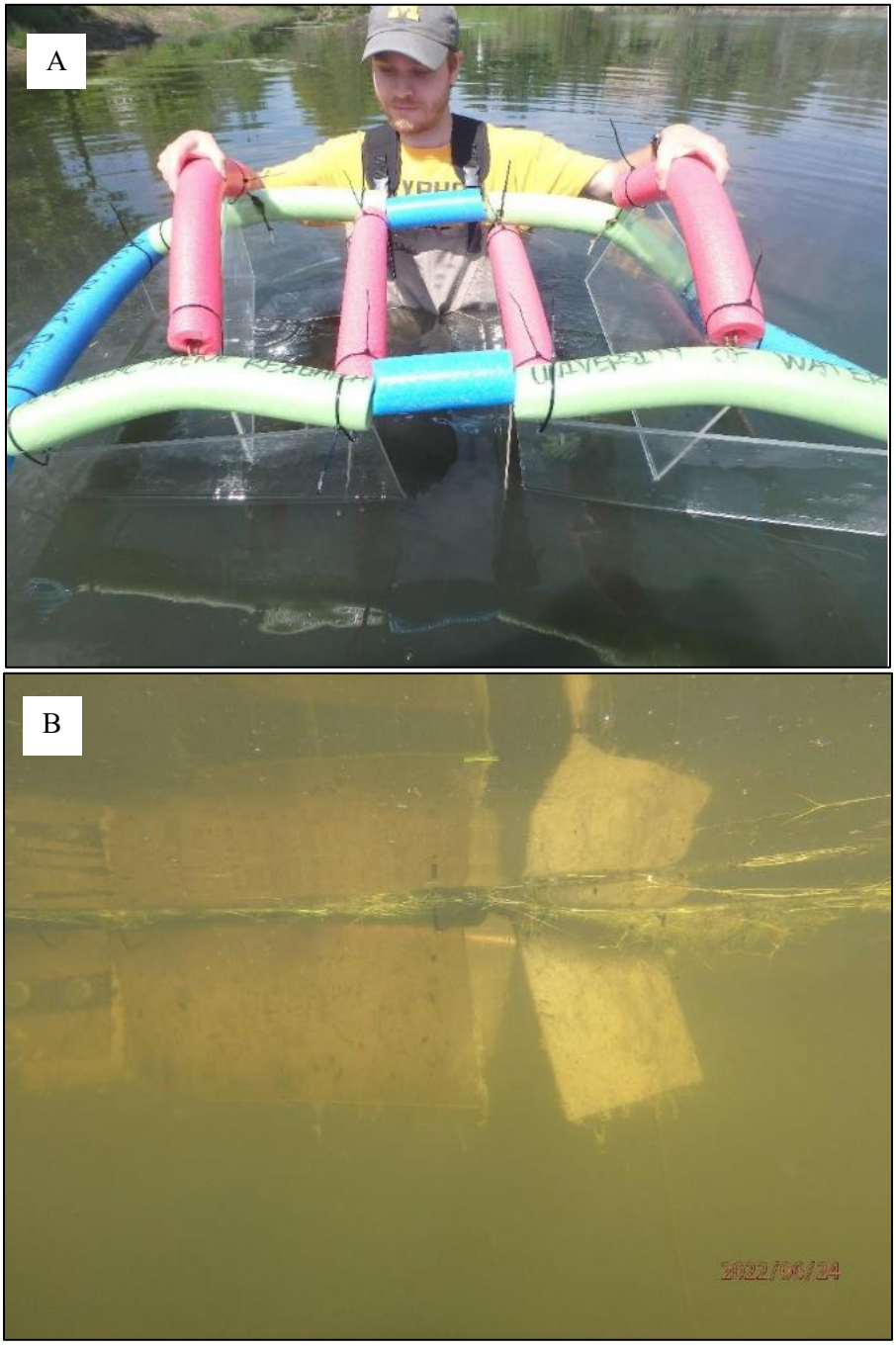


Figure B-1. Biofilm samplers deployed at stormwater pond sites in Brampton, ON. Acrylic plates were suspended from floats (A) and submerged in the water where they were colonized by biofilms (B).

Table B-2. Pesticide detections in stormwater and in biofilm by pesticide type and use.

Category		Total # of pesticides	Total overall detections	Detections in stormwater		Detections in biofilm	
				# of pesticides	# of hits	# of pesticides	# of hits
Type	Herbicides	14	177	13	139	6	37
	Insecticides	5	43	5	43	0	0
	Fungicides	8	62	3	10	7	52
	Other	5	28	2	13	3	15
Use	Current-use	26	282	21	195	12	87
	Legacy	1	5	1	5	0	0
	Unknown	5	22	1	5	4	17

Pesticide detections and concentrations in water samples:

Available on Figshare: https://figshare.com/s/27341527655e5b2b258a

Pesticide detections and concentrations in biofilm samples:

Available on Figshare: https://figshare.com/s/31e4a23e5819b2517979

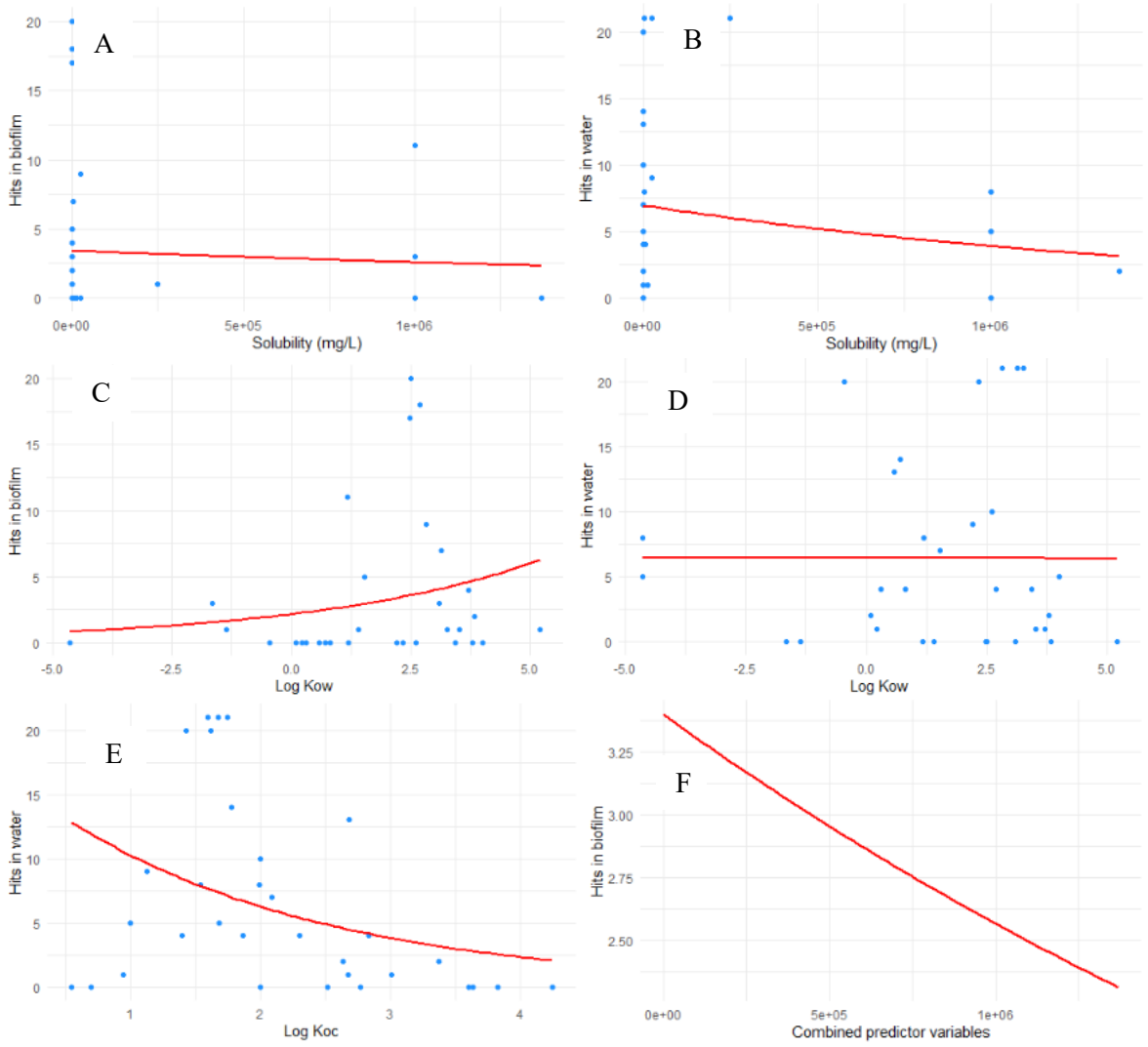


Figure B-2. Generalized linear models of the solubility (mg L^{-1}) of pesticides and their detections ("hits") in biofilm (A) and water (B); Log K_{ow} of pesticides and their detections in biofilm (C) and water); Log K_{oc} of pesticides and their detections in water (E); and the number of pesticide detections in biofilm using a combined model (F).

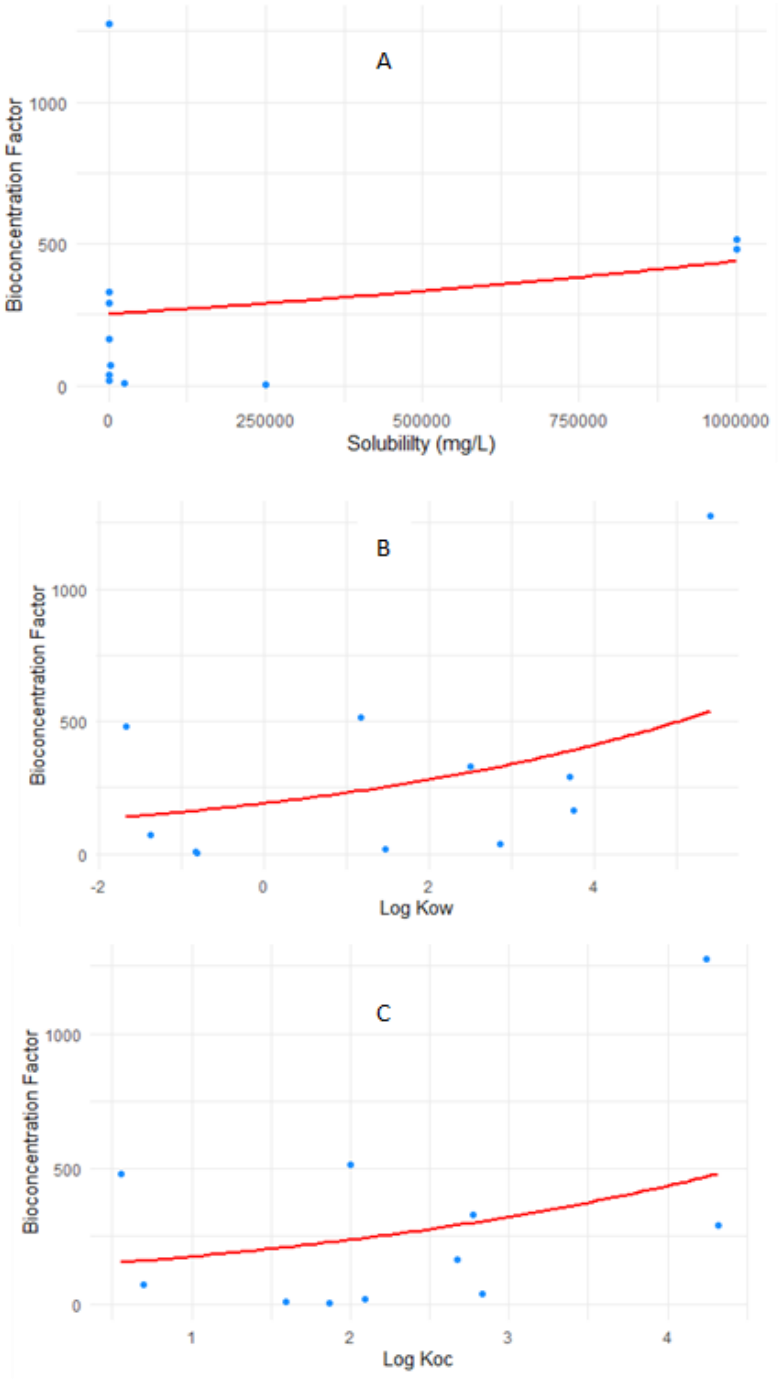


Figure B-3. Generalized linear regressions showing the relationship between pesticide physicochemical properties and their BCF_{DW} (n = 11) in biofilm samples. A) Water solubility (mg L^{-1}); B) Log K_{ow} (octanol-water partition coefficient); and C) Log K_{oc} (soil adsorption coefficient)

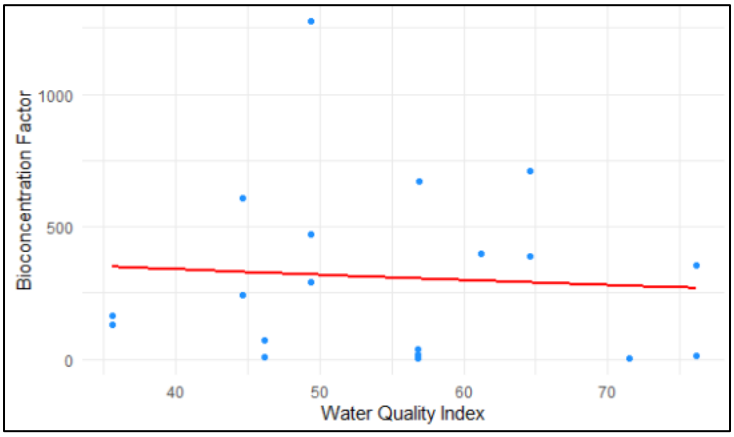


Figure B-4. Generalized linear model of the CCME Water Quality Index Score and BCF_{DW} values (n = 17, p = 0.768).

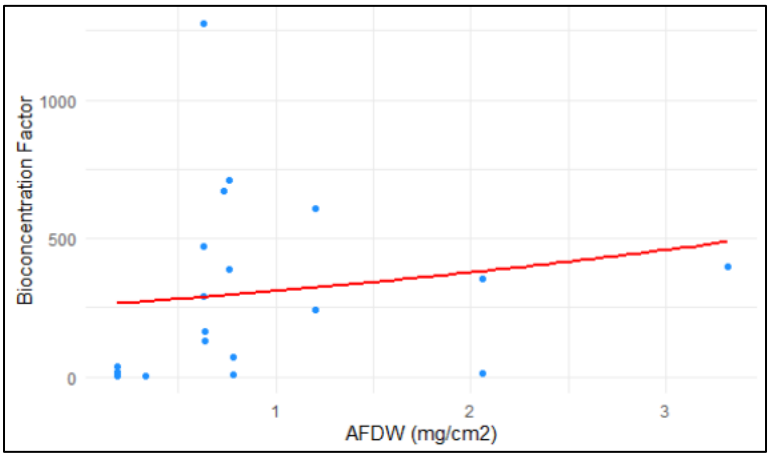


Figure B-5. Relationship between ash-free dry weight (mg cm^{-2}) and calculated pesticide BCF_{DW} values (n = 17; p = 0.586) using a generalized linear model.

Other information:

Site locations: https://figshare.com/s/8ad9316ca7c42c1928ff

Site characteristics: https://figshare.com/s/3f17702070cb8baa26d3

Water quality assessment results: https://figshare.com/s/62c747135f94d0672a3d

E. coli and coliforms (AFL): https://figshare.com/s/e5cfd674c74bb9aaed70

Nutrients in water (ALS): https://figshare.com/s/1bcf2ed9f695a6bdb23d

CCME WQI Calculations: https://figshare.com/s/c8aa8abacae79d0ac48c

Biofilm analysis (chlorophyll-*a* and AFDW) results: https://figshare.com/s/76756a987ee8f1590c1c

Appendix B

Supplemental information for Chapter 4

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1. List of pesticide analytes and their MDLs/MQLs in biofilm samples
2. Methodology for pesticide analysis
3. Table C-1. Test conditions for two dietary bioassays
4. Table C-2. Water quality in test vessels during *N. triangulifer* trial
5. Tables C-3 and C-4. Water quality in test vessels during *P. pilsbryi* trial
6. Pesticide detections and concentrations in biofilm samples
7. Table C-5. Properties of pesticides detected in biofilm samples
8. Table C-6. Survival of *N. triangulifer* during acute dietary bioassay
9. Table C-7. Growth and biomass production of *N. triangulifer* during acute dietary bioassay
10. Table C-8. Survival of *P. pilsbryi* during chronic dietary bioassay
11. Table C-9. Growth and biomass production of *P. pilsbryi* during chronic dietary bioassay
12. Behaviour tests (methods, results, and Table C-10.)
13. Table C-11. Spearman's rank correlations with refined pesticide burden.

1. List of pesticide analytes and their MDLs/MQLs in biofilm samples

Freeze-dried biofilm samples collected from the 15 stormwater pond sites were submitted to the Agriculture and Food Laboratory (AFL) in Guelph, Ontario. These samples were screened for ~500 current-use and historical pesticides. Lists of analytes and their respective limits of detection and quantification in environmental media can be accessed at this FigShare link: https://figshare.com/s/703b8b647d8a1adbe75a.

2. Methodology for pesticide analysis:

Adapted from Brian Atkinson, Agriculture and Food Lab, Guelph, Ontario.

Subsampling/Preprocessing

Biofilm samples are homogenized to a powder with dry ice and a small food processor to ensure test portion taken for analysis is representative of the entire sample. Stormwater samples are thawed and shaken for one minute by hand before being subsampled for each test. No filtering of the water was used. Meaningful residue data can only be obtained when a representative sample is obtained.

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3. Test conditions for two dietary bioassays

Table C-1. Test conditions for two dietary bioassays with *Neocloeon triangulifer* and *Planorbella pilsbryi*

Test	**Bioassay with mayfly nymphs**	**Bioassay with juvenile snails**
Test species	*Neocloeon triagulifer*	*Planorbella pilsbryi*
Test duration	7-days (acute)	28-days (chronic)
Endpoints	Survival, growth, biomass production	Survival, growth, biomass production
Test type	Static	Static
Renewal	Day 3 and 5	Half water-change every other day; full water-change on day 0, 7, 14, and 21
Number of treatments	15 + 3 controls	15 + 3 controls
Number of replicates per treatment	5	5
Number of organisms per test vessel	3	5
Age of organisms	< 24 h	4 weeks
Test vessel	30 mL glass beakers	250 mL glass jars
Test solution volume	15 mL	200 mL
Test water composition	Dechlorinated, reverse-osmosis water	Dechlorinated, reverse-osmosis water
Aeration	None	None
Photoperiod	16:8	16:8

4.Water quality measurements in test vessels in *N. triangulifer* trial

Table C-2. Water quality measurements on test vessel water on Day 1 and 7 of acute dietary bioassay with *Neocloeon triangulifer*. Treatment vessel numbers represent the stormwater pond sites from which biofilm diets were collected and are listed in order of increasing pesticide burden. CF = control fed; CS = control starved; CB = control biofilm. Water quality was not measured (NM) on Day 7 in test vessels with 0% survival on Day 5.

Treatment vessel	Test day	DO (mg L^{-1})	Conductivity (µS cm^{-1})	pH
CF	1	8.18	483.7	8.457
	7	8.62	589.5	8.533
CS	1	8.28	390.8	8.339
	7	NM	NM	NM
CB	1	8.24	368.9	8.461
	7	8.02	475.8	8.289
14	1	8.33	364	8.212
	7	NM	NM	NM
113	1	8.21	367.5	8.308
	7	7.80	359.7	8.281
58	1	8.15	369.6	8.339
	7	7.76	571.5	8.431
85	1	7.84	365.7	8.326
	7	8.01	214.4	8.379
174	1	8.51	392.4	8.224
	7	8.06	494.3	8.394
188	1	8.08	379.8	8.319
	7	7.71	509.7	8.444
26	1	8.19	370.3	8.350
	7	8.29	448.8	8.419
49	1	8.15	370.3	8.352
	7	8.04	479.3	8.394
56	1	8.15	372.4	8.197
	7	8.12	522.7	8.441
156	1	8.10	367.9	8.351
	7	7.96	477.2	8.378
46	1	8.19	373.3	8.129
	7	NM	NM	NM
51	1	6.14	368.2	8.300
	7	7.83	502.4	8.395
109	1	8.15	370.6	8.335
	7	7.62	547.1	8.273
106	1	8.24	364.4	8.295
	7	7.73	511.6	8.344
116	1	8.23	370.9	8.298

	7	NM	NM	NM

5.Water quality measurements in test vessels in *P. pilsbryi* trial

Table C-3. Water quality measurements on test vessel water on Day 0, 7, 14, 21, and 28 of the chronic dietary bioassay with *Planorbella pilsbryi*. Treatment vessel numbers represent the stormwater pond sites from which biofilm diets were collected and are listed in order of increasing pesticide burden. CF = control fed; CS = control starved; CB = control biofilm. Water quality was not measured (NM) in test vessels with 0% survival.

Treatment vessel	Water temperature (C)	Conductivity (µS cm^{-1})	pH	DO (mg L^{-1})
		Day 0		
	21.2	264	7.32	8.38
		Day 7		
CF	21.6	331	6.8	4.54
CS	21.5	267	6.89	7.82
CB	21.6	358	6.68	4.97
14	22.3	368	6.95	4.48
113	22.3	360	6.93	3.52
58	22.5	347	6.85	4.71
85	22.9	324	7.05	4.67
174	22.7	342	6.96	4.67
188	22.3	386	6.73	4.92
26	22.9	357	6.98	3.72
49	22.5	343	6.89	4.62
56	22.5	335	6.9	5.31
156	22.9	406	6.91	1.28
46	22.6	341	6.88	5.56
51	22.4	356	6.78	4.28
109	22.1	367	6.72	3.64
106	22.6	340	6.94	5.62
116	21.5	376	6.52	2.17
		Day 14		
CF	20.9	296	6.882	5.69
CS	20.9	2652	7.428	8.16
CB	21	406	7.115	4.9

14	21	399	7.219	5.33
113	21.2	383	6.718	3.62
58	21	412	7.281	4.77
85	21.2	332	6.764	4.74
174	21	360	7.217	5.47
188	21.1	488	7.047	0.75
26	21	381	7.319	5.84
49	21	374	7.136	4.96
56	21.1	365	6.899	4.36
156	21.3	425	6.725	1.01
46	21	380	7.157	4.3
51	21	395	7.124	4.58
109	20.9	366	7.291	5.51
106	21	359	7.197	5.5
116	21.1	386	6.809	3.2
		Day 21		
CF	21.5	344	6.835	1.17
CS	NM	NM	NM	NM
CB	21.3	407	7.044	4.34
14	21.3	407	7.133	4.27
113	21.4	374	7.031	3.96
58	21.2	433	7.048	2.11
85	21.6	320	6.876	3.63
174	21.1	372	7.049	4.36
188	21.4	421	6.822	0.4
26	21.4	381	7.08	4.29
49	21.2	371	7.009	3.65
56	21.3	347	6.894	3.94
156	21.3	402	6.972	3.35
46	21.4	383	6.866	3.69
51	21.2	420	6.904	1.51
109	21.7	363	7.273	4.48
106	21.4	354	6.848	4.04
116	21.5	376	6.845	2.96
		Day 28		
CF	21.7	385	6.644	4.32
CS	NM	NM	NM	NM

CB	21.7	433	7.181	5.61
14	22	441	7.044	3.39
113	22.2	389	6.922	3.92
58	21.8	443	6.96	2.01
85	21.8	474	6.858	4.01
174	21.9	390	6.986	2.91
188	21.9	461	6.792	1.09
26	21.8	416	7.316	3.86
49	21.8	415	7.069	3.95
56	22	352	6.943	3.22
156	21.7	475	6.943	4.77
46	21.8	393	6.931	3.61
51	21.9	373	7.067	2.17
109	21.8	391	7.082	4.81
106	21.8	357	6.945	4.89
116	21.7	383	6.714	3.12

Table C-4. Ammonia concentrations (mg L^{-1}) in test vessel water on days 0, 7, 11, 14, 18, 21, 25, and 28 during chronic bioassay with *Planorbella pilsbryi.* Treatment vessel numbers represent the stormwater pond sites from which biofilm diets were collected and are listed in order of increasing pesticide burden. CF = control fed; CS = control starved; CB = control biofilm. Water quality was not measured (NM) in test vessels with 0% survival.

Treatment	Day 0	Day 7	Day 11	Day 14	Day 18	Day 21	Day 25	Day 28
CF	1.5	2	2	2	2	0.25	1	2
CS	1.5	0	0	0	0	NM	NM	NM
CB	1.5	2	1.5	1	1.5	0.75	1	1.5
14	1.5	2	1.5	2	1.5	1.5	1.5	2
113	1.5	0	0.25	0	0	0.25	0	0.5
58	1.5	0	0.25	0	0	0	0	0
85	1.5	0.5	0.25	0.75	0.75	0.75	0.5	1
174	1.5	0	0.25	0	0	0	0	0
188	1.5	0	0.5	0	0.75	0	0.25	0.25
26	1.5	0	0	0.5	0.25	0.25	0.25	0.25
49	1.5	1	0.75	1.5	1	1	0.5	1
56	1.5	1.5	1	2	1.5	1	1	1.5
156	1.5	0	0	0	0	0	0	0

46	1.5	0.75	0.5	2	1.5	0	1.5	2.5
51	1.5	0.25	0.5	0.5	0.75	0	0.5	1
109	1.5	1.5	0.5	0.75	0.5	1	1	1
106	1.5	0.5	0.5	0.75	0.5	0.75	0.75	0.5
116	1.5	0.25	0.25	0.25	0	0.25	0	0

6. Pesticide detections and concentrations in biofilm samples

Data is publicly available online at: https://figshare.com/s/caa147298376057e2883

7. Properties of pesticides detected in biofilm samples

Table C-5. Properties of pesticides detected in biofilm bioassay diets. Information sourced from the Pesticide Properties Data Base (PPDB) and the Pesticide Product Information Database (PPID).

Pesticide	Type	CAS	Mode of Action	Possible urban sources
2,4-D	Herbicide	94-75-7	Synthetic auxin	Lawn/turf
Chlorpropham	Herbicide	101-21-3	Mitosis inhibitor	Food storage (potato crops)
Diuron	Herbicide	330-54-1	Photosystem II inhibitor	Ditches/municipal land
HEPA	Herbicide	22987-21-9	Unknown	Unknown
MCPP	Herbicide	7085-19-0	Synthetic auxin	Lawn/turf
Pendimethalin	Herbicide	40487-42-1	Cell division and elongation inhibitor	Forest/woodlot
2-phenylphenol	Microbiocide	90-43-7	Biosynthesis disruptor in microbes	Food storage (fruits)
Azoxystrobin	Fungicide	131860-33-8	Respiration inhibitor	Lawn/turf
Carbendazim	Fungicide	10605-21-7	Mitosis and cell division inhibitor	Lawn/turf
Imazalil	Fungicide	35554-44-0	Sterol biosynthesis inhibitor	Veterinary uses, food storage (fruit and wheat)
Tebuconazole	Fungicide	107534-96-3	Sterol biosynthesis inhibitor	Wood preservative
Thiabendazole	Fungicide	148-79-8	Cytoskeleton compromiser	Industrial, construction, forestry
Thiophanate-methyl	Fungicide	23564-05-8	Mitosis and cell division inhibitor	Lawn/turf
Nicotine	Other	54-11-5	Nicotinic acetylcholine receptor agonist	Litter
Melamine	Other	108-78-1	Unknown	Plastic, cleaning products

8. Survival of *N. triangulifer* during acute dietary bioassay

Table C-6. Mayfly nymph mean survival from Day 0 to Day 7. CF = Control Fed, CS = Control Starved, CB = Control Biofilm. Brackets indicate standard deviation. Test diets are listed in order of increasing pesticide burden. Test diets which were significantly different from the CF or CB control diets on Day 7 are denoted with a superscript [1] (for CF) or [2] (for CB).

Treatment	Day 0	Day 1	Day 3	Day 5	Day 7
CF	100 (±0)	100 (±0)	100 (±0)	100 (±0)	100 (±0)
CS [1,2]	100 (±0)	100 (±0)	0 (±0)	0 (±0)	0 (±0)
CB	100 (±0)	100 (±0)	93.3 (±14.9)	93.3 (±14.9)	90 (±22.4)
14 [1,2]	100 (±0)		26.7 (±27.9)	0 (±0)	0 (±0)
113 [1,2]	100 (±0)		100 (±0)	73.3 (±14.9)	0 (±0)
58 [1,2]	100 (±0)		53.3 (±18.3)	20.0 (±29.8)	0 (±0)
85	100 (±0)		93.3 (±14.9)	93.3 (±14.9)	93.3 (±14.9)
174 [1,2]	100 (±0)		73.3 (±14.9)	20.0 (±18.3)	6.7 (±14.9)
188 [1,2]	100 (±0)		53.3 (±29.8)	13.3 (±18.3)	0 (±0)
26 [1,2]	100 (±0)		100 (±0)	60.0 (±43.5)	30.0 (±29.8)
49 [1,2]	100 (±0)		100 (±0)	40.0 (±14.9)	26.7 (±27.9)
56 [1,2]	100 (±0)		73.3 (±14.9)	13.3 (±18.3)	0 (±0)
156	100 (±0)		80.0 (±18.3)	60.0 (±27.9)	40 (±14.9)
46 [1,2]	100 (±0)		86.7 (±18.3)	6.7 (±14.9)	0 (±0)
51 [1,2]	100 (±0)		73.3 (±27.9)	33.3 (±23.6)	26.7 (±14.9)
109	100 (±0)		93.3 (±14.9)	53.3 (±18.3)	46.7 (±18.3)
106 [1,2]	100 (±0)		60.0 (±14.9)	13.3 (±18.3)	13.3 (±18.3)
116 [1,2]	100 (±0)		33.3 (±0)	0 (±0)	0 (±0)

9. Growth and biomass production of *N. triangulifer* during acute dietary bioassay

Table C-7. Average mayfly growth and biomass production (± standard dev) following the end of the test. CF = control fed diet; CS = control starved diet; CB = control biofilm diet. Test diets are listed in order of increasing pesticide burden.

Treatment diet	Average growth (µm)	Average body biomass production (µm)
CF	534.8 (±68.5)	534.8 (±49.8)

Treatment diet	Average growth (µm)	Average body biomass production (µm)
CS	0 (±0)	0 (±0)
CB	155.5 (±112.9)	134.8 (±99.2)
14	0 (±0)	0 (±0)
113	0 (±0)	0 (±0)
58	0 (±0)	0 (±0)
85	384.5 (±218.2)	322.0 (±97.9)
174	29.6 (±0)	2.0 (±4.4)
188	0 (±0)	0 (±0)
26	37.4 (±26.8)	10.0 (±14.4)
49	28.9 (±24.8)	7.7 (±11.4)
56	0 (±0)	0 (±0)
156	166.4 (±77.3)	62.3 (±33.4)
46	0 (±0)	0 (±0)
51	158.4 (±59.2)	42.2 (±29.1)
109	90.5 (±44.1)	42.2 (±13.9)
106	178.4 (±127.6)	23.8 (±38.9)
116	0 (±0)	0 (±0)

10. Survival of *P. pilsbryi* during chronic dietary bioassay

Table C-8. Snail survival (%) during the 28-chronic dietary bioassay. Brackets indicate standard deviation. CF = control fed diet; CS = control starved diet; CB = control biofilm diet. Test diets are listed in order of increasing pesticide burden. Test diets which were significantly different from the CF or CB control diets on Day 7 are denoted with a superscript [1] (for CF) or [2] (for CB).

Treatment	Day 0	Day 7	Day 14	Day 21	Day 28
CF	100 (±0)	100 (±0)	100 (±0)	100 (±0)	100 (±0)
CS [1,2]	100 (±0)	76 (±16.7)	28 (±11.0)	0 (±0)	0 (±0)
CB	100 (±0)	100 (±0)	100 (±0)	100 (±0)	100 (±0)
14	100 (±0)	100 (±0)	92 (±11.0)	92 (±11.0)	88 (±11.0)
113	100 (±0)	100 (±0)	100 (±0)	100 (±0)	100 (±0)
58	100 (±0)	100 (±0)	100 (±0)	100 (±0)	100 (±0)
85	100 (±0)	100 (±0)	100 (±0)	100 (±0)	100 (±0)
174	100 (±0)	96 (±8.9)	96 (±8.9)	96 (±8.9)	96 (±8.9)
188 [1,2]	100 (±0)	92 (±17.9)	80 (±24.5)	60 (±20.0)	52 (±11.0)
26	100 (±0)	100 (±0)	100 (±0)	100 (±0)	100 (±0)
49 [1,2]	100 (±0)	88 (±11.0)	80 (±14.1)	52 (±22.8)	24 (±16.7)

Treatment	Day 0	Day 7	Day 14	Day 21	Day 28
56 [1,2]	100 (±0)	72 (±17.9)	44 (±16.7)	28 (±11.0)	24 (±8.9)
156 [1,2]	100 (±0)	76 (±16.7)	60 (±14.1)	48 (±11.0)	32 (±11.0)
46 [1,2]	100 (±0)	92 (±11.0)	80 (±20.0)	60 (±20.0)	48 (±11.0)
51	100 (±0)	100 (±0)	96 (±8.9)	96 (±8.9)	92 (±11.0)
109	100 (±0)	84 (±16.7)	84 (±16.7)	72 (±11.0)	72 (±11.0)
106 [1,2]	100 (±0)	80 (±20.0)	72 (±11.0)	44 (±16.7)	28 (±11.0)
116 [1,2]	100 (±0)	88 (±11.0)	80 (±14.1)	64 (±8.9)	48 (±11.0)

11. Growth and biomass production of *P. pilsbryi* during chronic dietary bioassay

Table C-9. Average snail growth (mg) and biomass production (mg) for each biofilm treatment at Day 28 (± st dev)

Treatment	Average growth (mg)	Average biomass production (mg)
CF	15.51 (±1.70)	15.51 (±1.70)
CS	0 (±0)	0 (±0)
CB	22.58 (±2.92)	22.58 (±2.92)
14	15.79 (±1.78)	13.94 (±2.54)
113	25.74 (±3.28)	25.74 (±3.28)
58	14.52 (±1.93)	14.52 (±1.93)
85	35.57 (±3.89)	35.57 (±3.89)
174	16.97 (±1.88)	16.25 (±2.14)
188	18.07 (±6.40)	8.94 (±1.78)
26	19.28 (±1.35)	19.28 (±1.35)
49	10.35 (±6.19)	3.25 (±2.60)
56	34.23 (±7.07)	7.73 (±0.73)
156	23.06 (±7.13)	6.94 (±1.53)
46	19.05 (±3.33)	9.09 (±2.32)
51	17.79 (±2.88)	16.23 (±2.51)
109	17.08 (±3.65)	12.15 (±2.09)
106	13.65 (±1.57)	3.75 (±1.25)
116	21.60 (±2.51)	10.44 (±2.99)

12. Behaviour tests on *Planorbella pilsbryi*

Methods

On day 27 of the chronic dietary exposure test on *Planorbella pilsbryi*, we performed a behaviour test following the methods described by Alonso & Camargo (2009) and Romero-Blanco et al. (2021). We randomly selected and removed one organism from each test vessel, placed the snail on its side in a glass dish filled with dechlorinated test water, and timed how long it took to undergo normal movement, defined as a sliding movement using the foot (Alonso & Camargo, 2009). This test resulted in two measured endpoints: time to start normal movement (TSNM) and percent immobility. Snails were considered immobile if TSNM was longer than 180 seconds, meaning death or close to death, and percent immobility was the number of individuals per treatment who experienced immobility. We used a Spearman's ranking analysis using R in RStudio to investigate the relationship between behavioral endpoints and other test endpoints.

Results

Table C-10. Snail behaviour at Day 27 of chronic dietary bioassay. Average time to start normal movement (seconds ± stdev) and immobility (%) calculated for treatments and controls with surviving individuals. CF = control fed diet; CS = control starved diet; CB = control biofilm diet. Sites are listed in order of increasing pesticide burden.

Treatment	Average time to start normal movement (s)	Immobility (%)
CF	16.6 (±3.2)	0
CS	N/A	N/A
CB	29.8 (±4.9)	0
14	40.6 (±6.5)	0
113	48.8 (±5.8)	0
58	53.8 (±10.7)	0
85	35.6 (±3.0)	0
174	43 (±6.5)	0
188	120.4 (±28.2)	0
26	41 (±5.6)	0
49	137 (±11.3)	60
56	122 (±13.3)	0
156	103 (±19.2)	20
46	73.25 (±8.0)	20
51	51.8 (±7.5)	0
109	47.6 (±6.9)	0
106	54.2 (±8.9)	0
116	53.4 (±8.0)	0

We found a significant negative relationship between mean snail survival and average time to start normal movement ($p = 0.0000962$), meaning treatments exhibiting higher survival had lower TSNM. We also found a significant negative relationship between TSNM and biomass production ($p = 0.000129$) but not with growth ($p = 0.758$). These results indicate behavioral studies are valuable for use as test endpoints, especially in trials with high survivorship across treatments where a more sensitive endpoint may be needed. Additionally, should researchers wish to study life-cycle effects (e.g., egg mass production), behavioural tests can be administered at regular check points throughout a longer-term trial. This method is minimally invasive: snails are only away from their test vessels for a maximum of 3 mins and are subsequently gently returned to their test vessels. With careful handling to ensure no damage is done to their shells, the temporary stress of being moved around likely has a minimal impact on the health and wellbeing of individuals.

We found that TSNM was not significantly correlated to our selected metrics of nutritional quality, however, pesticide burden was positively related with TSNM ($p = 0.0266$). This means the snails consuming biofilm diets containing a higher number of pesticides took more time to right themselves and begin their normal movement. If consuming contaminated food hinders snail movement, individuals may be less likely to escape predation, compete for resources, or simply right themselves following unexpected hydraulic movements (such as in storm events with faster flows).

Immobility was negatively related to survival ($p = 0.0301$) and biomass production ($p = 0.0233$), but not to growth ($p = 0.693$). We did not decern any significant relationships between immobility and nutritional quality or pesticide burden. This endpoint was not as sensitive as TSNM, and did not provide a useful amount of information, however it was simple to calculate and so may still be a useful endpoint for chronic studies.

14. **Table S11. Spearman's rank correlations with refined pesticide burden**

Table C-11. Results of Spearman's rank correlations with a refined set ($n = 8$) of pesticides to determine pesticide burden. Asterisks indicated a significant ($p < 0.05$) result.

Test species	Endpoint	Spearman's rank correlation results	
		rho	p-value
Neocloeon triangulifer	Survival	-0.353	0.165
	Growth	-0.226	0.383
	Biomass production	-0.324	0.204
Planorbella pilsbryi	Survival	-0.783	0.000204*
	Growth	-0.0909	0.729
	Biomass production	-0.705	0.00158*

Appendix C

Supplemental information for Chapter 5

Contents:

1. Information and data available in Figshare repository
2. Supplemental figures: **D-1** – Map of SWP sites; **D-2** – Schematic of climate conditions during sampling period; **D-3** – Biofilm sampling rack; **D-4** – o-DGT sampler set-up.
3. Supplemental tables: **D-1** – Summary of measured water quality parameters; **D-2** – Results of water quality measurements.

Information and data available in Figshare Repository:

1) Stormwater pond site locations and features: https://doi.org/10.6084/m9.figshare.27629475
2) Results of all measured water chemistry parameters:
   a) https://doi.org/10.6084/m9.figshare.23907963 (within pond measurements)
   b) https://doi.org/10.6084/m9.figshare.23907840 (bacterial contamination)
   c) https://doi.org/10.6084/m9.figshare.23907939 (nutrient contamination)
3) Chemical analysis in water and biofilm samples:
   a) A list of all chemical analytes from all pesticide screens, along with their detection and quantification limits: https://doi.org/10.6084/m9.figshare.27629508
   b) Raw analytical results: https://doi.org/10.6084/m9.figshare.27629520
4) Chemical analysis in o-DGT samplers:
   a) Details of the target analytes, including their limits of detection (LODs), limits of quantification (LOQs), multiple reaction monitoring (MRM) transitions, and retention times. Include raw analytical results: https://doi.org/10.6084/m9.figshare.27629538
5) Metal analysis in biofilm:
   a) The method detection limits (MDL) and method recovery limits (MRL) for metal and metalloid analytes: https://doi.org/10.6084/m9.figshare.29149691

b) Raw analytical results: https://doi.org/10.6084/m9.figshare.29149769

Supplemental figures:

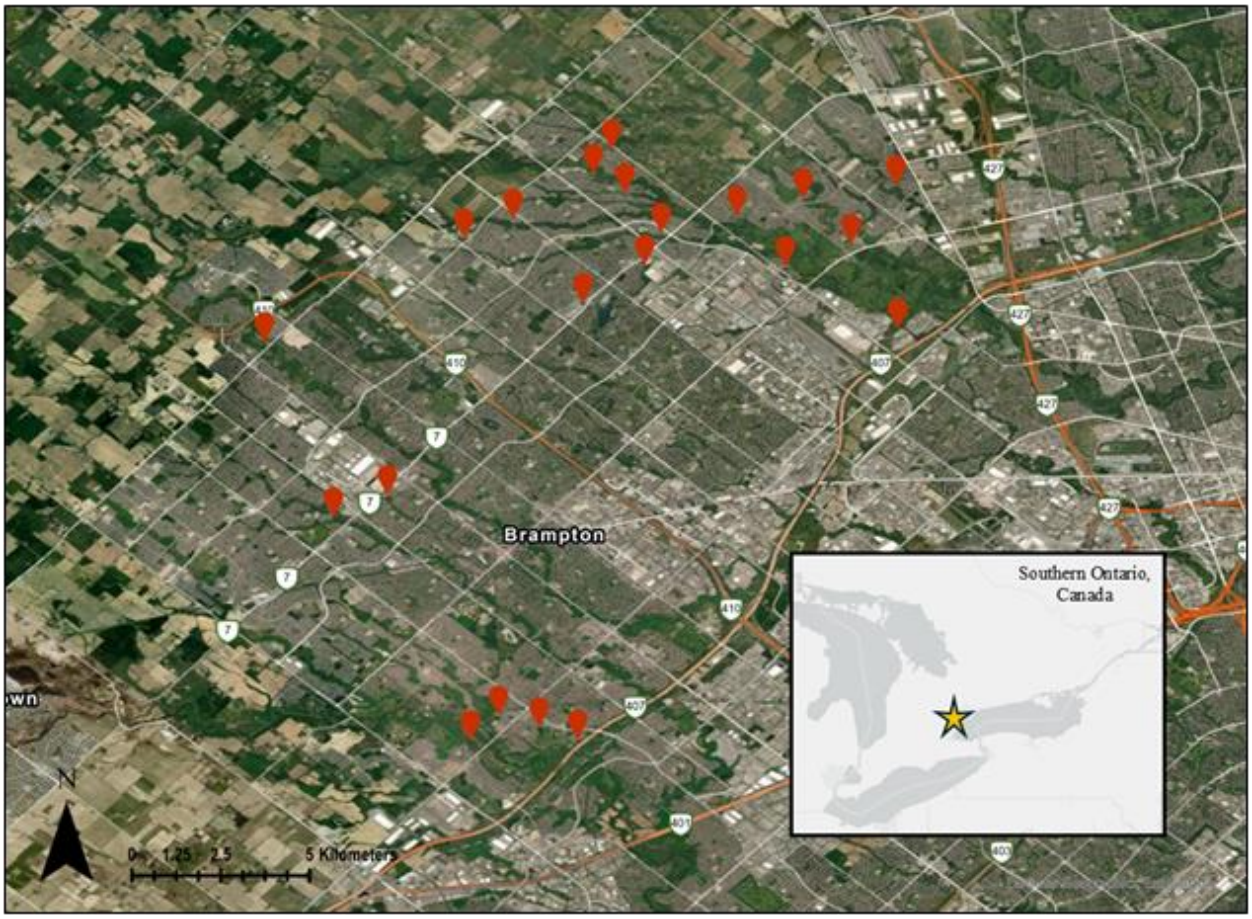


Figure D-1. Map of stormwater pond sites (represented by red pins) in Brampton, Ontario, Canada. Map adapted from Izma et al., (2024).

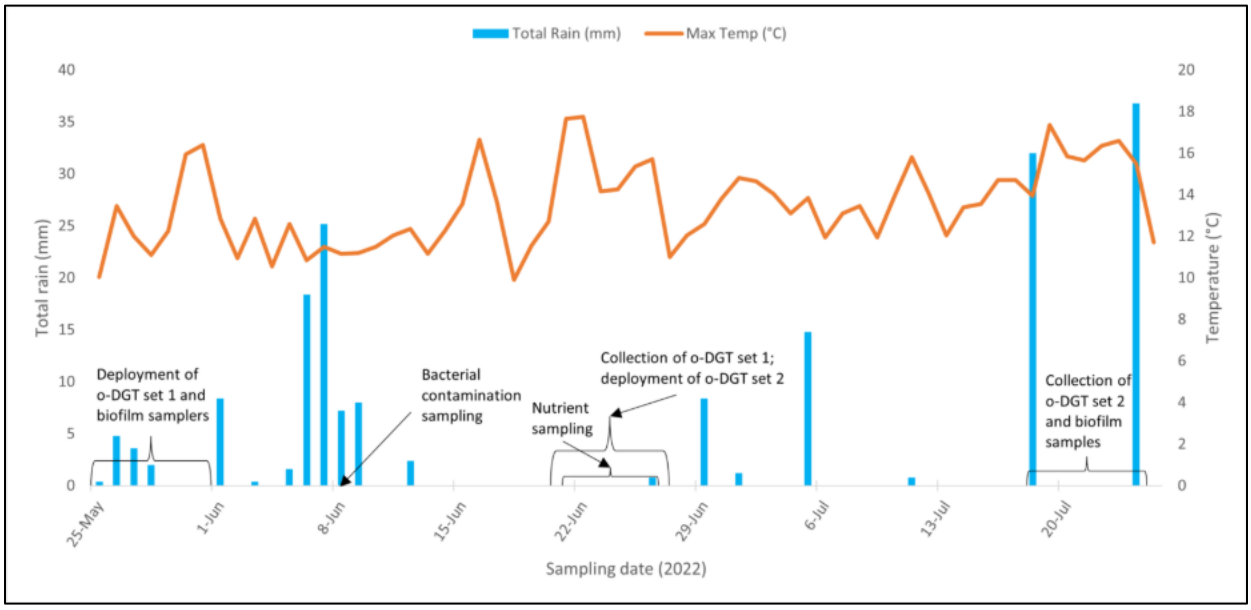


Figure D-2. Schematic of climatic conditions during the sampling period from May 25 – July 25, 2022, showing daily maximum air temperatures and total rainfall amounts. Water quality parameters assessed on a weekly basis included temperatures, dissolved oxygen, chloride, conductivity, and total suspended solids. Samples for analysis of bacterial contamination were taken once on June 8 after a significant rainfall event from the previous day, and samples for analysis of nutrient contamination were taken throughout the week of June 21 – June 17, as indicated on the chart.



Figure D-3. The biofilm sampling rack, showed from above and below the water surface.



Figure D-4. Two o-DGT samplers mounted on a custom-built holder.

Supplemental tables:

Table D-1. Summary of water quality parameters measured at 21 stormwater pond sites in Brampton, Ontario.

Parameter	Units	Sampling frequency	Method
Temperature	°C	Weekly	Hach multi-probe meter
Conductivity	mS cm^{-1}	Weekly	Hach multi-probe meter
DO	mg L^{-1}	Weekly	YSI DO meter
TSS	mg L^{-1}	Weekly	Gravimetric
Cl^-	mg L^{-1}	Weekly	Colorimetric
NH_3	mg L^{-1}	Once	Fluorometric
NO_2	mg L^{-1}	Once	Ion chromatography
NO_3	mg L^{-1}	Once	Ion chromatography
PO_4	mg L^{-1}	Once	Colorimetric
Coliform bacteria	cfu/100 mL	Once	Plate counts
E. coli	cfu/100 mL	Once	Plate counts

Table D-2. Summary of water quality measurements taken at 21 stormwater ponds in Brampton, Ontario. Half of the MDL value was used to calculate the means for parameters with values under the MDL. For parameters containing observations above the MQL, the mean was not calculated.

Parameter	Range	Mean	Standard deviation
Chloride	61 mg L^{-1} – <MQL (200 mg L^{-1})	N/A	N/A
TSS	7.3 – 135.4 mg L^{-1}	38.6 mg L^{-1}	26.9 mg L^{-1}
DO	2.87 – 14.1 mg L^{-1}	9.30 mg L^{-1}	3.00 mg L^{-1}
NH_3	<MDL – 2.50 mg L^{-1}	0.162 mg L^{-1}	0.538 mg L^{-1}
NO_2	<MDL – 0.0110 mg L^{-1}	0.0272 mg L^{-1}	0.0130 mg L^{-1}
NO_3	<MDL – 0.132 mg L^{-1}	0.0553 mg L^{-1}	0.0304 mg L^{-1}
PO_4	<MDL – 0.0403 mg L^{-1}	0.00610 mg L^{-1}	0.0107 mg L^{-1}
Coliform bacteria	120 – <MQL (1.00 E5)	N/A	N/A
E. coli	30 – <MQL (1.00 E+5)	N/A	N/A

Appendix D

Supplementary information for Chapter 6

Figshare project files:

1. Site locations: 10.6084/m9.figshare.23907972
2. Site features (landscape and local variables): 10.6084/m9.figshare.23907993
3. Water quality measurements: 10.6084/m9.figshare.23907963
4. Nutrient analysis in water: 10.6084/m9.figshare.23907939
5. Bacterial contamination in water: 10.6084/m9.figshare.23907840
6. Pesticide analytes and their MDLs and MQLs in water and biofilm samples: 10.6084/m9.figshare.27629508
7. Pesticide concentrations in water and biofilm samples: 10.6084/m9.figshare.27629520
8. Raw diatom counts and relative abundances of genera: 10.6084/m9.figshare.30546383
9. Raw invertebrate counts and relative abundances of taxa: 10.6084/m9.figshare.30546398

Table E-1. Principal components analysis (PCA) axis loadings for environmental variables.

PCA	Variable	Axis 1 Loading	Axis 2 Loading
Landscape Drivers	Canopy	-0.3707	0.8903
	Impervious	0.5544	-0.0128
	Road	0.5530	0.3687
	Water	-0.4993	-0.2667
Local Drivers	DT	0.5418	0.1631
	NLE	0.4356	-0.04284
	RE	-0.2121	0.6635
	Shrub	0.5392	0.2138
	Size	0.3280	0.3587
	Vegprop	-0.2710	0.5974
Water Quality	Chl-a	0.2633	0.3430
	Chloride	0.01671	0.4571
	Coliform	0.3063	0.02413
	Conductivity	0.01180	0.5275
	CP_ratio	-0.2452	0.1511
	DO	-0.3972	0.1755
	Ecoli	0.3121	0.01787
	NH3	0.1654	-0.07595

PCA	Variable	Axis 1 Loading	Axis 2 Loading
	pH	-0.3420	0.3240
	Pheo	0.2854	0.3397
	PO4	0.3825	-0.05659
	Temp	-0.3380	0.06888
	TSS	0.1867	0.3265
Pesticides in Water	2,4-D	0.4245	-0.2769
	2,4-DP	0.06298	-0.1385
	2,4,5-T	-0.07202	-0.1364
	Bentazon	-0.2419	-0.3461
	Chlorate	0.08994	0.1413
	Clothianidin	0.01121	-0.2487
	Dicamba	0.4962	-0.09903
	Flonicamid	-0.2003	-0.4818
	Flupyradifurone	0.1142	-0.04530
	Imidacloprid	-0.07701	-0.6039
	MCPA	0.3751	0.04084
	MCPP	0.3636	-0.2681
	Triclopyr	0.4069	0.04058
Pesticides in Biofilm	2,4-D	0.2278	0.2440
	Azoxystrobin	0.5743	-0.4061
	Diuron	0.1106	-0.2823
	HEPA	0.6229	-0.2552
	Nicotine	-0.2995	-0.5828
	Tebuconazole	-0.3583	-0.5395

Table E-2. Biological metrics for periphytic diatom assemblages from 21 SWP sites. Richness represents the total number of genera identified at each site.

Site	Richness	% *Achnanthidium*	% Motile	% Dominant
10	12	9.0	13.3	73.0
14	14	45.3	37.5	45.3
26	15	62.8	31.0	62.8
46	11	32.8	57.0	50.8
49	9	15.8	72.5	56.5
51	19	18.3	65.5	41.0
56	16	3.5	73.8	28.5
58	18	60.3	21.3	60.3
78	10	77.8	8.0	77.8
85	9	12.8	42.8	39.5
96	9	35.0	6.3	35.0

106	9	79.0	13.8	79.0
109	7	86.8	12.3	86.8
113	17	9.3	74.8	57.5
116	14	18.0	40.0	28.3
130	11	57.8	14.5	57.8
153	19	3.8	81.5	34.8
156	12	35.3	13.3	39.3
174	13	61.8	27.3	61.8
188	15	9.5	47.3	29.0
191	9	64.3	2.0	64.3

Table E-3. Biological metrics for aquatic invertebrate assemblages from 21 SWP sites. Richness represents the total number of taxa identified at each site.

Site	Richness	%EPT	%Worms	%Diptera	%Dominants
10	13	0.3	0.0	1.9	56.7
14	18	9.3	2.7	11.9	60.0
26	9	0.0	10.6	9.0	52.9
46	12	3.7	5.0	39.6	39.6
49	7	0.0	0.0	55.1	55.1
51	10	3.0	0.0	20.6	29.2
56	14	0.0	1.1	5.8	62.2
58	18	15.0	6.3	22.7	22.7
78	26	6.1	24.7	12.8	15.2
85	21	0.0	4.3	12.4	32.1
96	17	2.2	4.0	2.1	47.0
106	15	0.0	0.7	12.4	38.6
109	7	0.0	0.0	20.1	71.8
113	16	1.2	2.3	10.9	55.6
116	8	0.0	0.0	43.0	42.7
130	21	0.0	7.8	4.5	38.8
153	12	0.0	0.0	16.1	35.1
156	15	0.0	31.6	2.6	31.6
174	16	8.4	0.3	26.4	31.7
188	11	0.0	6.2	1.7	65.6
191	14	0.0	9.2	72.1	69.9

Table E-4. Taxon codes for diatom and invertebrate taxa.

Diatom taxa		Invertebrate Taxa	
Code	**Name**	**Code**	**Name**
ACHN	*Achnanthidium*	AESH	Aeshnidae
AMPH	*Amphora*	AMPH	Amphipoda
CALO	*Caloneis*	ASEL	Asellidae
COCC	*Cocconeis*	BAET	Baetidae
CRAT	*Craticula*	BELO	Belostomatidae
CTEN	*Ctenophora*	CAEN	Caenidae
CYMB	*Cymbella*	CALO	Calopterygidae
CYPH	*Cyclostephanos*	CERA	Ceratopodonidae
DIAT	*Diatoma*	CHAO	Chaoboridae
ENCA	*Encyonema*	CHIR	Chironomidae
ENCS	*Encyonopsis*	COEN	Coengrionidae
ENTO	*Entomoneis*	CORD	Corduliidae
EOLI	*Eolimna*	CORI	Corixidae
EPIT	*Epithemia*	CULI	Culicidae
EUNO	*Eunotia*	DYTI	Dytiscidae
FRAG	*Fragilaria*	ELMI	Elmidae
GOMP	*Gomphonema*	GERR	Gerridae
GRUN	*Grunowia*	GOMP	Gomphidae
GYRO	*Gyrosigma*	GYRI	Gyrinidae
HALA	*Halamphora*	HALI	Haliplidae
HIPP	*Hippodonta*	HIRU	Hirudinea
LEMN	*Lemnicola*	HYCH	Hydrachnidia
NAVC	*Navicymbula*	HYPH	Hydrophilidae
NAVI	*Navicula*	HYPT	Hydroptilidae
NITZ	*Nitzschia*	LEPC	Leptoceridae
PLAN	*Planothidium*	LEPH	Leptohyphidae
PSEU	*Pseudostaurosira*	LIBE	Libellulidae
SELL	*Sellaphora*	LYMN	Lymnaeidae
STEC	*Stephanocyclus*	NEMA	Nematoda
STEP	*Stephanodiscus*	NOTE	Noteridae
SYNE	*Synedra*	NOTO	Notonectidae
TABU	*Tabularia*	OLIG	Oligochaeta
THAL	*Thalassiosira*	PHYS	Physidae
ULNA	*Ulnaria*	PLAA	Planariidae

Diatom taxa		Invertebrate Taxa	
Code	**Name**	**Code**	**Name**
		PLAO	Planorbidae
		PLEI	Pleidae
		PSYC	Psychodidae
		PYRA	Pryalidae
		SALD	Saldidae
		SCI	Scirtidae
		SMIN	Sminthuridae
		SPHA	Sphaeriidae
		STRA	Stratiomyidae
		VELL	Vellidae
		VIVI	Viviparidae

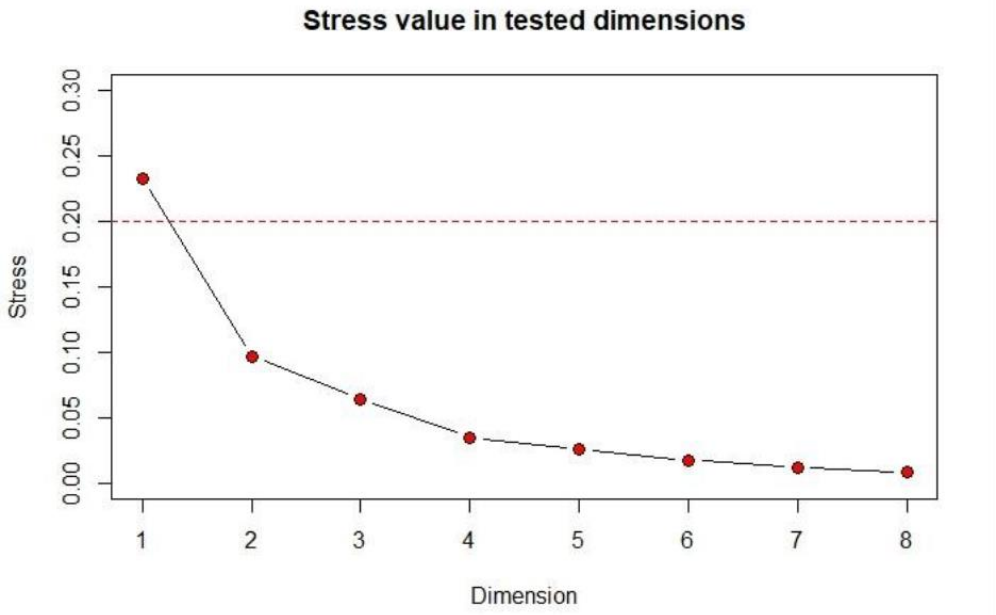


Figure E-1. Scree plot for a non-metric multidimensional scaling (NMDS) ordination with periphytic diatom assemblages.

Table E-5. The results of non-metric multidimensional scaling (NMDS) ordination with periphytic diatom and aquatic invertebrate assemblages.

NMDS	Dimensions	Stress	# of iterations	Variance explained	
Diatoms	2	0.098	100	NMDS1	0.7305
				NMDS2	0.2695
Invertebrates	3	0.089	100	NMDS1	0.4276
				NMDS2	0.3526
				NMDS3	0.2198

Table E-6. Correlations between environmental variables (vectors) and axes of a non-metric multidimensional scaling (NMDS) ordination of periphytic diatom assemblages. Asterisks denote vectors included in the NMDS ordination.

Vector	NMDS1	NMDS2	R^2	p-value
Developed Cover	0.49217	0.87050	0.0531	0.624
Canopy Cover	0.14209	-0.98985	0.0372	0.710
Naturalized Basin	0.33263	-0.94306	0.0045	0.959
Reed and Cattail Cover	-0.99862	0.05254	0.0773	0.480
Nutrient Pollution	0.14440	0.98952	0.2057	0.130*
Road Runoff	-0.56269	0.82667	0.4362	0.011*
Synthetic Auxins	-0.09064	-0.99588	0.4081	0.010*
Non-Point Source Pesticides	-0.84063	-0.54161	0.0968	0.413
Increasing 2,4-D	0.82778	0.56106	0.0468	0.665

Table E-7. Correlations between biological metrics (vectors) and axes of a non-metric multidimensional scaling (NMDS) ordination of periphytic diatom assemblages. Asterisks denote vectors included in the NMDS ordination.

Metric	NMDS1	NMDS2	R^2	p-value
Richness	-0.6226	0.7825	0.3310	0.021*
%Achn	0.9831	0.1832	0.9233	0.001*
%Motile	-0.7995	0.6006	0.8421	0.001*
%dominant	0.9642	-0.2654	0.4441	0.009*

Table E-8. Results of simple regression analysis between PC axis scores (representing environmental gradients) and biological metrics calculated from diatom assemblages. Asterisks denotes significant relationships ($p < 0.05$).

Biological metric	Coefficient	Std. error	t-value	Adjusted R^2	F-stat (1 and 19 DF)	*p*-value
Predictor variable: *Road Runoff*						
Richness	0.5348	0.4622	1.157	0.01665	1.339	0.2616
% *Achnanthidium*	-7.270	3.216	-2.261	0.1705	5.111	0.03570*
% motile	8.164	2.851	2.864	0.2647	8.201	0.009938*
% dominant	-3.023	2.215	-1.365	0.04137	1.863	0.1882
Predictor variable: *Synthetic Auxins*						
Richness	-0.1338	0.4499	-0.2970	-0.04775	0.08844	0.7694
% *Achnanthidium*	-3.932	3.295	-1.193	0.02077	1.424	0.2474
% motile	-2.925	3.146	-0.930	-0.00681	0.8647	0.3641
% dominant	-0.007734	2.1883	-0.004	-0.05263	1.249e-5	0.9972
Predictor variable: *Nutrient Pollution*						
Richness	0.1456	0.4106	0.355	-0.04571	0.1257	0.7268
% *Achnanthidium*	2.116	3.082	0.687	-0.02715	0.4714	0.5006
% motile	0.7067	2.9338	0.241	-0.04943	0.05802	0.8122
% dominant	-0.7842	1.9909	-0.394	-0.0441	0.1552	0.698

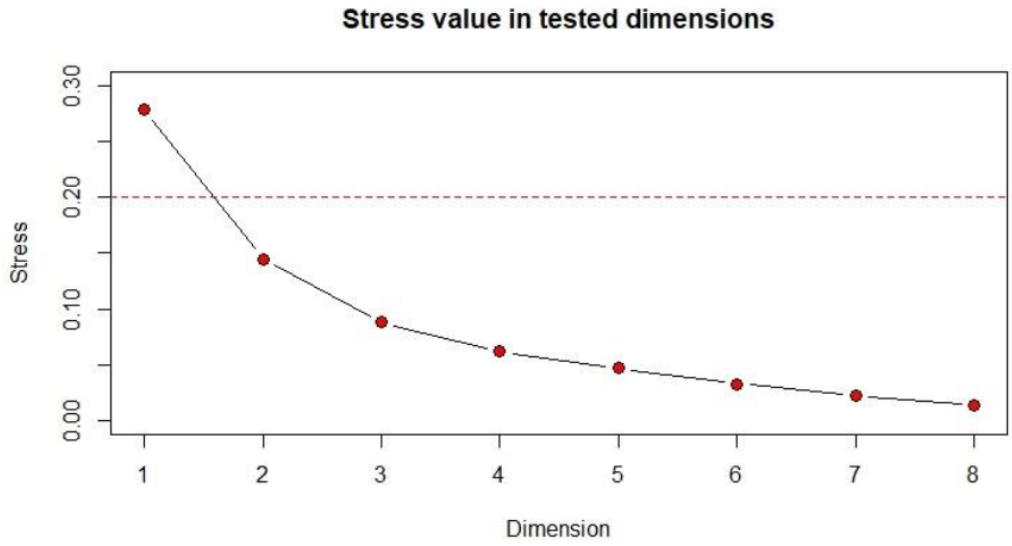


Figure E-2. Scree plot for a non-metric multidimensional scaling (NMDS) ordination with aquatic macroinvertebrate assemblages.

Table E-9. Correlations between environmental variables (vectors) and axes of a non-metric multidimensional scaling (NMDS) ordination of aquatic macroinvertebrate assemblages. Asterisks denote vectors included in the NMDS ordination.

Vector	NMDS1	NMDS2	NMDS3	R^2	p-value
Developed Cover	0.28493	-0.39597	0.87294	0.1969	0.281
Canopy Cover	0.30529	0.75416	0.58142	0.2408	0.223
Naturalized Basin	0.13814	0.67985	0.72022	0.4430	0.024*
Reed and Cattail Cover	-0.00635	-0.16119	0.98690	0.1102	0.555
Nutrient Pollution	0.15006	-0.96748	-0.20364	0.3152	0.090*
Road Runoff	-0.63275	0.13474	-0.76255	0.3657	0.051*
Synthetic Auxins	-0.14352	-0.39570	0.90710	0.0452	0.835
Non-Point Source Pesticides	-0.07081	-0.32695	-0.94238	0.0786	0.697
Increasing 2,4-D	0.38020	-0.21130	0.90044	0.2009	0.255

Table E-10. Correlations between biological metrics (vectors) and axes of a non-metric multidimensional scaling (NMDS) ordination of aquatic macroinvertebrate assemblages. Asterisks denote vectors included in the NMDS ordination.

Metric	NMDS1	NMDS2	NMDS3	R^2	*p*-value
Richness	0.7657	-0.2067	0.6092	0.2544	0.154*
%EPT	0.04549	0.5968	0.8011	0.6196	0.006*
%worms	0.7358	0.1587	0.6583	0.4421	0.030*
%diptera	0.5800	0.8138	-0.03606	0.3525	0.061*
%dominant	-0.6401	-0.7683	0.005110	0.08490	0.688

Table E-11. Results of simple regression analysis between PC axis scores (representing environmental gradients) and biological metrics calculated from aquatic macroinvertebrate assemblages. Asterisks denotes significant relationships ($p < 0.05$).

Biological metric	Coefficient	Std. error	t-value	Adjusted R^2	F-stat (1 and 19 DF)	*p*-value
Predictor variable: Local_PC1 (Naturalized Basin)						
Richness	0.1404	0.6948	0.202	-0.05037	0.04086	0.842
% EPT	1.2374	0.5021	2.465	0.2024	6.074	0.02342*

% worms	-0.1431	1.1689	-0.122	-0.0518	0.01499	0.9038
% diptera	1.198	2.644	0.453	-0.04137	0.2054	0.6555
% dominant	0.322	2.249	0.143	-0.0515	0.02051	0.8876
Predictor variable: WaterChem_PC1 (Nutrient Pollution)						
Richness	-0.1054	0.5555	-0.19	-0.05064	0.036	0.8515
% EPT	-0.4821	0.4477	-1.077	0.007927	1.160	0.2950
% worms	0.05737	0.9348	0.061	-0.05242	0.003766	0.9517
% diptera	-0.3774	2.1236	-0.178	-0.05088	0.03158	0.8608
% dominant	-0.03618	1.799	-0.02	-0.05261	0.0004047	0.9842
Predictor variable: WaterChem_PC2 (Road Runoff)						
Richness	-1.7235	0.5102	-3.378	0.3423	11.41	0.003159*
% EPT	-0.6687	0.5129	-1.304	0.03382	1.70	0.2079
% worms	-1.996	0.984	-2.028	0.1347	4.114	0.05678
% diptera	1.761	2.434	0.723	-0.02442	0.5233	0.4782
% dominant	3.482	1.929	1.805	0.1014	3.257	0.087

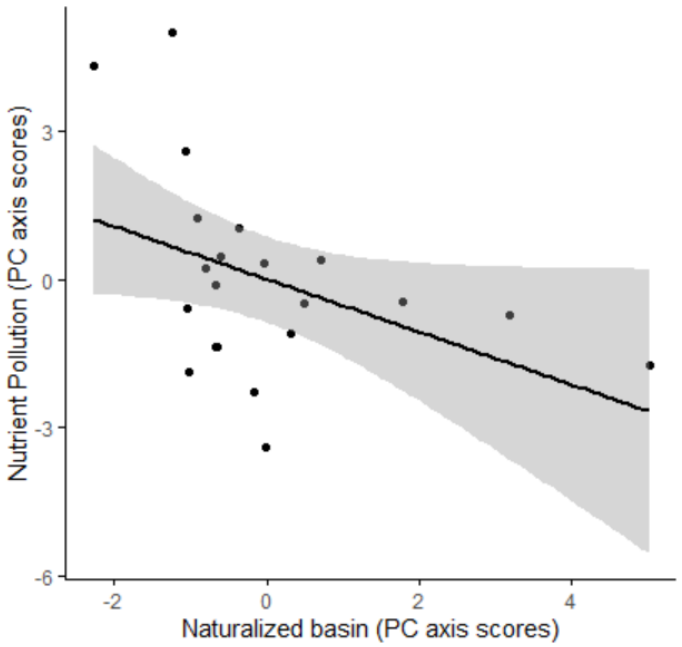


Figure E-3. Relationship between *Naturalized Basin* and *Nutrient Pollution* PC axis scores (p = 0.055).

Appendix E
Insights into improving the biofilm sampling method

Background

One of the most important limiting factors for the chemical analysis of biofilm is the amount of dry mass required for submission. For measuring pesticides, this requires 3-5 g (dry weight) per analysis screen, thus our target submission was ~15 g for the three analytical screens used. Biofilms are mostly water (~90-99%), so obtaining 15 g dry weight requires we harvest a substantial amount of biofilm from the field. To reasonably recommend the implementation of biofilm sampling into pesticide monitoring programs, I felt it was necessary to contribute to its practicality by exploring how different approaches affect total biomass available for harvest.

Reasoning

In 2023, I conducted field work locally in three SWPs located on campus at the University of Waterloo. My main objective was to test various biofilm sampling methods in order to optimize an approach for maximizing biofilm mass harvested. The two main parameters I aimed to test were 1) deployment duration, and 2) the orientation of the substrates. In my experience, biofilms typically take a week or two after deployment for initial colonization and four to six weeks for substantial growth on the artificial substrates. Longer deployments could result in more biomass, but lower the practicality and may also increase the risk of vandalism or theft. The main question I ask here is: how long do biofilm samplers need to be deployed to obtain adequate biomass for chemical analysis? I define adequate biomass as 15 g dry weight per sampling rack (containing 10 acrylic plates). Substrate orientation was also an important consideration, since vertically-oriented substrates are more space-efficient, yet receive less direct sunlight than the top side of horizontally-oriented substrates. Similarly, I asked: Which orientation maximizes biomass production?

My approach was mainly exploratory and anecdotal, and I did not attempt to statistically analyze the results. This work is simply intended to provide some insight into optimal sampler design, and can provide context for future research questions. Future research wishing to test various sampler designs should set up a minimum of 10 test sites, with multiple duplicate sampling racks in each, in order to draw statistically-sound conclusions.

Methods

I sampled three SWPs for 10 weeks (Figure F-1). These were: Day Care Pond (DC), situated near the day care; Open Text Pond (OT), situated near the Open Text building; and Claire's Pond (CP), situated near my lab mate's residence on campus.

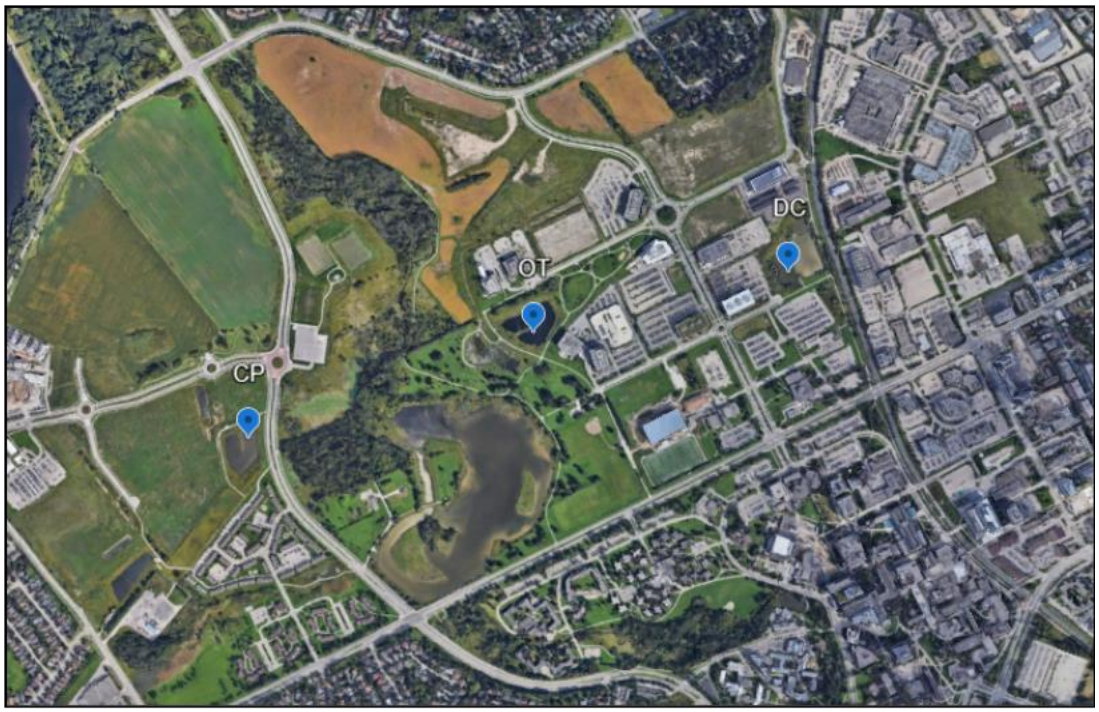


Figure F-1. Map of the three SWP sites on University of Waterloo campus. Image made from Google Earth (2025).

At each SWP, I deployed two different sampling racks. Rack A had 10 acrylic plates suspended vertically, similar to the design used in the Brampton SWPs the year prior (Figure F-2). I used Rack A to explore the effect of deployment duration on the amount of biomass collected. I collected biofilm from two acrylic slides every two weeks (i.e., Week 2, Week 4, Week 6, Week 8, and Week 10) and harvested the biofilm grown on both sides of the two plates into a sample representative of that deployment time. This totaled five samples per SWP site, which were kept frozen until the end of the survey period.



Figure F-2. Biofilm sampler Rack A – in which all acrylic plates (n = 10) were suspended vertically. Two plates were harvested for biofilm every two weeks.

I used another rack type, Rack B, to compare the orientation of acrylic plates (Figure F-3). Rack B had two acrylic plates positioned vertically and two positioned horizontally, both suspended in the water column at a depth of about 10 cm below the surface. After 6 weeks of deployment, I harvested the biofilm from all four plates, separating the samples by orientation for a total of two samples per site.



Figure F-3. Biofilm sampler Rack B – in which two acrylic plates were positioned to sit vertically, and two were positioned to sit horizontally in the water column. Biofilm was harvested after 6 weeks of deployment.

Following the survey period, I homogenized and separated each biofilm sample into three subsamples. I measured the total wet weight and dry weight for each subsample to calculate average biomass values per acrylic plate for each pond. I calculated the ratio of wet biofilm to dry biofilm for each sample in order to estimate the total amount of dry mass harvested from each sampling time on each sampler rack.

Findings

Rack A - Deployment duration

The amount of biomass harvested at each sampling point was variable across sites (Figure F-4). The DC pond likely provided ideal conditions for biofilm growth, promoting rapid colonization and biomass production throughout the survey period. After only two weeks, nearly 3 g (dry weight) of biomass per plate was produced; extrapolating to the entire sampling rack (~30 g), this would far exceed the minimum

requirements for sample submission for chemical analysis (15 g dry weight). The amount of biomass rose substantially each sampling time, peaking at 8 weeks, after which it fell back to levels similar to week 6.

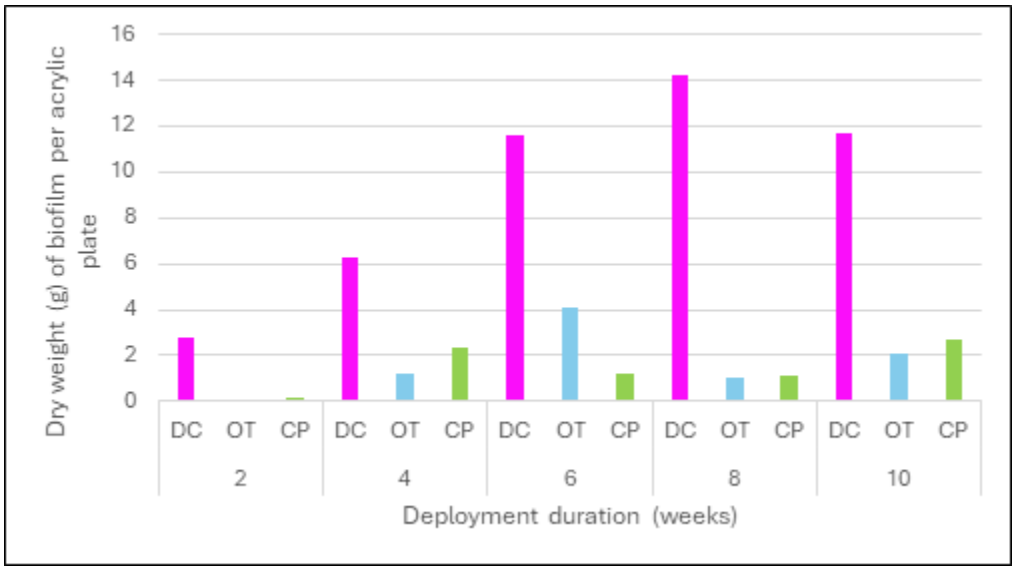


Figure F-4. Dry weight (g) of biofilm harvested per acrylic plate deployed in SWPs across a 10-week survey period.

The OT and CP ponds showed a more variable growth pattern. Biomass from the OT pond peaked at week 6, only surpassing 2 g (dry weight) for that sole week. Biomass from the CP site peaked at week 10 but showed a bimodal growth pattern. This pattern might be the result of biofilm sloughing, a "boom/bust" dynamic where biofilm builds, sloughs off, and then regrows, which can occur at random or because of sudden changes in the surrounding environment (e.g., dramatic oxygen depletion; Brading et al., 1995)). Both the OT and CP sites had increases in biomass in the last week of sampling, compared to the week before. These variable growth patterns suggest that biomass production in these ponds may be dependent on limiting factors, such as nutrient availability, which may have dynamic and sporadic occurrences and levels in the ponds.

Biomass from the OT and CP sites only met the mass requirements for a hypothetical submission for chemical analysis (15 g dry weight total, or 1.5 g per plate) on week 10. Thus, a conservative approach would deploy the sampling racks for a minimum of 10 weeks to ensure even ponds with slow-

growing biofilms can still be sampled and analyzed. A longer sampling time may not be as practical, but it also sheds more light on exposures in a wider temporal range.

Rack B - Substrate orientation

Average dry mass per acrylic plate was higher on vertically-oriented acrylic plates compared to horizontal at the DC and OT ponds, and was roughly equal in both orientations at the CP site (Figure F-5). Although there was relatively good growth on the top side of the horizontally-oriented plates, likely due to the even access to direct sunlight, the bottom side of the plates were often sparsely covered. The vertical plates tended to have more even growth on both sides of the plates. Since vertical plates are more spatially efficient, and seem to result in either higher or similar levels of biomass compared to horizontal plates, I would recommend the use of vertical plates in biofilm sampler racks.

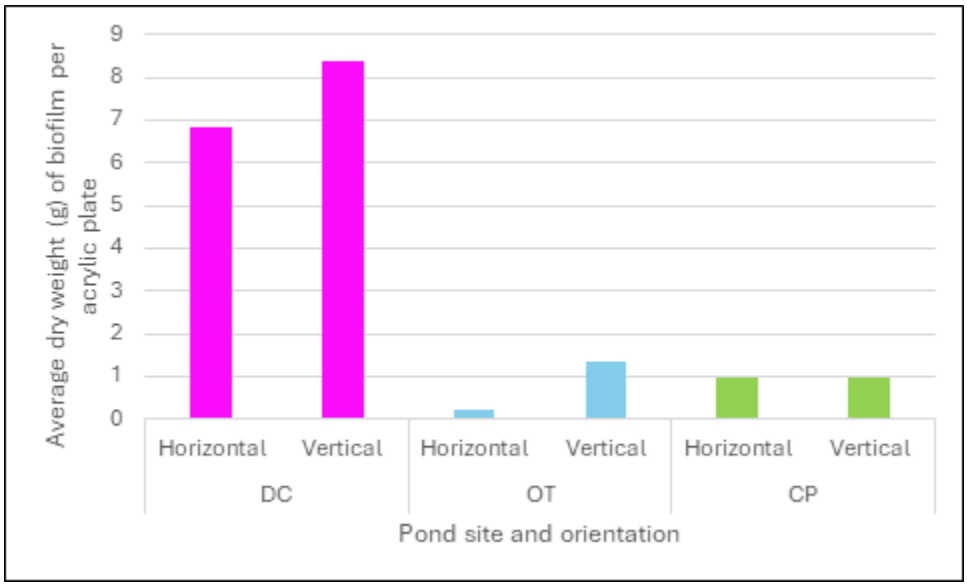


Figure F-5. Dry weight (g) of biofilm harvested from either horizontally- or vertically-oriented acrylic plates.