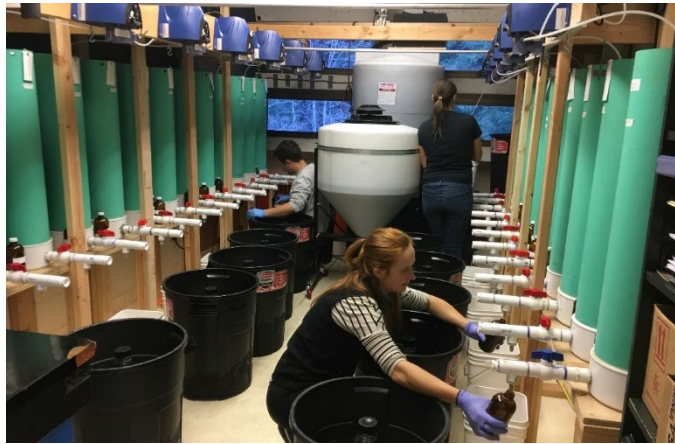

Testing Removal of 6PPDQ and Coho Salmon Lethality by High- performance Bioretention Media Blends: Final King County Report



May 2025



King County

Department of Natural Resources and Parks
Water and Land Resources Division
Science and Technical Support Section
King Street Center, KSC-NR-5600
201 South Jackson Street, Suite 5600
Seattle, WA 98104
206-477-4800 TTY Relay: 711
www.kingcounty.gov/EnvironmentalScience

Alternate Formats Available
206-477-4800 TTY Relay: 711

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Citation

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Executive Summary

In 2020, a team of researchers in Washington answered a two-decade-long question plaguing environmental managers in the Puget Sound region—why are coho salmon dying before they can spawn in urban creeks? Their discovery was a previously unknown chemical derived from tire rubber called 6PPD-quinone (6PPDQ) that makes its way into streams via stormwater runoff. 6PPDQ is a transformation product of the antiozonant 6PPD, which has been used in virtually all car tires in the U.S. since the 1960s. 6PPDQ can kill half of a test population of coho salmon at concentrations as low as 41 parts per trillion in just a few hours and is also acutely toxic to rainbow trout and brook trout, with median lethal concentrations that have been measured in some surface waters.

The extreme toxicity of 6PPDQ to salmon has spurred an urgent regional need to identify stormwater treatment technologies that remove 6PPDQ and protect salmon from acutely toxic stormwater. Bioretention is expected to be one of the best treatments for 6PPDQ because of its proven effectiveness for protecting coho salmon from toxic stormwater and its flexibility as a stormwater best management practice (BMP). The purpose of this study was to determine the 6PPDQ treatment effectiveness of three configurations of high performance bioretention soil mixes (HPBSMs) that are newly adopted by the Washington State Department of Ecology (Ecology) and King County and compare these to the default sand and compost-based bioretention soil mix (BSM) widely used in Washington. This is the first study to quantify 6PPDQ treatment by bioretention soil mixes used in Washington State. The major findings of this study are:

- All tested HPBSM configurations and the default BSM completely protected juvenile coho salmon from acutely toxic stormwater.
- While all tested media reduced 6PPDQ concentrations by at least 10-fold, the HPBSMs provided small, significant improvements in 6PPDQ treatment.
- Stormwater filtered through the HPBSMs always had 6PPDQ concentrations below Ecology's adopted acute aquatic life criteria for 6PPDQ of 12 ng/L (12 parts per trillion). This was not the case for the default soil mix.

Bioretention has not been previously allowed for stormwater quality treatment in King County's Surface Water Design Manual (2024) because of concerns over leaching of nutrients and metals from the compost in the default BSM. HPBSM was adopted by Ecology in 2021. Informed by the research reported here, King County added bioretention designs using HPBSM for water quality treatment and flow control BMPs in 2024. The adoption of HPBSM expands the use of bioretention in King County, allowing use of an effective 6PPDQ treatment as well as use for basic (i.e., solids), metals, and phosphorus treatments without the nutrient and metals leaching associated with compost.

1 Introduction

Bioretention is a widely used and flexible stormwater treatment practice used for managing the volume and to some extent the water quality of stormwater. It consists of a shallow depression in the landscape where stormwater is collected and filtered through engineered soil mixtures that infiltrate water, diverting some portion of runoff from the stormwater conveyance network. Washington State Department of Ecology (Ecology) has two specifications for non-compost-based bioretention media in Western Washington: 60:40 BSM and HPBSM.

King County's Surface Water Design Manual (SWDM) (2024) currently allows the use of bioretention as a flow-control Best Management Practice (BMP). However, bioretention that is under-drained and diverts water back into the stormwater conveyance network was previously not allowed as a water quality treatment in King County because of the potential for compost in the bioretention media to act as a pollution source. Pollutant export is undesirable, but its net effect is particularly problematic where nutrient and metals loading in stormwater runoff is relatively low, such as in the less-urbanized areas of unincorporated King County. In this situation, the nutrients and metals exported from compost can result in negative treatment effectiveness or increased pollutant concentrations in effluent relative to influent. Where stormwater influent is more contaminated, the reduction in metals and nutrients from treatment can outweigh a smaller total export from compost. Ideally, a treatment option is not a source of any pollutant export.

Ecology's default specification—60:40 BSM—widely used for decades, is a mixture of 60 percent sand and 40 percent compost by volume. While the standard 60:40 BSM effectively infiltrates stormwater, removes select contaminants (e.g., suspended solids, hydrocarbons), and supports plants, it has also been implicated as a source of nutrient and metals pollution.

Ecology issued guidance in 2013 documenting the release of nitrogen, phosphorus, and dissolved copper from the 60:40 BSM, and recommended this media not be used in areas with phosphorus-sensitive receiving waters. In 2016 Ecology updated this guidance, recommending that the 60:40 BSM should not be used within one quarter mile of phosphorus-sensitive receiving waters (Ecology 2016). In 2019, Ecology updated the stormwater manual to reflect this guidance.

In 2020, the City of Redmond, King County, Herrera Environmental Consultants, and several other jurisdictions and researchers completed a nearly 10-year effort to design a high performance bioretention soil mix (HPBSM) that meets Ecology criteria for 1) basic treatment (i.e., solids), 2) enhanced treatment (i.e., dissolved copper and zinc), and 3) phosphorus treatment (Herrera Environmental Consultants 2020). Ecology published the specifications for the HPBSM configurations tested in this study (Ecology 2021), which are included in the 2021 King County SWDM as amended in 2024 (King County 2024), as flow control BMPs and water quality treatment options.

Bioretention is the best-studied treatment to date for mitigating 6PPD-quinone (6PPDQ) pollution in stormwater. Researchers identified 6PPDQ as the cause of coho salmon Urban Runoff Mortality Syndrome (URMS) in 2020 (Tian et al. 2021), although stormwater runoff was implicated in killing returning coho salmon spawners in Western Washington decades ago (Scholz et al. 2011). However, even before the discovery of 6PPDQ, studies showed that passing stormwater through a simple sand and compost mixture protected coho salmon from its toxic effects (McIntyre et al. 2015; Spromberg et al. 2016). Shortly after its discovery, Ecology released a report that synthesized current knowledge on 6PPDQ's physicochemical properties, fate and transport, and sources to suggest which stormwater BMPs would be expected to remove 6PPD and 6PPDQ from stormwater runoff (Navickis-Brasch et al. 2022). This report ranked BMPs that promote dispersion, infiltration, biofiltration, or sorption as having the highest 6PPDQ treatment potential. BMPs utilizing bioretention media were among the highest ranked for removing 6PPD and 6PPDQ because the physicochemical properties of these chemicals (i.e., moderately non-polar, high log K_{ow} and K_{oc}) suggested that these chemicals would be likely to attach (i.e., sorb) to organic matter and particles.

In response to the discovery of 6PPDQ, King County conducted the study described in this report to determine whether this new HPBSM would provide effective 6PPDQ treatment and protect coho salmon from the toxic effects of stormwater runoff. Three configurations (i.e., types) of HPBSM and the 60:40 BSM were tested in a laboratory setting during three simulated storm events. This report describes the pre-trial tests of the tested bioretention medias, the dosing of bioretention test columns with stormwater, and the 6PPDQ concentrations, conventional water quality parameters, and impacts to juvenile coho salmon associated with untreated and bioretention-treated waters.

1.1 Project Goals and Study Questions

1.1.1 Project Goals

This project aimed to study the relative effectiveness of the three different Ecology-approved configurations of the HPBSM in reducing concentrations of 6PPDQ in stormwater (Ecology 2021) and thereby reducing risk of URMS in fresh waters within King County. The 6PPDQ treatment effectiveness of HPBSMs was compared to 60:40 BSM.

Researchers defined the following goals in the quality assurance project plan (QAPP) for this study (King County 2023):

The **primary goal** of this bench-scale study was to determine the extent to which the three HPBSM configurations reduce the concentration of 6PPDQ in stormwater to below levels toxic to coho salmon, eliminate coho salmon toxicity (via any protective water quality characteristics [see secondary goal]), or both; and if any of the HPBSMs perform better or worse at this function than 60:40 BSM. This project includes both chemical analysis of treated and untreated stormwater for 6PPDQ and direct toxicity tests with juvenile coho salmon.

In addition, a **secondary goal** of the study was to identify and measure stormwater constituents and conditions (e.g., pH, dissolved oxygen) that may be dynamic in stormwater and may also be affected by these approved bioretention media types. We evaluated how common water quality characteristics change during each cycle in the experiment, and the degree to which they change due to passing through the BSM columns.

Addressing the **secondary goal** included measuring dissolved organic carbon (DOC) and total suspended solids (TSS) in untreated and treated stormwater effluents to evaluate whether these parameters affected the outcome of toxicity tests, for example through binding 6PPDQ.

2 Methods

2.1 Study Design Overview

This study consisted of multiple types of sampling activities and tests to 1) prepare and test the bioretention materials for potential leachable pollutants, 2) measure 6PPDQ treatment effectiveness of the media mixes, and 3) evaluate impacts of other water quality parameters and storage and transport on 6PPDQ concentrations and toxicity. Following is an overview of the study design and purposes of each activity:

1. Testing medias for leachable pollutants
 - a. Column flushing
 - i. Measure whether the media export nutrients, metals, or 6PPDQ when flushed with clean water to determine if they are a source of these pollutants.
 - b. Fathead minnow tests
 - i. Measure whether metals documented to be exported from 60:40 BSM at levels above Washington Aquatic Life Criteria are sufficient to cause toxicity in a standard, acute fish (fathead minnow) toxicity test. Only 60:40 BSM was evaluated here because the HPBSM materials had previously been demonstrated to not leach metals.
 - ii. To ensure any toxicity observed in toxicity tests was from contaminants originating from stormwater and not from bioretention media.
2. Dosing columns and sampling
 - a. Dose columns with stormwater, and sample to measure effectiveness in terms of 6PPDQ removal and toxicity reduction.
 - i. These tasks directly support the study goal to evaluate effectiveness of the HPBSMs at removing 6PPDQ and preventing toxicity to coho salmon.
 - b. Dose columns with other, easier to obtain stormwater to simulate aging (1 water year).
 - i. Aging the columns with stormwater helps increase the realism of the treatment effectiveness testing by simulating the pollutant loading and wetting associated with real-life stormwater BMPs.

Water from a stormwater wet pond in Bellingham was used instead of the I-5 runoff used in the dosing with sampling events because it was available close to the location of the columns and was much easier to obtain.

3. Evaluate impacts of water parameters, storage, and transport on 6PPDQ.
 - a. Measure water quality parameters at different timepoints in each dosing cycle (from stormwater source, after compositing into influent, after treatment, and during toxicity tests).
 - i. TSS, DOC, redox potential, conductivity, and pH were all measured alongside 6PPDQ in water samples as potential explanatory variables that could potentially impact 6PPDQ bioavailability and toxicity.

2.1.1 Tested Bioretention Mixes

This study consisted of bench-scale soil column tests of three HPBSM types and the 60:40 BSM (Figure 1). The following specific bioretention media types have been approved for use by Ecology and were tested in this study for 6PPDQ removal (percentages are by volume):

1. **Type 1:** 18-inch HPBSM primary layer consisting of: 70% sand, 20% coir, 10% biochar, plus a 12-inch drainage layer of sand.
2. **Type 2:** 18-inch HPBSM primary layer plus 12-inch polishing layer. The polishing layer consists of 90% sand, 7.5% activated alumina, and 2.5% iron aggregate.
3. **Type 3:** Type 2 HPBSM, plus 2-inch compost surface layer meeting Ecology's bioretention compost specifications.
4. **60:40 BSM:** 60% sand/40% compost.

In the Ecology guidance (Ecology 2024), the biochar component is called *high carbon wood ash* (HCWA). In this document, we use the term *biochar* instead because HCWA is a type of biochar.

We eliminated one of the four BSM types when testing effectiveness in reducing or eliminating acute lethality to juvenile coho salmon in controlled laboratory toxicity tests due to practical space constraints (Type 2). There are several reasons we elected to eliminate HPBSM 2:

- The unique treatment components of the HPBSM are in the primary layer and the polishing layer. Type 1 is needed to test the primary layer alone. Types 2 and 3 contain both layers.
- The only distinction between HPBSM Type 2 and Type 3 is that the latter contains a compost mulch layer on top. The compost in Type 3 is only added to support plant aesthetics, if needed, not for additional treatment.
- Prior research told us the compost layer of Type 3 would release additional pollutants (nutrients and metals) to stormwater whereas that additional pollutant load would not be tested using Type 2.

- We expected to learn more when testing Type 3 instead of Type 2.

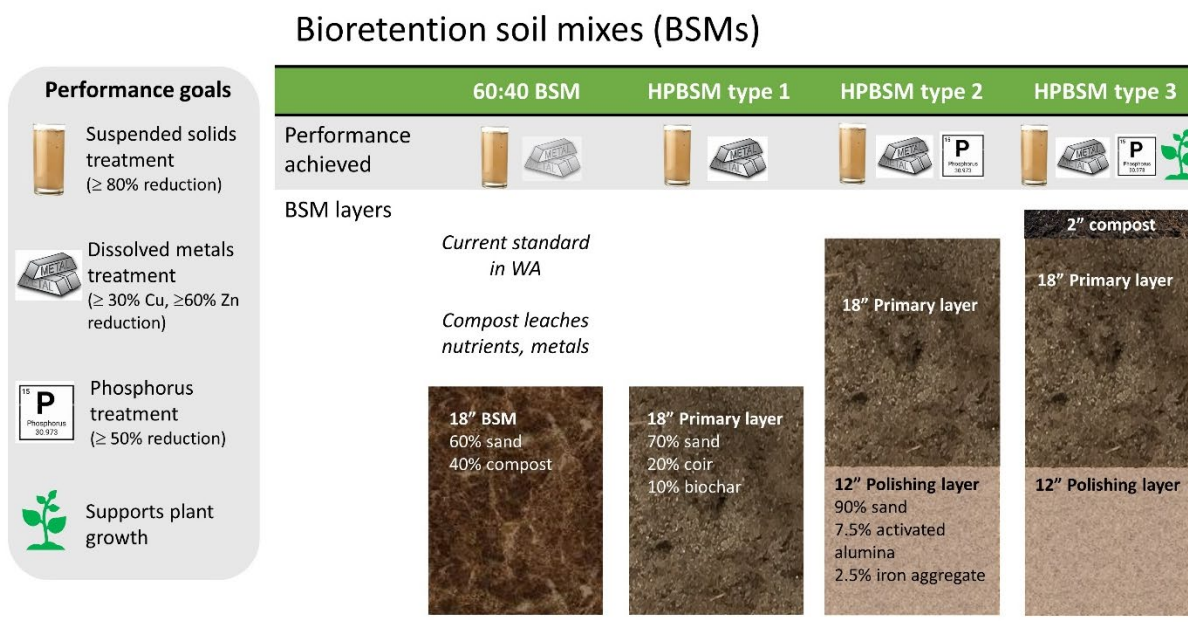


Figure 1. Bioretention mixes tested in this laboratory study include Washington’s default 60:40 BSM and three configurations of the high performance bioretention soil media. Ecology’s performance goals (Ecology 2021) achieved by each mix is displayed above the media components.

2.2 Bioretention Column Construction

The bioretention media column array (Figure 2) used to test the HPBSMs and 60:40 BSM was located in a laboratory at Western Washington University’s (WWU) Institute for Environmental Toxicology in Bellingham. The array consisted of a High-Density Polyethylene (HDPE) mixing tank (200-gallon cone bottom), HDPE distribution tank (20-gallon cone bottom), polyvinyl chloride (PVC) distribution manifold, Teflon™ delivery lines (1/8 in ID), peristaltic pumps (Pulsafeeder Chem-tech XP Series), and PVC columns (20.3 cm [8 in] diameter by 91.4 cm [36 in] tall). To prepare the media columns, old media from previous studies was removed. Columns were then washed with potable water, scrubbed, washed with Liquinox soap, and rinsed with deionized water. Each column was inspected for leaks or other defects.

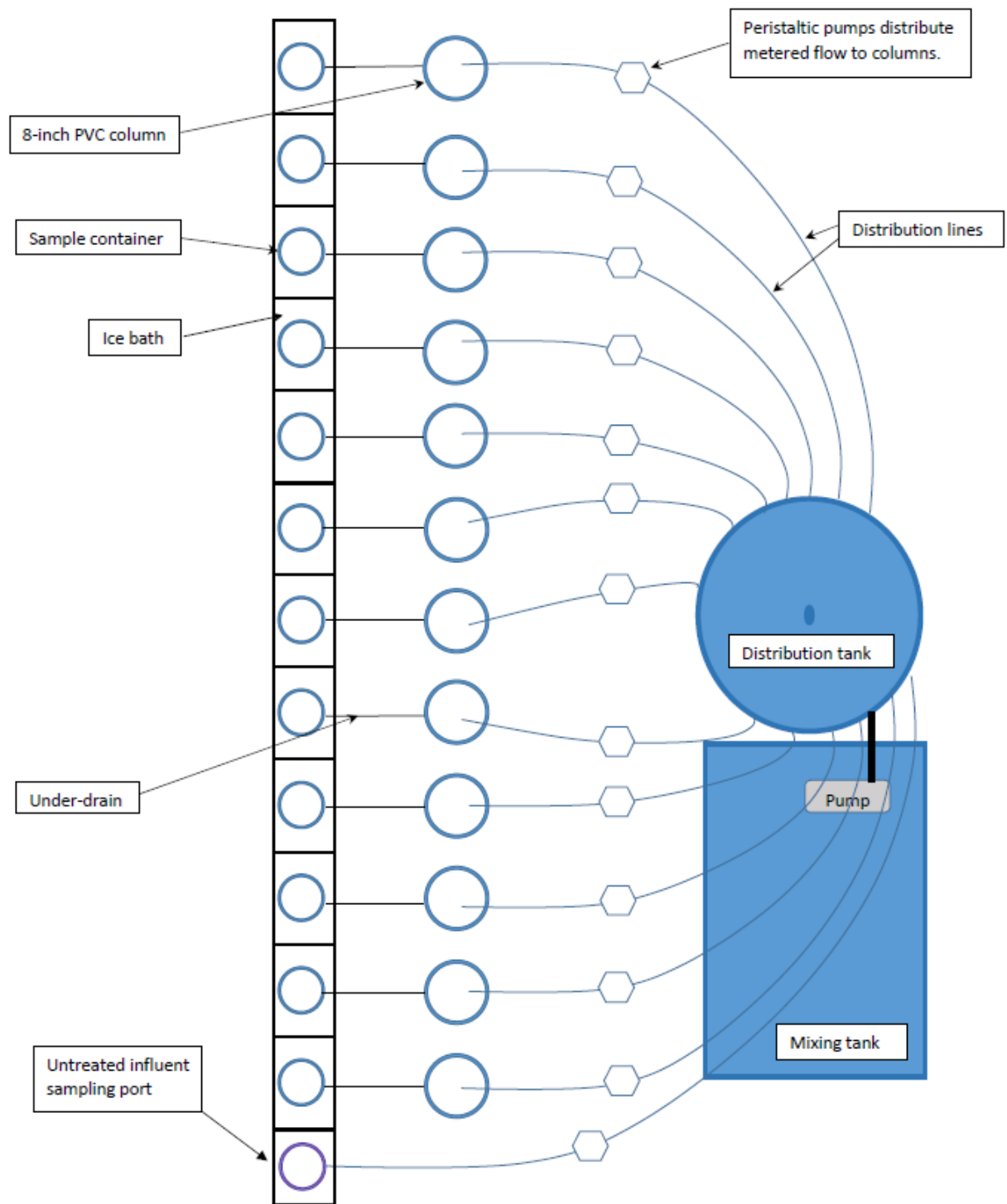


Figure 2. Schematic of bioretention column array from QAPP (King County 2023).

The mixing and distribution tanks were pressure washed to remove residue from previous experiments then washed with Liquinox and rinsed with deionized water. The distribution manifold was disassembled, washed with potable water and Liquinox, and rinsed with deionized water. All delivery lines from the distribution manifold to peristaltic

pumps were replaced with new Teflon tubing. Finally, each peristaltic pump was cleaned, lubricated, and calibrated. Maximum error recorded for peristaltic pumps was +3.84 percent and maximum spread of error was +3.84 to -3.57 percent at operating volume. See Peristaltic Pump Accuracy Checks and Calibration spreadsheet for pump accuracy.

2.2.1 Measuring Loss to and Release from Equipment

6PPDQ is known to adhere to plastics and silicone via sorption because of its moderate hydrophobicity (Hu et al. 2023). To determine the potential for 6PPDQ in stormwater influent to be lost to equipment, 6PPDQ-spiked water was rinsed through the column array. First, a sample of deionized water was spiked with 6PPDQ. We then rinsed this water through a single column of the bioretention column array before any soil media was loaded, and captured the rinsate/effluent in a glass container. Two 0.250 L samples were collected and analyzed for 6PPDQ: the influent and the effluent. Loss to equipment was calculated using 6PPDQ concentrations via the following equation:

$$\text{Loss-to-equipment} = [(6\text{PPDQ}_{\text{influent}} - 6\text{PPDQ}_{\text{effluent}})/6\text{PPDQ}_{\text{influent}}] * 100\%$$

Following the loss-to-equipment test, four equipment rinsate blanks were collected to determine whether 6PPDQ could be released from the columns used in the loss-to-equipment test or from unused columns or sampling containers:

1. The column used in the loss-to-equipment test underwent rinsing with deionized water, followed by collection of a rinsate blank.
2. An additional rinsate blank was performed on each of three columns not used in the loss-to-equipment test.
3. A rinsate blank was also collected of the fluorinated high-density polyethylene (FLDE) 20-L carboys to be used in transporting stormwater and treated effluent samples.

2.3 Bioretention Column Media Preparation and Testing

Once the column array was tested and met requirements described in the Quality Assurance Project Plan (King County 2023), the columns were packed with media. We first tested the quality of the media components using the Synthetic Precipitation Leaching Procedure (USEPA 1994) described in Department of Ecology's *Guidance on using new high performance bioretention soil mixes*, May 2021 (Ecology 2021) for total and dissolved copper, nitrate/nitrite-N, orthophosphorus (ortho-P), and total phosphorus Table 1. Researchers used consistent measurement (by volume) and packing methods on all columns. Individual media components were measured in graduated containers and the same vibration method was used to attain specified volumes. The components were placed in large plastic containers and mixed thoroughly to create specific blends. The media blends were placed in the columns and compacted using a graduated plunger every six inches. This method was used to approximate typical field compaction rates of 80 percent by penetrometer.

The media in the columns were then flushed with deionized water. The purpose of this test was to determine if specific contaminants (TSS, dissolved and total metals, and

6PPDQ) were leachable from the tested media, which would indicate the media could be a source of those contaminants. We flushed all 12 columns (four treatments replicated three times) with the equivalent of one Seattle water year of deionized water (378 liters/column). Fourteen flushing events at 27 liters per column per event were conducted over a 2-week period. See Section 2.4 for justification of this hydraulic loading rate. One-liter samples were collected from the primary collection container (24-liter glass carboys) at the first and last flushing events. 6PPDQ concentrations were only measured in the final flush effluents because we did not anticipate the media to export 6PPDQ but wanted to ensure no export before starting the experiment dosing with stormwater.

Table 1. Parameters measured and associated methods used in this study.

Parameter	Method	Laboratory
6PPDQ	KCEL SOP# 4077v0	KCEL
TSS	SM 2540-D	KCEL
DOC	SM 5310-B	KCEL
Dissolved metals	EPA 200.8	Exact Scientific
Total metals	EPA 200.8	Exact Scientific
Oxidation-reduction potential (ORP/redox)	Hanna H198190 user manual	KCEL
Specific conductance	KCEL SOP #2045v1	KCEL
pH	KCEL SOP #2045v1	KCEL
Temperature	KCEL SOP #2045v1	KCEL
Dissolved oxygen	KCEL SOP #2045v1	KCEL
SPLP - total and dissolved copper	EPA 200.8 UCT KED	ARI
SPLP - nitrate/nitrite-N	EPA 300.0	ARI
SPLP - total phosphorus, ortho-phosphorus	SM 4500-P E-99	ARI

2.4 Hydraulic Loading Rate

The same hydraulic loading rate was applied for all column dosing (i.e., flushing deionized water, I-5 stormwater, and Bellingham aging stormwater). Researchers dosed each column with 27 liters of stormwater over a 2.5-hour period, which was equivalent

to a 10-year, 24-hour storm in Seattle, Washington (assuming a 15:1 contributing area: facility surface area, 90 percent contributing area effectiveness, and a runoff treatment requirement of 91 percent). Given that Ecology's 2024 Stormwater Management Manual for Western Washington requires a target precipitation depth equivalent to a 6-month, 24-hour storm (Ecology 2024), the dosing rate used in this study can be considered a rigorous test of the media's treatment effectiveness.

2.5 Dosing and Sampling

After the flushing phase of the study was complete, the columns were dosed with highway runoff from Interstate 5 (I-5) in Seattle and Bellingham, Washington.

The Seattle stormwater sampling was conducted at a Washington State Department of Transportation (WSDOT) runoff test site located under the I-5 Ship Canal Bridge (Figure 3). This site receives runoff from a 12.8-hectare (31.6-acre) drainage area, including 9.2 hectares (22.7 acres) of pavement and 3.6 hectares (8.9 acres) of roadside landscaping. It is not subject to treatment upstream of the sample collection port.



Figure 3. A) Location of the I-5 WSDOT Ship Canal Bridge runoff test site used to collect stormwater for column dosing with sampling. B) King County's Field Science Unit collecting stormwater for column dosing.

The highway runoff from Seattle (collected directly off the highway) had high concentrations of 6PPDQ, was sampled for 6PPDQ plus other parameters, and was used for toxicity testing on coho salmon.

The Bellingham runoff was collected from a Washington Department of Transportation (WSDOT) stormwater pond adjacent to I-5 and was only sampled for 6PPDQ and not used for toxicity tests on the coho salmon. The Bellingham stormwater was used to age the media columns and attain the target dosing volume of 81 percent of a Seattle water year as prescribed in the QAPP (King County 2023). See Table 2 for the dosing schedule.

Table 2. Dosing and sampling schedule for lab study. TSS = total suspended solids, DOC = dissolved organic carbon, DO = dissolved oxygen, ORP = oxidation-reduction potential.

Record Number	Type of Event	Date	Volume Applied (liters/column)	Collection Location and Sample Parameters
1	1 st dosing	4/18/23	27	I-5 Seattle (6PPDQ, TSS, DOC, pH, temperature, DO, ORP, specific conductance, toxicity)
2	1 st aging	4/25/23	27	I-5 Bellingham (no samples)
3	2 nd aging	4/26/23	27	I-5 Bellingham (no samples)
4	3 rd aging	5/3/23	27	I-5 Bellingham (6PPDQ)
5	2 nd dosing	9/26/23	27	I-5 Seattle (6PPDQ, TSS, DOC, pH, temperature, DO, ORP, specific conductance, toxicity)
6	4 th aging	10/16/23	27	I-5 Bellingham (6PPDQ)
7	5 th aging	10/17/23	27	I-5 Bellingham (no samples)
8	6 th aging	10/25/23	27	I-5 Bellingham 6PPDQ (no samples)
9	7 th aging	10/26/23	27	I-5 Bellingham (6PPDQ)
10	8 th aging	11/6/23	27	I-5 Bellingham (6PPDQ)
11	9 th aging	11/7/23	27	I-5 Bellingham (no samples)
12	3 rd dosing	3/13/24	27	I-5 Seattle (6PPDQ, TSS, DOC, pH, temperature, DO, ORP, specific conductance, toxicity)

2.5.1 Stormwater Collection, Column Dosing, and Sampling

King County Environmental Lab (KCEL) staff collected Seattle stormwater in 20-liter, fluorinated HDPE carboys, iced, and driven to the bioretention media lab in Bellingham. Contents of the 20-liter containers were poured into the HDPE mixing tank and experiments would begin at approximately 9 a.m. Sub-samples to evaluate 6PPDQ concentrations were taken at the point of collection (Seattle) and at the beginning, mid-point, and end of the dosing experiment (Table 3). The purpose of the 6PPDQ influent sub-samples was to determine if concentrations decreased over the sampling process. Researchers collected a 27-liter volume for each dosing experiment in 24-liter glass carboys placed in ice under each of the 12 media columns. A 13th glass carboy collected untreated influent directly from the delivery system tubing (i.e., did not run through a column).

Table 3. Sample processing and transport time points.

Time Point	Definition	Location
T ₀	Time stormwater was collected.	Sampling location in Seattle
T ₁	Time that stormwater sample was composited and homogenized. T _{1A} , T _{1B} , and T _{1C} are time points during column dosing (start, middle, end) when influent samples were collected.	Bioretention Laboratory
T ₂	Time that treatment was complete and effluents were sampled.	Bioretention Laboratory
T ₃	Time all samples arrived at KCEL.	KCEL Receiving
T ₄	Time that toxicity tests were conducted.	KCEL Toxicity and Chemistry Labs

At the end of dosing experiments (2.5 hours), the glass carboys were stirred on a large stir plate, pressurized, and sub-samples taken from each column for 6PPDQ analysis (Figure 4). The 12 sub-samples were iced, transported to KCEL, and analyzed for 6PPDQ, DOC, and TSS. For toxicity testing, we composited the three 24-liter carboys from each treatment into 20-liter fluorinated carboys (the 20-liter carboy filled 1/3 from each column sample), iced, and transported to KCEL. Finally, the following parameters were measured from sub-samples for each column using sondes: pH, temperature, DO, ORP, and specific conductance. Two different sondes were used for measurements: 1) YSI EXO 1s (temperature, DO, pH, and specific conductance) and 2) Hanna HI98190 (ORP). The number of samples and associated measurements taken at the various time points in the study are shown in Appendix A, Table A-1.



Figure 4. Sub-sampling apparatus using positive pressure and continuous stirring for homogenous sub-sampling.

2.5.2 Aging the Bioretention Media

To simulate aging of the bioretention media between the dosing event storm, stormwater from a WSDOT stormwater pond in Bellingham, Washington was applied to the columns. This location was close to the WWU bioretention media laboratory enabling quick collection of high volumes of stormwater. Researchers pumped stormwater from a WSDOT stormwater pond into a 280-gallon HDPE tank and transported to the WWU bioretention media laboratory. The water was pumped up to the lab from the collection tank to the mixing tank. The same volume (27 liters/column) and time frame (2.5 hours) was used for the aging events as for the dosing experiments. Sub-samples were collected from the influent sampling pump for 6PPDQ, DOC, and TSS analysis at the 3rd, 4th, 7th, and 8th aging event.

2.6 Chemical Analyses

2.6.1 Laboratory Chemical Analyses

The following laboratory methods are summarized from this study's QAPP (King County 2023).

Researchers quantified 6PPDQ by liquid chromatography/triple quadrupole mass spectrometry using an isotopically labeled internal standard (D5-6PPDQ) method as in Hunt et al. (2021) and as documented in KCEL SOP# 4077v0. The LCMS/MS system consists of an Agilent 1290 Infinity II LC system equipped with an Agilent Infinity Lab

Poroshell 120 EC-C18 analytical column coupled to an Agilent Technologies 6470 triple quadrupole mass spectrometer. A 6PPDQ precursor ion and three of its products were monitored in positive multiple reaction monitoring (MRM) mode. The presence and ratio of these ions was used to confirm 6PPDQ identification. Quantification is achieved using 6PPDQ calibration standards spiked with an isotopically labeled internal standard, D5-6PPDQ.

DOC was analyzed by KCEL SOP #3036 and SM 5310-B. DOC samples were first filtered through a 0.45 µm filter.

Total suspended solids (TSS) were determined by KCEL SOP #3009 and SM 2540-D. A measured volume of a well-mixed sample was filtered through a glass fiber filter to determine TSS. The residue retained on the glass fiber filter was dried to a constant weight at 103°C to 105°C. The resulting net weight represents the TSS.

2.6.2 Stormwater Quality Characteristics

At various points throughout the experimental cycle performed for each storm (Table 3), we collected information on the stormwater quality characteristics that may be affected by bioretention media or other factors (Table 1). At each point during an experimental cycle that collected water quality characteristics, an aliquot of up to 0.3 to 0.5 L of the water to be tested was put into a wide-mouth container. One or more single- or multi-parameter probes were used to measure the following characteristics:

1. Temperature (°C), SOP #2045v1
2. Specific conductance (umhos/cm), SOP #2045v1
3. pH (unitless), SOP #2045v1
4. Oxidation-reduction potential (mV), Hanna H198190 user manual
5. Dissolved oxygen (mg/L), SOP #2045v1

Data collection was performed in accordance with the KCEL Field Sciences Unit's (FSU's) SOPs for field measurement of each of these parameters. A multiparameter probe was used for temperature, pH, specific conductance, and DO; the appropriate SOP was applied (e.g., Attended YSI EXO Multiprobe Operations, SOP# 2045v1 2017). For ORP measurements, we used a standalone field meter. All calibration procedures, record keeping, and instrument use were consistent with SOPs or manufacturer's instructions.

2.7 Toxicity Testing

Toxicity testing was conducted to 1) test for acute toxicity of the default 60:40 BSM rinsate to fathead minnow and 2) test for toxicity of stormwater to juvenile coho salmon before and after treatment by bioretention media.

2.7.1 Fathead Minnow Exposures

To confirm that any fish toxicity observed in coho salmon toxicity tests from the 60:40 BSM effluent was attributable to stormwater (i.e., 6PPDQ), flush water from the media was tested using fathead minnow (*Pimephales promelas*) acute exposures prior to the

start of stormwater dosing. One 2-L sample of flush water from the 60:40 BSM column following the final flush was tested for acute toxicity to fathead minnow as part of preparation of the bioretention media columns. We conducted the fathead minnow test according to EPA Test Method 2000.0: Fathead Minnow, *Pimephales promelas*, Acute Toxicity Tests with Effluents and Receiving Waters (USEPA 2002) and KCEL standard operating procedure (SOP) #4014v3.

2.7.2 Coho Salmon Exposures

Toxicity tests with coho salmon were conducted on effluent composited from the 60:40 BSM, Type 1 and Type 3 HPBSM, untreated stormwater and a laboratory control (laboratory well water). We performed toxicity tests according to EPA Test Method 2019.0: *Rainbow Trout*, *Oncorhynchus mykiss*, and *Brook Trout*, *Salvelinus fontinalis*, *Acute Toxicity Tests with Effluents and Receiving Waters* (modified by using coho salmon as the test organism).

Coho salmon embryos are only available December to January of each year. Modifications of EPA 2019.0 (fish age, number of organisms, and loading rate) were necessary to meet the project objectives within this constraint. Toxicity test conditions used for the coho salmon exposures are detailed in Table 4.

Table 4. Coho salmon acute toxicity test conditions.

Condition	Specification
Organism	Coho salmon (<i>Oncorhynchus kisutch</i>)
Test Type	Static non-renewal
Sample Hold Time	Initiate within 36 hours of sample collection.
Temperature	12 ± 2°C
Control Water	Well Water hardness approximately 40 to 100 mg/L as CaCO ₃ .
Light Intensity	500 to 1000 lux
Photoperiod	16 h light:8 h dark
Test Chamber Size	18L glass jars
Renewal of Test Solution	None
Age of Test Organisms/Loading rate	58d (.69 g/L), 290d (2.64 g/L), 35d (.31 g/L)
Test Concentrations	100% sample
Number of Organisms	5
Number of Replicates	4
Feeding	None during test and ceased 48 hrs. prior to test initiation.
Oxygen/Aeration	None, unless DO concentration falls below 6.0 mg/L or loading rate > .8 g/L.
Positive Control	6PPDQ (one LC ₅₀ test per batch of test organisms)
Test Duration	24 hours

Condition	Specification
Endpoint	Mortality and observational notes of suspected URMS symptoms (disorientation, swimming in circles, gaping, etc.) will be made in the first 12 hours of exposure.
Test Acceptability	≥90% control survival
Measurements	Temperature, pH, dissolved oxygen (daily for each).
Water Quality	Hardness, alkalinity, specific conductance, redox (0-hour for each).

2.8 Data Analysis

All data analyses were conducted in the R statistical programming language (R Core Team 2023) using R studio.

2.8.1 Chi-square Test of Fathead Minnow Count Data

The fathead minnow toxicity tests using 60:40 BSM flush water resulted in counts of the number of fish alive after 48 hours of exposure. Researchers used a Chi-square test to determine if the number of fish alive at each concentration of 60:40 BSM flush water (0%, 6.25%, 12.5%, 25%, 50%, and 100%) was the same (null hypothesis) or different across concentrations.

2.8.2 6PPDQ Summary Statistics

Summary statistics, including the mean, median, and standard deviation, were calculated for untreated and treated stormwater sample 6PPDQ concentrations. Untreated stormwater groups included I-5 runoff grab samples, homogenized stormwater influent from the dosing tank, and the aging stormwater taken from a wet pond in Bellingham.

We grouped the treated stormwater (bioretention effluent) samples by bioretention mix using data from individual column effluent samples at timepoint T2 (after dosing was completed). The 6PPDQ concentration data was visually determined to fit a lognormal distribution using log-transformed normal quantiles plots. The treated stormwater data contained many non-detects (<MDL); thus, prior to summary statistic calculations, non-detect data were imputed via robust regression order statistics (“robust” ROS) using the `ros()` function from the NADA package in R (Lee 2020). Robust ROS imputation is appropriate for small data sets (<50 observations) with <50 to 80 percent censoring. It uses a distributional assumption (lognormal in this case) to impute non-detect data from a probability distribution of detected data (Helsel 2011). Summary statistics are then calculated using this imputed data.

2.8.3 Comparing Effluent 6PPDQ Concentrations

Effluent 6PPDQ concentrations were compared across treatments to test for significant differences in treatment effectiveness and median effluent 6PPDQ concentrations. Researchers ran a non-parametric, censored Peto-Peto test (Peto and Peto 1972) on

6PPDQ data. The function `cen1way()` from the *NADA2* package (Helsel 2024) in R was used to run this Peto-Peto test and pairwise comparisons using a Benjamini-Hochberg false discover rate to account for multiple comparisons.

2.8.4 6PPDQ Concentration Reduction

To calculate the treatment effectiveness of the bioretention mixes, censored data (<MDL) were substituted with the MDL value of 2 ng/L. Therefore, achieving 100 percent concentration reduction was not possible, and reduction rates calculated from non-detect data are right-censored and represent a lower limit of reduction rather than an exact value. For example, if the influent 6PPDQ concentration was 100 ng/L and the effluent was <MDL, the concentration reduction would be reported as >98% because the effluent concentration would be substituted with 2 ng/L, and the true effluent concentration is known to be between 0 and 2 ng/L. The equation used for concentration reduction calculations was:

$$\% \text{ 6PPDQ concentration reduction} = [(C_i - C_e)/C_i] * 100\%,$$

where C_i is the untreated stormwater influent concentration and C_e is the treated bioretention effluent concentration.

Because reduction rates were right-censored and did not fit normal or lognormal distributions, we used a Peto-Peto test with pairwise comparisons as described in section 2.8.3. We flipped the reduction rate values to transform them into left-censored values by subtracting them from 100 prior to running the Peto-Peto test because the `cen1way()` function only accommodates left-censored data (Helsel 2011).

2.8.5 6PPDQ and Water Quality Covariates

Several other water quality parameters with potential relevance to 6PPDQ were measured alongside 6PPDQ concentrations. These parameters included total suspended solids (TSS), dissolved organic carbon (DOC), specific conductance, pH, temperature, and oxidation reduction (redox) potential. To explore relationships between 6PPDQ and these parameters, scatterplots of each parameter and 6PPDQ concentration were first generated for all samples. Not all parameters were measured at every timepoint. The number of samples analyzed for each of these parameters is listed by sample type in Table A-1.

3 Results

The results of the 6PPDQ treatment tests and coho salmon toxicity tests as well as related statistical analyses on 6PPDQ data are detailed below. Summary statistics for all timepoints, parameters, and treatments are shown in Table A-1. Results for preliminary column testing, including 6PPDQ loss to equipment tests, bioretention media leaching tests (SPLP), BSM leachate toxicity tests, and column flushing tests, are detailed in Appendix B.

3.1.1 6PPDQ in Treated and Untreated Stormwater

Filtering the stormwater influent through the tested bioretention medias resulted in at least a 10-fold decrease in 6PPDQ concentration. The concentration range for 6PPDQ in untreated stormwater influent samples was 226 ng/L to 808 ng/L while the concentration range for 6PPDQ in treated bioretention effluents was <MDL (2 ng/L) to 22.5 ng/L.

The 6PPDQ MDL for this study was 2 ng/L. All 60:40 BSM effluent samples had detectable 6PPDQ concentrations (Table 5). HPBSM Type 1 had three samples (33.3%) where 6PPDQ concentrations were below the MDL while the HPBSMs Types 2 and 3 had six of nine samples (66.6%) where 6PPDQ concentrations were below the MDL.

Table 5. Summary statistics for 6PPDQ concentrations (ng/L) in untreated (I-5 stormwater runoff grab samples, influent stormwater, and Bellingham wet pond water used for aging columns) and treated stormwater (bioretention effluents).

Bioretention treatment	N	Detection frequency ¹ (%)	Median	Mean	Standard Deviation	Min	Max
I-5 Stormwater runoff grab	3	100	577	654	347	286	856
Influent stormwater	9	100	503	590	361	226	808
Bellingham wet pond	4	100	15.9	19.9	15	9.9	50.2
All effluent samples	36	58.3	2.8	3.8	5.0	<MDL	22.5
60:40 BSM effluent	9	100	3.8	8.8	8.1	2.8	22.5
HPBSM Type 1 effluent	9	66.7	2.2	2.5	1.3	<MDL	5
HPBSM Type 2 effluent	9	33.4	1.6	2.1	1.4	<MDL	5.1
HPBSM Type 3 effluent	9	33.4	2.2	2.6	1.3	<MDL	5.3

1. For groups with non-detect concentrations (HPBSM effluents), robust ROS was used to estimate summary statistics, means, and medians. Calculated summary statistics may be estimated below the 6PPDQ MDL of 2 ng/L because of ROS imputation. Effluent summary statistics only include data from individual columns (timepoint T2). Effluent composite data from the toxicity test (T4) are presented in Table 13.

For all tested bioretention mixes, the highest 6PPDQ concentrations in effluents were observed in Storm 3 (Figure 5). Effluent concentrations did not appear to be driven by influent concentrations—Storm 1 had the highest influent concentrations but the lowest effluent concentrations (Figure 5).

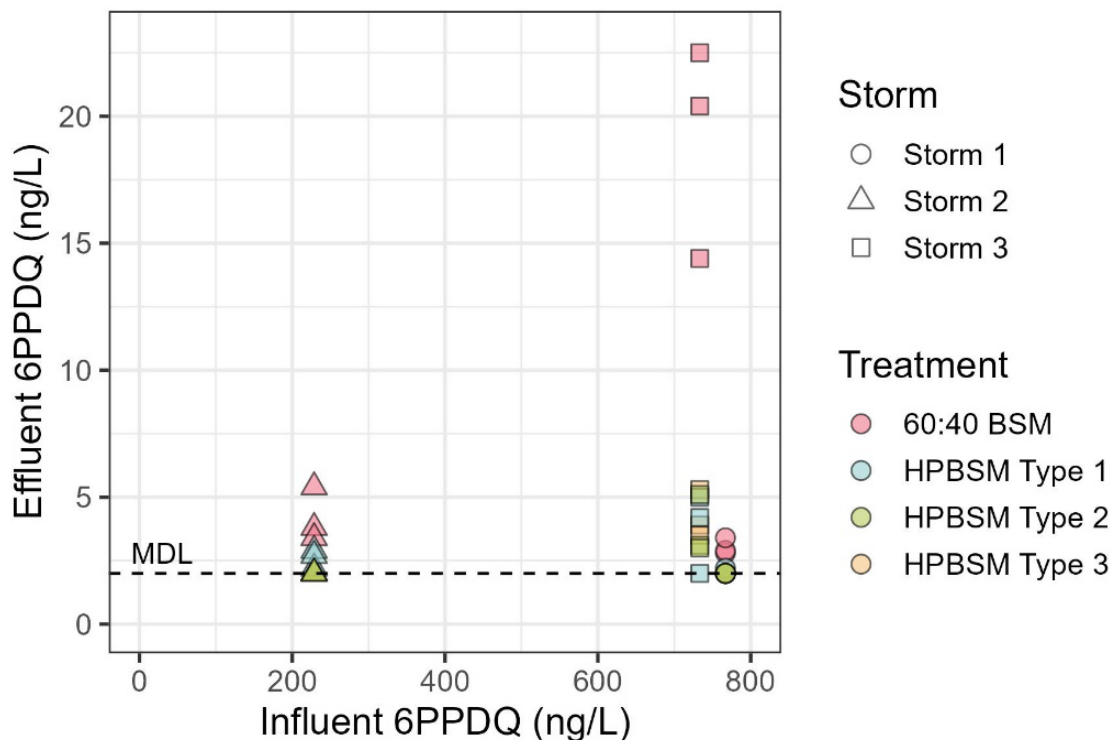


Figure 5. Influent vs effluent 6PPDQ concentrations (ng/L) for the three storm dosing events. Influent values represent the average of T1A, T1B, and T1C samples as in % concentration reduction calculations.

Researchers ran a censored and non-parametric Peto-Peto test of the 6PPDQ data (Chisquare = 15.85, $p = 0.0012$). Results showed that significantly lower 6PPDQ concentrations were measured in the HPBSM effluents than 60:40 BSM effluents (pairwise Peto-Peto tests: HPBSM Type 1, $p = 0.023$; HPBSM Type 2, $p = 0.020$; HPBSM Type 3, $p = 0.031$) (Figure 6).

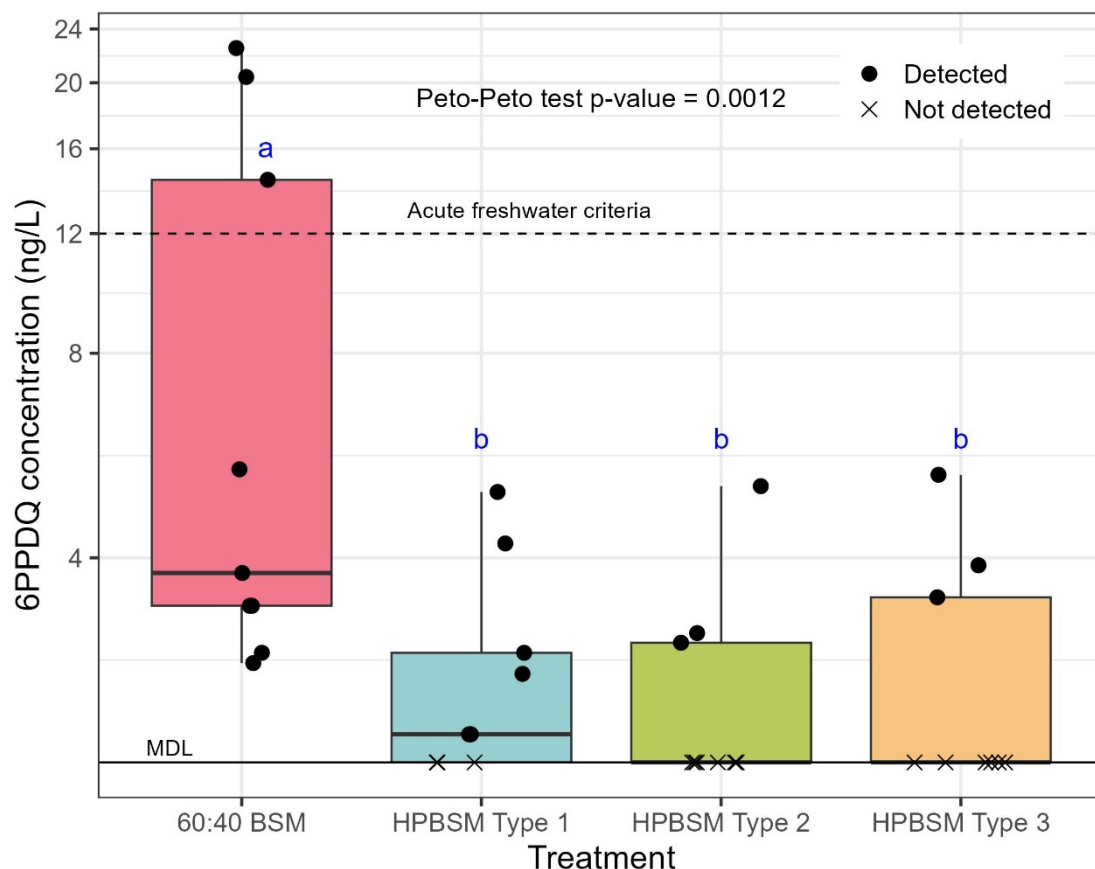


Figure 6. Censored boxplots showing observed distribution and medians (thick black line in box) of data above the MDL (solid line). The y-axis is shown on a log-scale to better show treatment differences. The proportion of censored data below the MDL (2 ng/L) is represented by the portions of the interquartile range that are omitted. The dashed black line represents the acute aquatic life criteria (12 ng/L) that has been developed by Ecology. Statistically different groups are denoted as a and b.

Ecology recently adopted an acute, freshwater aquatic life criteria value for 6PPDQ of 12 ng/L ([WAC 173-201A-240](#))¹. Notably, in every HPBSM effluent sample, 6PPDQ concentrations were below Ecology's proposed aquatic life criteria of 12 ng/L. Effluent samples from the 60:40 BSM exceeded this criterion for three of nine samples.

¹ This acute, freshwater criterion of 12 ng/L is subject to change before the final rulemaking and must be approved by EPA before they can be used for Clean Water Act programs. See Ecology's [Aquatic Life Toxics Criteria Rulemaking Concise Explanatory Statement Chapter 173-201A WAC](#) for details on rulemaking.

3.1.2 6PPDQ Concentration Reduction by Bioretention Mixes

All the tested bioretention mixes reduced 6PPDQ concentrations by at least 96.9 percent during each storm event. Mean 6PPDQ concentration reduction (%) values are shown in Table 6.

Table 6. Mean and standard error 6PPDQ concentration reduction (%) for each tested bioretention mix. Note that for data <MDL, the MDL of 2 ng/L was used as the value, thus 100% removal was not a possible value.

Bioretention Mix	6PPDQ Concentration Reduction (%)			
	Mean	Standard error (n = 9)	Min	Max
60:40 BSM	98.4	0.346	96.9	99.6
HPBSM Type 1	99.4	0.136	98.7	>99.7
HPBSM Type 2	99.5	0.093	99.1	>99.7
HPBSM Type 3	99.4	0.091	99.1	>99.7

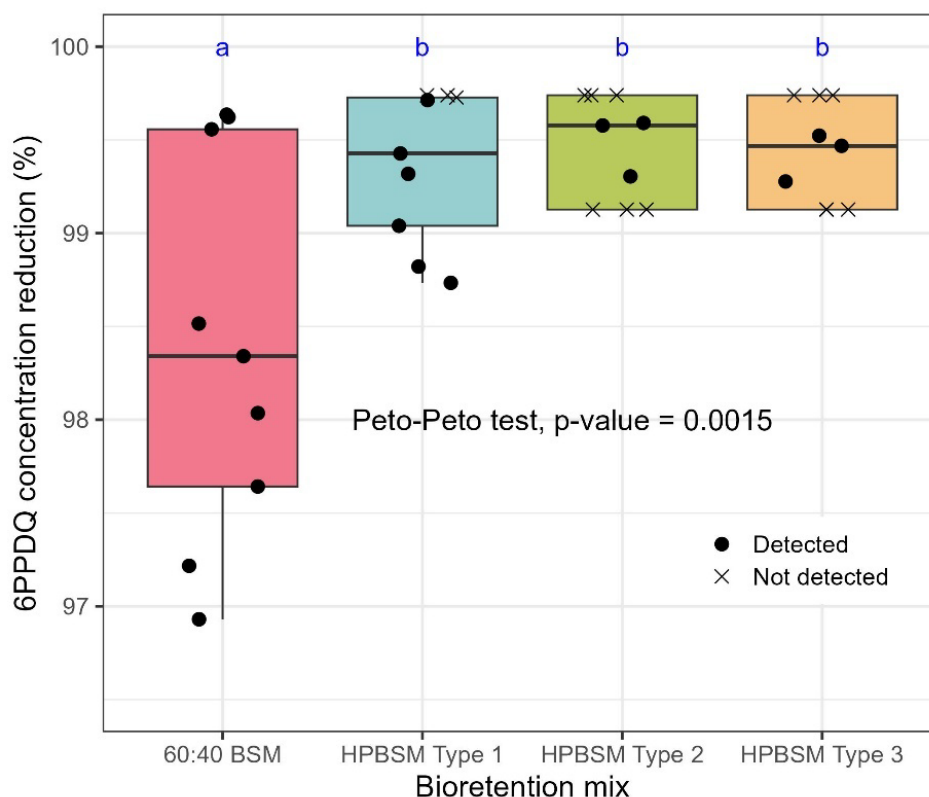


Figure 7. Percent concentration reduction of 6PPDQ by the different bioretention soil mixes. Values <MDL were substituted with the MDL of 2 ng/L prior to calculating removal efficiency. See Section 2.8.4 for details on % reduction calculations and the Peto-Peto statistical test.

HPBSM mixes achieved significantly greater 6PPDQ concentration reduction than the 60:40 BSM ($p \leq 0.05$). We found no significant differences in 6PPDQ concentration reduction between HPBSM types (Figure 7).

3.2 Coho Salmon Exposures

3.2.1 Coho Salmon Survival

Untreated stormwater influent was acutely toxic to exposed coho salmon during 24-hour static exposure tests for all three storms. Coho salmon survival was 0 to 5 percent in untreated stormwater influent compared to 100 percent survival across all treated effluents (Table 7). The concentration range for 6PPDQ in untreated stormwater influent was 226 ng/L to 808 ng/L.

Table 7. Coho salmon survival and 6PPDQ concentrations in coho salmon exposure waters for toxicity tests.

Exposure Treatment n = 20	Coho Salmon Survival (%)			6PPDQ Concentration (ng/L)		
	Storm 1	Storm 2	Storm 3	Storm 1	Storm 2	Storm 3
Well water control	100	100	100	< MDL	<MDL	<MDL
Untreated stormwater influent	5	0	5	754	225	640
60:40 BSM (composite)	100	100	100	4.4	3.0	26.8
HPBSM Type 1 (composite)	100	100	100	2.5	<MDL	7.8
HPBSM Type 3 (composite)	100	100	100	<MDL	<MDL	4.7

Water quality parameters remained within acceptable limits throughout each test, control survival met acceptability criteria of ≥ 90 percent (U.S. EPA 2002).

The relative sensitivity of coho salmon over the course of the study was evaluated by conducting reference toxicant tests of a 6PPDQ analytical standard (HPC Standards). We performed three tests and LC50s were calculated using analytically verified test concentrations. Results were compared to calculated laboratory control limits (mean $LC50 \pm 2SD$). Results of 56.9, 92.5, and 50.6 ng/L 6PPDQ were within current control limits of 35.68 to 130.65.

3.2.2 Relationships between Other Water Quality Parameters and Coho Salmon Toxicity

Except for one individual in each of the first and third storm events, all coho salmon exposed to untreated stormwater died during exposures. Conversely, all coho salmon exposed to lab control water or treated stormwater survived. Because of this binary outcome, it was not possible to determine if any of the conventional parameters had protective or antagonistic effects on 6PPDQ.

3.3 Other Water Quality Parameters

A secondary goal of the study was to identify and measure stormwater constituents and conditions (e.g., pH, dissolved oxygen) that may be dynamic in stormwater and may also be affected by the tested media mixes. We evaluated how common water quality characteristics change during each cycle in the experiment and the degree to which they change due to passing through the BSM columns.

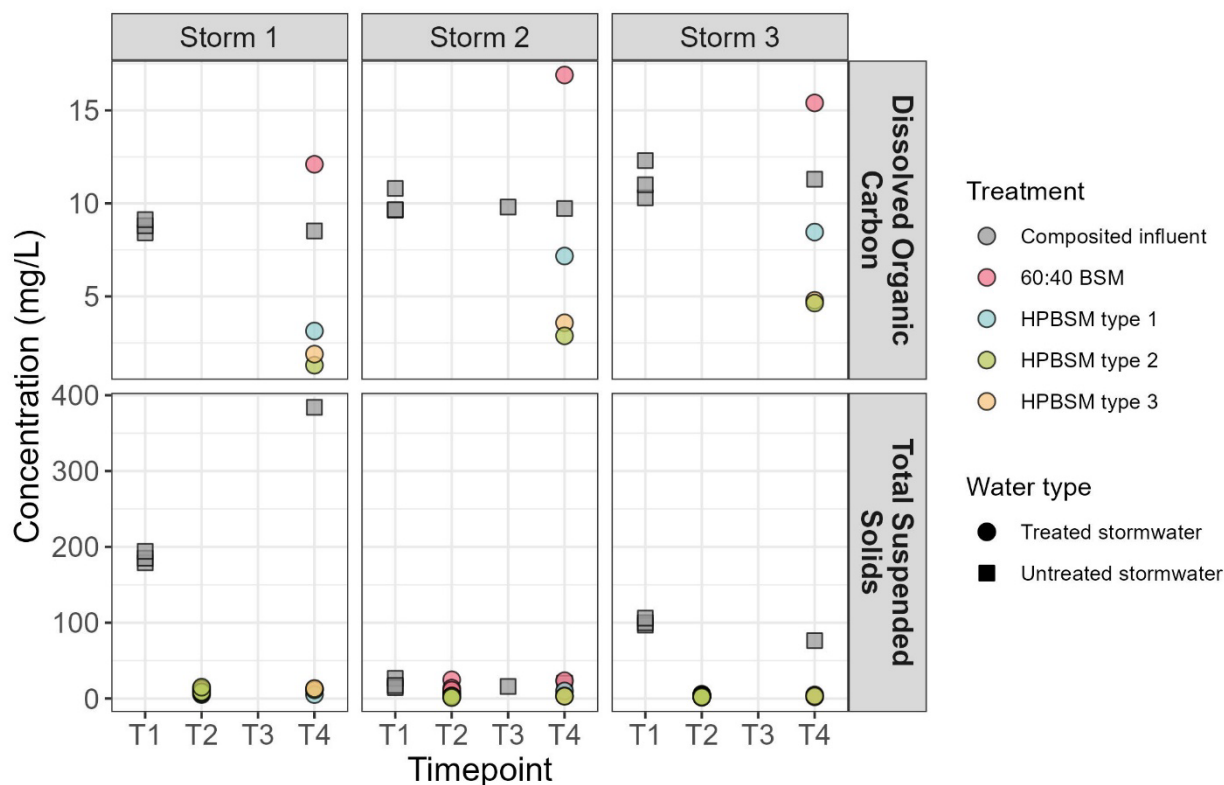


Figure 8. TSS and DOC concentrations in the various untreated and treated stormwater samples at all storm timepoints. Timepoints: T₁ = Time that stormwater sample was composited, T₂ = Time that treatment was complete, and effluents were sampled, T₃ = Time all samples arrived at KCEL, T₄ = Time that toxicity tests were conducted.

DOC concentrations in the composited influent (untreated) were stable across timepoints (T₁ and T₄), with only slight variability across T₁ samples taken at the beginning, middle, and end of column dosing (Figure 8). DOC was also measured in the influent at T₃ during Storm 2 and showed results consistent with other influent samples. Bioretention treatment impacted DOC differently across the different mixes, increasing after treatment with the 60:40 BSM and decreasing after treatment with the HPBSMs. The lowest DOC concentrations were observed in HPBSM Types 2 and 3, which contained the polishing layer.

In storms with elevated TSS levels (Storms 1 and 3), TSS in composited influent was variable across timepoints and on average an order of magnitude higher than in treated effluent samples (Figure 8 – squares vs. circles). TSS concentrations were similar

across treated effluents within each storm event, except in Storm 2 where TSS in 60:40 BSM effluents were slightly elevated compared to the HPBSMs.

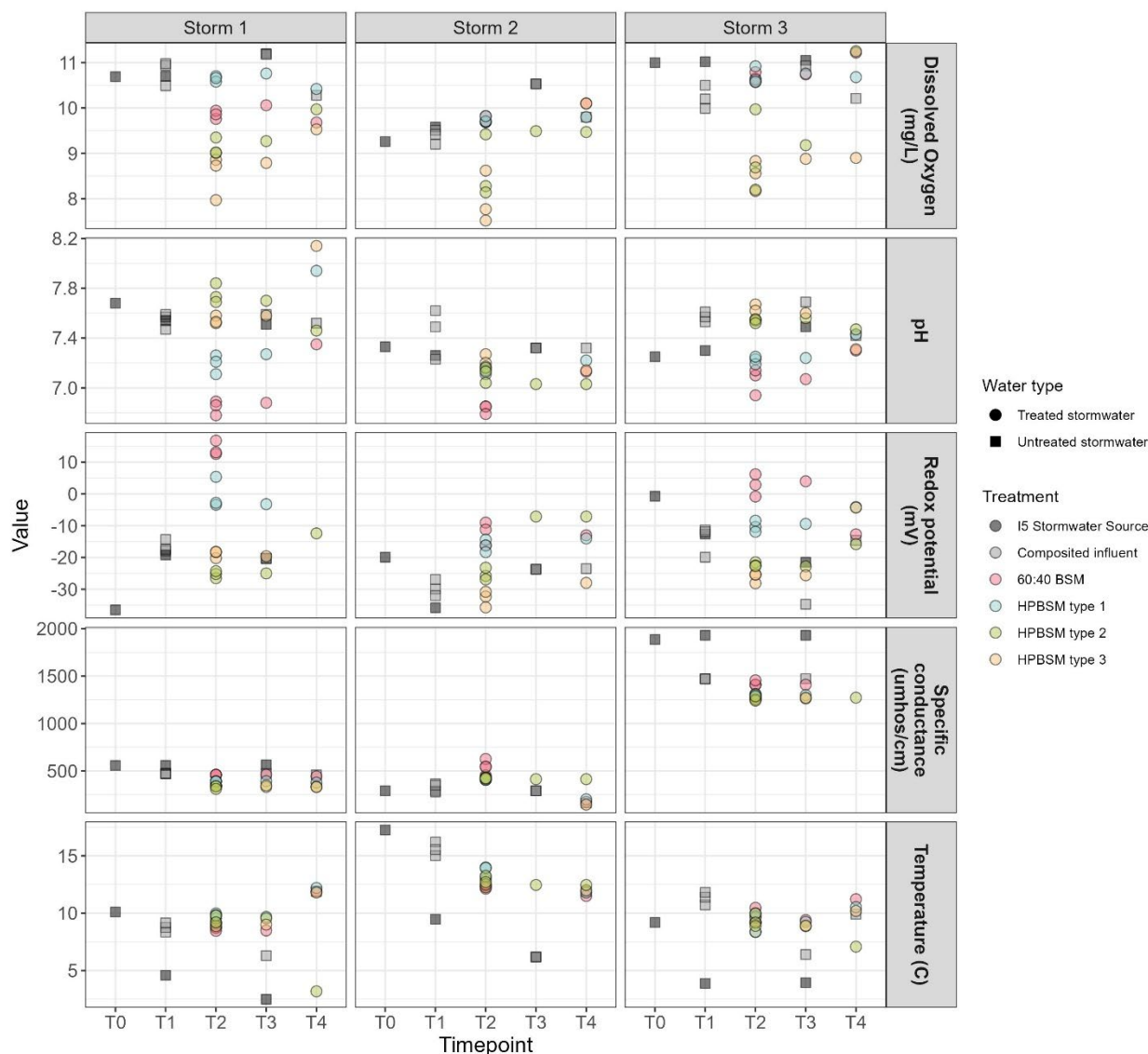


Figure 9. Dissolved oxygen, pH, redox potential, specific conductance, and temperature in the various untreated and treated stormwater samples at all storm timepoints.

Lower dissolved oxygen levels were observed in treated effluents (range 7.5 to 11.25 mg/L) than untreated stormwater (9.2 to 11.2 mg/L) (Figure 9). We observed lower dissolved oxygen in HPBSM Types 2 and 3 effluents (7.5 to 11.3 mg/L) than in the 60:40 BSM and HPBSM Type 1 effluents (9.7 to 11.2 mg/L).

No clear timepoint trend in pH data (Figure 9) was observed. The only consistent trend across treatments was that 60:40 BSM effluents tended to have the lowest pH (6.78 to 7.35) and was almost always lower than the pH in untreated stormwater (7.23 to 7.69).

The bioretention mixes impacted redox potential in stormwater differently. Filtration through the 60:40 BSM and HPBSM Type 1 mixes tended to increase redox potential of stormwater (Figure 9), while filtration through HPBSM Types 2 and 3 tended to range from no impact to a slight decrease in redox potential.

Specific conductance was notably higher across all samples in Storm 3 (Figure 9). In Storm 3, treatment by bioretention decreased specific conductance compared to untreated stormwater (from 1,470 to 1,931 umhos/cm to 1,242 to 1,456 umhos/cm). Specific conductance trends across all samples in Storms 1 and 2 were more subtle.

Temperatures across all samples ranged from 2.5°C to 17°C (Figure 9). We observed no effect of bioretention treatment on water temperature, but in some cases (Storms 2 and 3) a slight decrease in temperature can be observed across the timepoints, reflecting storage on ice during holding and transport.

Data exploration of potential relationships between 6PPDQ and water quality parameters are reported in Appendix D. Potential positive relationships with 6PPDQ were observed for TSS, specific conductance, DOC, and redox potential.

4 Uncertainty and Quality Assurance

This section addresses uncertainties underlying the data collected in this study as well as results from quality assurance steps taken to address those uncertainties. Uncertainties discussed here include the potential for 6PPDQ loss from contact with the bioretention column array, the potential for contaminants leaching from the 60:40 BSM to induce acute toxicity, and the degree to which contaminants are leached from HPBSM materials or flushed out during the initial applications of water to the mixes. This section also includes a description of the appropriateness of the hydraulic loading rates used in column dosing and comments on data verification and QAPP deviations (King County 2023).

4.1 6PPDQ Loss to and Release from Equipment

6PPDQ has an octanol-water partitioning coefficient ($\log K_{ow}$) of 4.3 (Hu et al. 2023), which indicates it is expected to favor partitioning to organic substances rather than remaining dissolved in water. Studies also show substantial loss of 6PPDQ during experiments (Lane et al. 2024; Herrera 2024), suggesting that sorption of aqueous 6PPDQ to sampling equipment may bias results of studies measuring 6PPDQ concentrations. 6PPDQ loss via sorption is more prevalent for porous, high surface area materials like rubber, silicone, and some plastics compared with glass, stainless steel, and chemically inert plastics (e.g., PTFE) (Hu et al. 2023).

To assess the potential for 6PPDQ loss to the bioretention column dosing array (i.e., two HDPE tanks, PTFE tubing, and PVC columns), we dosed an empty bioretention column with deionized water spiked with a high concentration of 6PPDQ (336 ng/L) prior to the start of the experiments and measured an 18.75 percent loss of 6PPDQ to the column array equipment. This loss is comparable to the loss to plastics observed during short contact times by Hu et al. (2023) who conducted batch sorption tests of lab

materials using ultrapure water spiked with very high concentrations of 6PPDQ (5,000 ng/L) to evaluate sorption to sampling materials. Though the observed loss to equipment in the present study was substantial, it may be an overestimate of how much the 6PPDQ in stormwater would sorb to the column array because stormwater is a complex matrix containing many other organics that may compete with 6PPDQ for attachment sites.

The primary concern for the loss-to-equipment biasing result would be if this loss impacted effluent much more than influent. This study design minimized this potential bias because influent was sampled after passing through most of the column array (HDPE tanks and PTFE tubing), except for the PVC columns (Figure 2). Prior to adding media to the PVC columns, they were abraded on their inner surfaces to minimize preferential flow along the media-column interface, ensuring that water passing through would have minimal contact with the PVC. Therefore, while loss-to-equipment may have had some impact on measured 6PPDQ concentrations in this study, it is unlikely to have impacted the study's findings.

4.2 Bioretention Soil Mix Leaching and Toxicity

4.2.1 Synthetic Precipitation Leaching Procedure (SPLP)

We conducted three different tests on the various bioretention mixes: fathead minnow acute toxicity tests were conducted on the 60:40 BSM flush water, leachate testing by SPLP was conducted on the components of the HPBSM primary layer, and bioretention soil column clean water flushing was conducted on all tested mixes to simulate one water year.

Because flush water from 60:40 BSM leachate has previously been shown to export levels of copper above Washington's water quality standards for the protection of aquatic life, an acute fathead minnow toxicity test was run on just the 60:40 flush water. The fathead minnow acute toxicity tests showed no significant difference in 24-hour survival across 60:40 flush water concentrations, suggesting there was no impact of the 60:40 flush water on fathead minnow survival.

SPLP tests run on the HPBSM primary layer materials (coconut coir, state sand, and biochar) showed that these materials were within Ecology's SPLP specification for dissolved copper and nitrate/nitrite (Ecology 2024). However, the coconut coir leachate exceeded Ecology's specifications for orthophosphorus (0.8 mg/L) by more than double with a leachate concentration of 1.74 mg/L. This coconut coir leachate orthophosphorus concentration was more than an order of magnitude higher than the concentration reported by Herrera (2020) of 0.033 mg/L.

4.2.2 Column Flushing

Prior to dosing the columns with stormwater, the columns were flushed over 14 storm cycles with the equivalent of one Seattle water year of deionized water. We collected column effluents during the initial and final flushing storms, and effluents from the initial and final flushes were composited by treatment. Effluents were analyzed for a suite of dissolved and total metals, TSS, and DOC. For most flushing samples, the 60:40 BSM

had the highest contaminant concentrations, followed by HPBSM Type 1, and HPBSMs Types 2 and 3.

Higher concentrations of metals were leached in the 60:40 BSM than any of the HPBSMs during both initial and final flushing events. This is consistent with results from earlier phases of HPBSM development, where bioretention mixes containing compost were generally reported to have higher metals concentrations in flush water compared with mixes without compost (Herrera 2016; Herrera 2020).

As expected, contaminant concentrations were much lower in samples from the final flush compared with the initial flush, except for total and dissolved lead (in all mixes) and total and dissolved zinc (in HPBSMs), which were slightly higher in final flush leachates compared with initial flush effluents. This is consistent with previous studies that showed metals may be exported from bioretention for up to approximately one water year (equivalent to this study's flushing period) following construction with new media (Herrera 2014; Taylor et al.; 2018, Mullane et al. 2015).

4.3 Representativeness of Column Dosing

The loading rate of stormwater used to dose the bioretention columns in this study was rigorous. During each dosing event (for sampling and aging), we dosed the bioretention columns with the equivalent of a 10-year, 24-hour storm in Seattle, Washington (assuming 15:1 contributing area: facility treatment area, 90 percent contributing area effectiveness, and a runoff treatment requirement of 91 percent). Ecology's Stormwater Management Manual for Western Washington (Ecology 2024) requires that bioretention facilities are sized to treat a target precipitation depth equivalent to a 6-month, 24-hour storm—a much smaller loading rate than used in this study. Additionally, the Seattle stormwater runoff source used in the dosing events drains an elevated bridge of a major interstate (I-5), and likely represents something close to a worst-case scenario for 6PPDQ loading and transport, with 6PPDQ concentrations measured in this study (across all samples) ranging from 225 ng/L to 1,109 ng/L. The Bellingham stormwater pond water used to age the columns throughout the study had much lower 6PPDQ concentrations, ranging from 9.9 ng/L to 50.5 ng/L 6PPDQ. While the columns were aged with water that was relatively low in 6PPDQ for stormwater, the intensive stormwater loading rates and high concentrations of the Seattle stormwater used during dosing with sampling indicate this was a rigorous test of the media's performance.

4.4 Data Verification

All results were compared to data quality objectives (DQOs) in the QAPP and reviewed for appropriateness of use in our analysis. Several deviations from the QAPP (King County, 2023) occurred, but none of them impacted results or interpretations in this report. All QAPP deviations for the bioretention media lab, chemistry, and toxicology procedures are documented in Appendix E specific to each of these categories. Although some procedures differed from the QAPP and holding times were exceeded for DOC and ammonia in 4 to 5 samples, researchers accepted all data as meeting DQOs and not anticipated to introduce significant bias.

5 Discussion and Findings

5.1 Overview

The primary objectives of this study were to evaluate the 6PPDQ treatment effectiveness of HPBSMs compared to the 60:40 BSM in terms of 6PPDQ concentration reduction and reduction of toxicity to coho salmon. We found that all three of the HPBSM types we tested, as well as the 60:40 BSM, completely prevented coho salmon death from acute stormwater toxicity. We also documented 6PPDQ concentration reduction by HPBSMs and 60:40 BSM for the first time, which all showed 6PPDQ reduction rates >96 percent. However, the HPBSM effluents had significantly lower 6PPDQ concentrations compared with the 60:40 BSM, demonstrating their improved treatment effectiveness for 6PPDQ.

In August 2024, Ecology issued revisions to the Water Quality Standards for Surface Waters of the State of Washington. In this revision Ecology adopted an acute, freshwater aquatic life criteria value for 6PPDQ of 12 ng/L ([WAC 173-201A-240](#)). We compared our effluent 6PPDQ concentrations to this acute criteria to provide context for how effective the different bioretention mixes were for 6PPDQ treatment.

5.2 Coho Salmon Toxicity Reduction by Bioretention Mixes

This study demonstrated that HPBSMs provide similar protection to coho salmon from stormwater as the 60:40 BSM. Untreated stormwater was acutely toxic to juvenile coho salmon, with 0 to 5 percent survival for exposed fish. In Storms 1 and 3, a single fish in the untreated stormwater exposure (n=20 per event) survived. Filtration of this stormwater through all tested media—the three types of HPBSM and the 60:40 BSM—completely protected juvenile coho salmon (100% survival) from this acute toxicity. This finding is consistent with previous studies conducted prior to the discovery of 6PPDQ which found that filtering acutely toxic stormwater through sand and compost bioretention soil (referred to here as the 60:40 BSM) prevents the acute toxicity observed when coho salmon are exposed to untreated stormwater (McIntyre et al. 2015; Spromberg et al. 2016; McIntyre et al. 2023).

5.3 6PPDQ Concentration Reduction by Bioretention Mixes

The major novel finding of this study was that all tested HPBSM configurations were highly effective at mitigating 6PPDQ, reducing concentrations in the effluent to often undetectable levels of 6PPDQ. The HPBSMs performed better in terms of 6PPDQ concentration reduction than the 60:40 BSM, which was previously shown to protect coho salmon from toxic stormwater (McIntyre et al. 2015; Spromberg et al. 2016; McIntyre et al. 2023), but had not yet been tested for 6PPDQ treatment effectiveness as these studies were conducted prior to 6PPDQ's discovery. Though concentration reduction efficiencies were only marginally higher in HPBSMs (range: 98.7 to >99.7%) compared to the 60:40 BSM (range 96.9 to 99.6%), these small effectiveness improvements may be important because of 6PPDQ's acute toxicity at very low concentrations. The highest effluent 6PPDQ concentration in this study was 26.8 ng/L from the 60:40 BSM composite used in the Storm 3 toxicity testing. The lowest

published 6PPDQ median lethal concentration (LC_{50}) for coho salmon as of this report's publishing is 41 ng/L for juvenile coho salmon (Lo et al. 2023)—only 1.5 times higher. All tested HPBSMs (Types 1, 2, and 3) reduced 6PPDQ concentrations to below Washington State's acute, freshwater aquatic life criteria for 6PPDQ of 12 ng/L in every simulated storm, suggesting these newly developed BSMs may be better suited for meeting regulatory compliance than the 60:40 BSM.

This study was not designed to test for longevity of the bioretention media's 6PPDQ treatment effectiveness and only encompassed one Seattle water year of stormwater dosing. We did observe higher 6PPDQ concentrations in effluent samples during Storm 3 compared to Storms 1 and 2; this was most notable for the 60:40 BSM but also observable in HPBSMs Types 1 and 2, which only had detectable levels of 6PPDQ in their effluents during Storm 3. This apparent elevated effluent 6PPDQ in Storm 3 did not appear to be driven by influent 6PPDQ concentrations, as concentrations in Storm 1 were higher than in Storm 3. This also does not appear to be driven by wetting and drying cycles in the columns as there were prolonged dry periods (128 and 145 days) prior to both Storms 2 and 3. 6PPDQ is expected to be removed from stormwater primarily via sorption to organic materials in the bioretention media (Hu et al. 2023; Hildebrandt et al. 2024). Loss of treatment effectiveness over time could result from using up adsorption sites or reaching equilibrium sorption capacity (Hildebrandt et al. 2024). An ongoing study of the longevity of bioretention depths (McIntyre et al. 2019) simulated 13 water years of stormwater application via accelerated dosing of bioretention columns containing the 60:40 BSM to evaluate how long contaminant treatment endured in aging bioretention media. Even after eight water years, bioretention columns that were still physically functioning continued to completely protect coho salmon from toxic stormwater (McIntyre et al. 2022). McIntyre et al. focused only on 60:40 BSM, and further study of the longevity of HPBSMs is warranted.

The observed differences in 6PPDQ concentration reduction among the tested media are likely driven by the different physical characteristics of the mixture components (e.g., surface area, sorption capacity, hydrophobic attraction, etc.). All HPBSM configurations yielded small but significant improvements in 6PPDQ removal compared with the 60:40 BSM, which suggests that the HPBSM primary layer components (70% sand, 20% coconut coir, 10% biochar) are better at capturing 6PPDQ than the 60:40 BSM (60% sand, 40% compost). Differences in physical and chemical characteristics of organic media components between the HPBSM primary layer and the 60:40 BSM likely drive the observed differences in 6PPDQ effectiveness. Some media characteristics that are important to sorption of organic contaminants include organic carbon content, surface area, strength of hydrophobic attractions, and hydraulic retention time (Okaikue-Woodi et al. 2020).

Compost is the organic component of the 60:40 BSM. The organic components of the primary layer of the HPBSM mixes include coconut coir (20% by volume) and high carbon wood ash, also known as biochar (10% by volume). Biochar is a highly porous, carbonaceous material with vast internal surface area and hydrophobic surfaces (Mohanty et al. 2018). These characteristics are known to enhance sorption of organic contaminants by increasing the sorption capacity of media mixes (via larger surface

area) and increasing the strength of hydrophobic attachments (Mohanty et al. 2018). A recent study by Hildebrandt et al. (2024) evaluated the 6PPDQ sorption capacities and kinetics of various sands, soils, and sorbent media, including the same compost used in the 60:40 BSM and a softwood biochar like the HCWA/biochar used in the HPBSMs in the present study. They found that softwood biochar was the most effective 6PPDQ sorbent in their experiments, with sorption equilibrium coefficients more than 50 times higher than the compost used in the 60:40 BSM. Notably, biochar demonstrated nearly irreversible 6PPDQ sorption, distinguishing it further from the other organic sorbents (Hildebrandt et al. 2024).

Coconut coir in the HPBSM primary layer may also impact 6PPDQ removal due to its high porosity and surface area (Tirpak et al. 2021). Coconut coir has typically been used as an organic matter replacement for compost in bioretention soil mixes because it offers similar properties with minimal leaching of nutrients (Tirpak et al. 2021). Esfandiar et al. (2024) found that adding 5 percent coconut coir fibers to a bioretention soil mix (80% sand, 10% silt, 6% clay, and 4% compost) increased removal of polycyclic aromatic hydrocarbons (PAHs) from synthetic stormwater.

5.4 Relationship between Water Quality Parameters on 6PPDQ Concentrations

Several water characteristics and conventional parameters were measured alongside 6PPDQ to discern how they might impact toxicity and concentrations of 6PPDQ (King County 2023). Because nearly all fish died from untreated stormwater exposure and all survived with treated stormwater exposure, we could not explore the impact of water parameters on toxicity of 6PPDQ. The relationships between water quality parameters and 6PPDQ concentrations were visualized with scatterplots. Potential positive relationships with 6PPDQ were observed for TSS (untreated stormwater), DOC (treated water), redox potential (treated water), and specific conductance.

In untreated stormwater, we observed higher 6PPDQ concentrations in samples with higher levels of TSS. This makes sense because 6PPDQ has a moderately high octanol-water coefficient ($K_{ow} = 4.30 \pm 0.02$) and low water solubility (38.4 µg/L), and can readily bind to particles in water (Hu et al. 2023). Additionally, 6PPDQ is transported in water both in a dissolved form and via tire wear particles (Hu et al. 2023), which, depending on particle size, may be measured as TSS.

Though we observed more 6PPDQ detects and higher concentrations at higher DOC concentrations we have too few DOC data points from this study to draw any conclusions on this relationship. However, recent studies of 6PPDQ absorption kinetics by Hildebrandt et al. (2024) indicate that DOC might impact and complicate 6PPDQ sorption dynamics in bioretention media. Given the impacts that stormwater filtration through different bioretention mixes have on DOC (see Section 3.3 and Figure 8), further study of the impacts of DOC on 6PPDQ sorption in stormwater facilities is warranted.

We observed that higher redox potential was associated with higher 6PPDQ concentrations (and more detections). Given that positive redox potential indicates oxidizing conditions, this observation might indicate continued 6PPD transformation impacting effluent 6PPDQ concentrations.

Future research should further examine relationships between 6PPDQ concentrations and water quality characteristics to help explain environmental conditions that influence 6PPDQ fate, transport, and formation in stormwater treatment systems. This information could help our understanding of which BMPs are likely to provide treatment of 6PPDQ.

5.5 Implications for Stormwater Management

Bioretention with the high performance bioretention soil mixes tested in this study has been adopted by King County as both a water quality treatment facility and as a flow control BMP in the 2024 amendment to the 2021 Surface Water Design Manual (King County 2024). Previously, bioretention had not been allowed for water quality treatment in King County because of concerns over the leaching of metals (Colton et al. 2014), arsenic (Batts 2025), and nutrients (Ecology 2016) from compost repeatedly observed in 60:40 BSM. However, in this study and in previous studies of 60:40 BSM (Spromberg et al. 2016; McIntyre et al. 2015), bioretention has been identified as an effective treatment for 6PPDQ that can protect coho salmon from the acute toxicity of 6PPDQ-laden stormwater. The adoption by King County of bioretention using HPBSM as a water quality treatment is therefore an important step toward managing stormwater to protect coho salmon and other sensitive salmonid species from 6PPDQ.

While 60:40 BSM—used widely in Washington—has been shown previously and in the present study to protect coho salmon from acutely toxic stormwater, this study demonstrated that HPBSM achieves better 6PPDQ treatment than 60:40 BSM. While these improvements in treatment appear small, they may be relevant given the extremely low levels of 6PPDQ that can kill coho salmon. HPBSM Types 1, 2, and 3 always reduced stormwater 6PPDQ concentrations to below Washington’s acute aquatic life criteria for 6PPDQ of 12 ng/L (WAC 173-201A-240). This suggests that HPBSM is a better water quality treatment option than 60:40 BSM for meeting statewide water quality goals for 6PPDQ. Further, as noted above, HPBSM does not suffer 60:40’s known net discharge of phosphorus, nitrate, and copper. Because this study was conducted in the lab over a relatively short period of time and does not represent full scale in-situ facility performance, more research is needed to confirm that HPBSM’s effectiveness for 6PPDQ treatment holds up under real-world conditions and longer periods of time.

5.6 Summary of Findings

- All tested HPBSM configurations and the default BSM completely protected juvenile coho salmon from acutely toxic stormwater.
- While all tested media reduced 6PPDQ concentrations by at least 10-fold, the HPBSMs provided small, significant improvements in 6PPDQ treatment.
- Stormwater filtered through the HPBSMs always had 6PPDQ concentrations below Washington State Department of Ecology’s (Ecology) adopted acute aquatic life criteria for 6PPDQ of 12 ng/L (12 parts per trillion). This was not the case for the default soil mix.

- Future work should involve field testing of HPBSMs at a full-scale stormwater treatment facility to ensure lab results hold up under real-world conditions.
- Future research is needed to determine how long HPBSMs last before clogging and/or losing treatment effectiveness.

6 Supplemental Data

The following data sets are available for download online:

Toxicology reports:

<https://your.kingcounty.gov/dnrp/library/2025/kcr4108.pdf>

Chemistry data:

<https://data.kingcounty.gov/d/6xti-bihh>

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Appendix A

Sample Numbers by Parameter and Timepoint

Table A-1. Number of samples with each measurement and analysis for the environmental process points in this study.

Parameter	Time Point	Flush Water (Metals, TSS, DOC)	6PPDQ	Coho Salmon Toxicity	Water Quality (pH, Temp, ORP, Cond, DO)	Toxicity Mitigation Factors (DOC, TSS)
Experimental process point						
<i>6PPDQ sorption loss to equipment</i>	Pre-trial	-	-	-	-	-
Spiked rinse, scale model of column equipment (pumps, tubes, etc.) (spiked D.I. water)		-	2	-	-	-
<i>Prior to preparing BSM/HPBSMx columns</i>						
Rinsate blank, subset of bioretention media column array (D.I. water)		-	1	-	-	-
Rinsate blank, bioretention media column used in loss-to-equipment test (D.I. water)		-	1	-	-	-
Rinsate blank, sampling vessels (D.I. water)		-	1	-	-	-
<i>Flush rinse, following column prep, prior to testing</i>						
Flush water tests, effluent composites from treatment types ready for testing (D.I. water)		8	4	1 FHM	0	0
<i>Untreated (Bellingham) stormwater for dosing-without-sampling (per storm event)</i>						
Grab of untreated influent during dosing without sampling		-	4	-	-	4
<i>Untreated stormwater grab (per storm event)</i>						

Parameter	Time Point	Flush Water (Metals, TSS, DOC)	6PPDQ	Coho Salmon Toxicity	Water Quality (pH, Temp, ORP, Cond, DO)	Toxicity Mitigation Factors (DOC, TSS)
Experimental process point						
Stormwater sample grab, delivered to KCEL immediately	T ₀	-	1	0	1	0
Stormwater sample grab in Bioretention Laboratory at time of influent compositing	T ₁	-	0	0	1	0
Stormwater sample grab upon arrival at KCEL after bioretention test	T ₃	-	1	0	1	0
Untreated stormwater composited into influent for treatment (per storm event)						
Composited stormwater influent in Bioretention Laboratory at time of compositing	T ₁	-	3	0	3	3
Untreated stormwater composite, upon arrival at KCEL after bioretention test	T ₃	-	0	0	1	0
At the point of toxicity testing	T ₄	-	1	1	1	1
Treated stormwater (per storm event)						
Post treatment effluent, at the time of sample collection in bioretention laboratory	T ₂	-	12	0	12	0
Treated stormwater composites, upon arrival at KCEL after bioretention test	T ₃	-	0	0	4	0
At the point of toxicity testing	T ₄	-	4	3	4	4

Appendix B

Bioretention Column Preparation Tests

B.1. Materials

The vendors and brand names of the materials used in the bioretention soil mixtures are provided in the table below.

Table B- 1. Materials used in bioretention mixes.

Material	Vendor, Location	Specific product name (if available)
<i>High performance bioretention mixes</i>		
Sand	Cal Portland Dupont, WA	
Coir	Botanicare IGS, Longview, WA	CocoGro®
Biochar (i.e., HCWA)	Walrath Landscape Supply (Tacoma, Gig Harbor)	Biological Carbon HPG (High Performance Grade) Stormwater Char
Activated Alumina	Axens North America, Houston, TX	ActiGuard® F
Iron aggregate	Connelly GPM, Chicago, IL	
<i>60:40 bioretention soil mix</i>		
Sand	Walrath Castle, Rock, WA	
Compost	Silver Springs Organics Rainier, WA	

B.1. Loss-to-Equipment and Rinsate Blank Results

After deionized water spiked with 6PPDQ was run through a single column of the bioretention array, the apparent loss-to-equipment was 18.75 percent (B-1). The rinsate blank of the column used in the loss-to-equipment test had slight contamination (Table B-1). The amount of 6PPDQ in the rinsate of the loss-to-equipment column (3 ng/L) is below the Reporting Detection Limit (10 ng/L) and any known toxicity threshold. The column used for the loss-to-equipment test was subsequently replaced with a new clean column prior to column packing. 6PPDQ was not detected in the rinsate/effluent composite of the unused columns or the rinsate blank of the FLDE carboy (Table B-1).

Table B-1. 6PPDQ Concentrations in the Loss-to-Equipment and Subsequent Rinsate Blanks.

Spike 6PPDQ (ng/L)			Rinsate 6PPDQ (ng/L)		
Influent	Effluent	Loss to Equipment (%)	Loss-to-equipment column	Unused column composite	FLDE carboy
336	273	18.75	3	<MDL	<MDL

B.2. Fathead Minnow Testing

Researchers observed fathead minnow survival rates of 95 to 100 percent following a 48-hour static exposure to various concentrations (0%, 6.25%, 12.5%, 25%, 50%, 100%) of 60:40 BSM flush water sample (Table B-2). Control survival (0% flush water) was 98 percent after 48 hours. The average ($\pm$ standard deviation) 48-hour survival across all other flush water concentrations was similar to the control at 97.8 percent ($\pm$ 1.78%) (Table B-2).

We conducted a Chi-square test of the 48-hour total number alive count data to test for differences in number of fish alive at the different flush water concentrations. Using a significance level of $\alpha = 0.05$ and 5 degrees of freedom, the critical value (CV) was determined to be 11.05. The calculated Chi-square value was 0.15, much less than the CV; thus, we could not reject the null hypothesis that survival was the same across flush water concentrations.

Table B-2. Fathead minnow toxicity test results performed on dilutions of 60:40 BSM flush water.

Concentration of 60:40 BSM Flush water sample (%)	24h				48h				Total # alive	Survival (%)
	A	B	C	D	A	B	C	D		
0 (control)	10	10	10	10	10	9	10	10	39	98
6.25	9	10	10	10	9	10	10	10	39	98
12.5	10	10	10	10	10	10	10	9	39	98
25	10	10	10	10	10	10	10	10	40	100
50	10	10	10	9	10	10	10	9	39	98
100	10	10	9	10	10	9	9	10	38	95

B.3. HPBSM Primary Layer Synthetic Precipitation Leaching Procedure

The HPBSM primary layer materials, state sand, coconut coir, and high carbon wood ash (biochar) were tested for their contaminant leaching potential using the Synthetic Precipitation Leaching Procedure (SPLP) (U.S. EPA 1994) and the results were compared to Ecology's SPLP specifications (Ecology 2024). Nitrate/Nitrite-N was not detected in any of the leached materials (Table B-3). The coconut coir leachate exceeded Ecology's specifications for ortho-phosphorus (0.8 mg/L) with a concentration of 1.74 mg/L (Table B-3). All other relevant SPLP results were within the Ecology specifications (Table B-3).

Table B-3. Synthetic precipitation leaching procedure (SPLP) results and relevant Ecology SPLP specifications for HPBSM primary layer materials.

Sample ID	Total copper (µg/L)	Dissolved copper (µg/L)	Nitrate-N (mg/L)	Nitrite-N (mg/L)	Ortho-P (mg/L)	Total phosphorus (mg-P/L)
<i>Detection limit</i>	0.346	0.346	0.1	0.1	0.004	0.008
<i>Ecology specification</i>		10	<i>Nitrate + Nitrite: 0.15</i>		<i>Sand: 0.15 Coconut coir, Biochar: 0.8</i>	
State Sand—leached*	6.27	0.784	<0.1	<0.1	0.083	0.023
Coconut coir botanicare—leached	5.17	N/A	<0.1	<0.1	1.74	1.72
High Carbon Wood Ash—leached	2.27	N/A	<0.1	<0.1	0.274	0.271

**Due to a high outlier for total copper in the State Sand leachate sample, this sample was reanalyzed for total copper and dissolved copper in the leachate. The re-analyzed total copper value is reported here. Dissolved copper was only analyzed in this sample. SPLP specifications for total copper and total phosphorus were removed between Ecology's 2021 HPBSM guidance (Ecology 2021) and the 2024 Stormwater Manual (Ecology 2024).*

B.4. Bioretention Column Flushing

In general, we observed a contaminant concentration pattern in column leachates where the 60:40 BSM had the highest contaminant concentrations followed by HPBSM Type 1, and HPBSMs Types 2 and 3 were similar (Table B-4). Typically, contaminant concentrations were much lower in the final flush compared with the initial flush, except

for total and dissolved lead (all mixes) and total and dissolved zinc (HPBSMs), which were slightly higher in final flush leachates compared with initial flush leachates. 6PPDQ was only analyzed for final flush samples and was not detected in any of the bioretention mix leachates.

Table B-4. Contaminant concentrations in leachates from the tested bioretention mixes during the first and final media flushing events. Column leachates (n = 3) were composited prior to analysis. Columns are colored by bioretention treatment to facilitate comparison between initial and final leachates.

Analytes	Initial Flush Leachates				Final Flush Leachates			
	60:40 BSM	HPBS M Type 1	HPBS M Type 2	HPBSM Type 3	60:40 BSM	HPBS M Type 1	HPBSM Type 2	HPBS M Type 3
6PPDQ (ng/L)					<2	<2	<2	<2
TSS (mg/L)	70.5	16	7.6	10.6	7.5	8.8	2.4	2.4
DOC (mg/L)	73.3	1.6	1.1	3.13	7.47	1.1	0.63	1
Diss. Cd (µg/L)	1.12	0.42	<0.12	<0.12	<0.06	<0.06	<0.06	<0.06
Total Cd (µg/L)	1.54	0.46	0.18	0.15	<0.06	<0.06	<0.06	<0.06
Diss. Ca (mg/L)	96.9	9.22	1.47	3.28	12.2	1.79	1.46	2.08
Total Ca (mg/L)	108	12.1	1.47	3.44	12.9	2.03	1.4	2.16
Diss. Cu (µg/L)	27.9	3.9	0.5	1.3	7.8	4.7	0.8	1.2
Total Cu (µg/L)	32.8	6.5	1	2.2	9.3	7.3	1.4	1.9
Diss. Pb (µg/L)	3	0.48	<0.060	0.13	7	7	0.1	0.2
Total Pb (µg/L)	4.7	0.82	0.1	0.24	10	10	0.2	0.3
Diss. Mg (mg/L)	33.7	3.66	0.31	0.73	4.2	1.11	0.381	0.625
Total Mg (mg/L)	36.9	4.34	0.38	0.86	4.47	1.69	0.456	0.793

	Initial Flush Leachates				Final Flush Leachates			
Analytes	60:40 BSM	HPBS M Type 1	HPBS M Type 2	HPBSM Type 3	60:40 BSM	HPBS M Type 1	HPBSM Type 2	HPBS M Type 3
Diss. Zn (µg/L)	17.7	3.78	<1.00	<1.00	11	11.8	<0.860	<0.860
Total Zn (µg/L)	27.8	11.7	<1.00	1.33	14.3	14.2	1.3	1.74

Appendix C

Summary Statistics of All Parameters by Timepoint

Table C-1. Mean and standard error of 6PPDQ and water quality characteristics throughout this study. The red X in the Client Locator column indicates characters in client locators that change based on storm event, column replicate, or grab replicate.

Sample Type	Experimental Process Point	Client Locator	Time Point	Mean (standard error)*							
				6PPD Q (ng/L)	TSS (mg/L)	DOC (mg/L)	Specific conductance (µS/cm)	DO (mg/L)	pH	Temp (°C)	OR P (mV)
Untreated stormwater (grab)	Stormwater sample grab	SXT0_U_I5_G	T0	643 (180)			911 (494)	10.3 (0.5)	7.42 (0.13)	12.2 (2.5)	-19.0 (10.3)
	Stormwater sample grab in bioretention laboratory at time of influent compositing	SXT1_U_I5_G	T1	541 (289)			926 (508)	10.4 (0.4)	7.37 (0.09)	6.0 (1.8)	-22.5 (6.9)
	Stormwater sample grab (KCEL after bioretention test)	SXT3_U_I5_G	T3	737 (269)			928 (508)	10.9 (0.2)	7.44 (0.06)	4.2 (1.1)	-21.9 (0.97)
Untreated stormwater composited into influent	Composited stormwater influent in bioretention laboratory at time of compositing	SXT1X_U_INF_G	T1A, T1B, T1C	576 (89)	102 (24)	10.0 (0.4)	758 (180)	10.1 (0.2)	7.52 (0.04)	11.8 (1.0)	-20.2 (2.6)
	Untreated stormwater composite (KCEL after bioretention test)	SXT3_U_INF_G	T3	259	15.9	9.8	745 (368)	10.8 (0.2)	7.53 (0.11)	6.3 (0.06)	-26.2 (4.4)
	During toxicity testing	SXT4_U_INF_E	T4	540 (161)	160 (113)	9.8 (0.8)	309 (148)	10.1 (0.2)	7.42 (0.06)	11.2 (0.66)	-19.1 (4.50)

Sample Type	Experimental Process Point	Client Locator	Time Point	Mean (standard error)*							
				6PPD Q (ng/L)	TSS (mg/L)	DOC (mg/L)	Specific conductance (µS/cm)	DO (mg/L)	pH	Temp (°C)	OR P (mV)
Bellingham stormwater using in-column aging	Collected from WSDOT stormwater pond adjacent to I-5 and used to age columns.	DoseX		20.8 (9.8)	19.4 (7.2)	10.5 (1.1)					
60:40 BSM	Post treatment effluent at the time of sample collection in bioretention laboratory	SXT2_T_60:40_X_E	T2	8.8 (2.7)	11 (2.2)		818 (153)	10.1 (0.2)	6.97 (0.04)	10.3 (0.56)	1.6 (3.9)
	Treated stormwater composites (KCEL after bioretention test)	SXT3_T_60:40_EC	T3				935	10.4	6.98	8.9	4.0
	During toxicity testing	SXT4_T_60:40_EC	T4	11.5 (7.7)	14 (5.4)	14.8 (1.4)	308 (135)	10.3 (0.5)	7.26	11.5 (0.20)	-12.9 (0.15)
HPBSM Type 1	Post treatment effluent at the time of sample collection in bioretention laboratory	SXT2_T_HP1_X_E	T2	2.1 (0.6)	3.9 (0.79)		701 (151)	10.4 (0.2)	7.19 (0.02)	10.9 (0.71)	-8.9 (2.5)
	Treated stormwater composites (KCEL after bioretention test)	SXT3_T_HP1_EC	T3				846	10.8	7.26	9.5	-6.3
	During toxicity testing	SXT4_T_HP1_EC	T4	3.4 (2.2)	6.1 (2.0)	6.3 (1.6)	288 (88)	10.3 (0.3)	7.53 (0.21)	11.5 (0.50)	-9.1 (5.0)
HPBSM Type 2	Post treatment effluent at the time of sample collection in bioretention laboratory	SXT2_T_HP2_X_E	T2	1.2 (0.7)	5.1 (1.5)		670 (148)	8.9 (0.2)	7.47 (0.10)	10.5 (0.60)	-24.3 (0.64)

Sample Type	Experimental Process Point	Client Locator	Time Point	Mean (standard error)*							
				6PPD Q (ng/L)	TSS (mg/L)	DOC (mg/L)	Specific conductance (µS/cm)	DO (mg/L)	pH	Temp (°C)	OR P (mV)
	Treated stormwater composites (KCEL after bioretention test)	SXT3_T_H P2_EC	T3				669 (298)	9.3 (0.1)	7.43 (0.20)	10.3 (1.1)	- 18.3 (5.6)
	During toxicity testing	SXT4_T_H P2_EC	T4	<MDL	5.9 (2.8)	2.9 (0.96)	673 (301)	10.2 (0.5)	7.32 (0.15)	7.58 (2.7)	- 11.8 (2.5)
HPBSM Type 3	Post treatment effluent at the time of sample collection in bioretention laboratory	SXT2_T_H P3_X_E	T2	1.4 (0.7)	7.7 (1.3)		685 (147)	8.3 (0.2)	7.46 (0.06)	10.2 (0.58)	- 26.0 (2.1)
	Treated stormwater composites (KCEL after bioretention test)	SXT3_T_H P3_EC	T3				810	8.8	7.6	9.0	- 22.6
	During toxicity testing	SXT4_T_H P3_EC	T4	1.6 (1.6)	6.3 (3.5)	3.4 (0.84)	237	9.5 (0.4)	7.53 (0.31)	11.3 (0.57)	- 16.5

Appendix D

Relationships between Water Quality Characteristics and 6PPDQ

As an extension of this project's secondary goal (see Section 1.1.1), we investigated potential relationships between 6PPDQ and other measured water quality parameters with scatterplot visualizations (Figure D-1) of each parameter versus 6PPDQ in samples of untreated and bioretention-treated stormwater. Identifying potential relationships between 6PPDQ and water quality characteristics could improve our understanding of the chemical's fate and transport in stormwater and stormwater treatment facilities. Relationships between 6PPDQ and water quality parameters differed between treated and untreated samples.

In untreated stormwater samples, 6PPDQ concentrations were higher at higher concentrations of TSS. No clear relationship was observed in treated stormwater samples where TSS and 6PPDQ values were low in general. We also observed increasing 6PPDQ concentrations with increasing specific conductance. This pattern was observed in both untreated and treated stormwater samples; however, in the treated stormwater samples this appears primarily driven by higher 6PPDQ and conductivity in samples from Storm 3. While we did not see signs of a relationship between redox potential and 6PPDQ in untreated stormwater, in the treated stormwater samples we saw higher 6PPDQ concentrations and more detections when redox potential was higher. Similarly, untreated stormwater samples showed no relationship between DOC and 6PPDQ but treated stormwater samples had more 6PPDQ detections at higher DOC levels. No relationships were observed between 6PPDQ and either pH or temperature in any water samples.

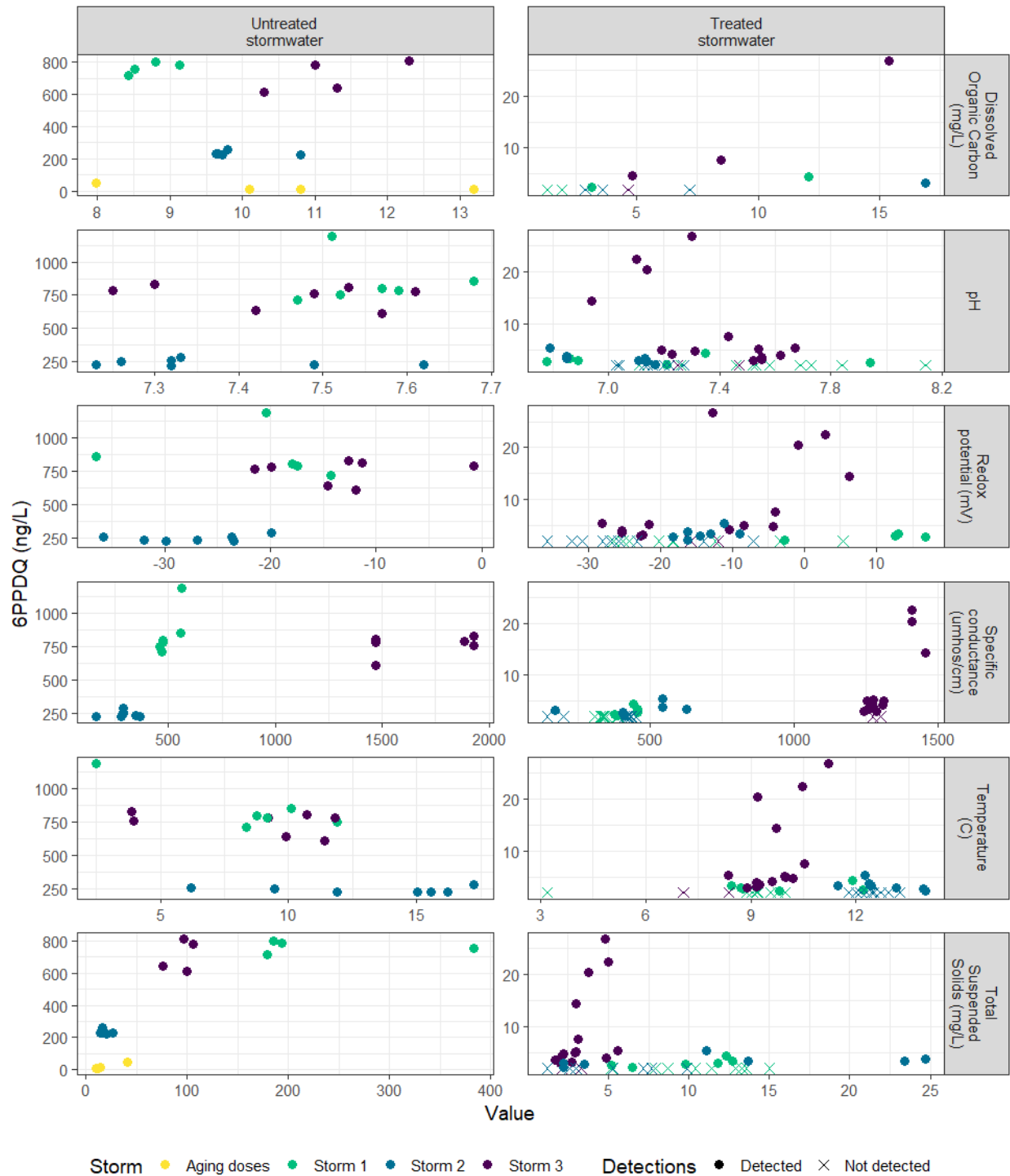


Figure D-1. Scatterplots of 6PPDQ and potential explanatory covariates in untreated (left) and treated (right) stormwater samples where both 6PPDQ and covariate were measured. Note that both X and Y axes are on different scales for each parameter and water type to best show relationships in the data.

Appendix E

Summary of QAPP Deviations

E.1. Field Procedures

- The QAPP stated that stormwater would be collected from a tap at the I-5 sampling site (King County 2023). This process was overly time-consuming due to the low-flow rate at the tap so the Field Science Unit (FSU) employed Contingency Plan Situation 1 (described in the QAPP, Appendix B: Field Sampling Plan) for all sampling events. This involved using a heavy-duty submersible bilge pump to pump water from the flow splitting vault into the stormwater sample containers.
- The QAPP stated that storms with at least 0.25 inches of rain would be targeted for sampling. However, constraints around scheduling and transportation necessitated a more opportunistic sampling approach. Thus, sampling did not always follow 0.25 inches of rain but always targeted rain events that produced flow at the sampling site, which was the primary criterion.
- A minor deviation was made for the handling of stormwater collected at timepoint T₀ (Table 1) where we separated aliquots used for 6PPDQ analysis and the measuring of water quality characteristics through the storm cycle. This adjustment was made after Storm 1 to minimize the exposure of the 6PPDQ analysis aliquot to air while water quality measurements were taken.
- Three grab samples of the influent (untreated stormwater composite) were collected at the beginning, middle, and end of the process of delivering influent to the bioretention columns (n = 3 for each influent parameter). The QAPP did not specify any specific timing of these samples. This influent sampling approach would not have impacted results because the 6PPDQ concentrations were very similar across these sampling timepoints within each storm.

E.2. Bioretention Media Lab Procedures

- Field parameters (temperature, conductivity, pH, redox potential, and dissolved oxygen) were scheduled to be collected for dosing events with sampling when compositing the three replicates for each treatment (Timepoint T₂, Table 1). Field scientists forgot to take these measurements for the second dosing event with sampling on 09/26/2023. Field parameters were instead measured from the composite samples when the Aquatic Toxicology lab started their toxicity analysis. We expect this omission to have little to no effect on interpretation of results.
- Before blending, the quality of the media components was tested using the Synthetic Precipitation Leaching Procedure (SPLP; EPA 1994) described in the HPBSM specifications (Ecology 2021). Copper was re-analyzed in the sand SPLP leachate because of a suspect high value of 63.4 µg/L. The re-analyzed sand leachate had a revised copper concentration of 0.748 µg/L. The coconut coir exceeded the ortho- and total phosphorus thresholds. The high carbon wood ash exceeded the threshold

for ortho-phosphorus. After review, the team decided to proceed with the materials acquired and tested for the following reasons:

- The materials were acquired from a reputable vendor and the same manufacturer that supplied components for developing the HPBSM.
 - These components have exceeded phosphorus thresholds in previous testing and still performed well to meet guidelines for filter media.
 - Phosphorus was not considered an important element impacting our planned coho salmon toxicity testing.
- The QAPP stated that samples would be collected from the untreated influent for the aging stormwater at the 3rd, 5th, 7th, and 9th dosing events. Aging stormwater samples were collected at the 3rd, 4th, 7th, and 8th dosing events (Table 1). The purpose of analyzing these samples was to qualitatively characterize the representativeness of one Seattle water year. This deviation did not impact the study because the 6PPDQ data obtained still characterize stormwater from the Bellingham collection site across a similar time period. The reasons for this deviation are:
 - The 5th event was mistakenly switched for the 4th event.
 - The courier responsible for transporting samples from Bellingham to Seattle was only available on specific dates for the 8th event but was free during the 9th event.

E.3. Analytical Chemistry Procedures

- Preservation holding times were exceeded by 1 day for ammonia for five samples and these were H-flagged. This may have biased ammonia results low, but this does not impact our results because we did not use ammonia in data analyses and control fish remained in good health throughout the study.
- Preservation holding times were exceeded by 1 day for DOC for five samples and these were H- and SH-flagged. This holding time exceedance is not expected to have meaningfully impacted the data because it was only 1 day out of hold, and others have reported no significant impact of exceeding DOC holding times for up to 21 days when samples are held at 4°C (Government of Newfoundland and Labrador 2010).
- A large discrepancy between specific conductance readings at timepoints T3 and T4 occurred in Storm 3. KCEL's investigation revealed that this was due to an issue with settings on the Aquatic Toxicology lab's instrument; thus, specific conductance readings for four samples were rejected and T3 specific conductance readings were reported in the Storm 3 toxicology report.
- During Storm 2, water quality characteristic data (pH, specific conductance, temperature, dissolved oxygen, and redox potential) were not collected at timepoint T3 because of analyst oversight. This did not impact results or interpretation of data because these characteristics were measured in samples at timepoints T1, T2, and T4.

E.4. Toxicology

- As expected, and stated in section 7.2 of the QAPP (King County 2023), fish exposed to samples from Storm 2 were older and experienced higher loading rates than specified in EPA Test Method 2019.0. Details on fish age and loading rates are summarized below (Table E-1). We don't expect this meaningfully affected the results because reference toxicity tests remained within two standard deviations of the mean LC50 throughout the study.

Table E-1. Age, size, and loading rate of juvenile coho salmon used in exposure tests.

Test #	Age (days-post swim-up at start of test)	Mean Standard Length (cm)	Mean Weight (grams)	Loading Wt./Vol. (g/L)
EPA 2019.0	Rainbow Trout: 15 to 30 Brook Trout: 30 to 60	—	—	—
Storm 1	58	3.9	0.69	0.69
Storm 2	290	5.66	2.64	2.64
Storm 3	35	3.26	0.31	0.31

- Because of dry weather in spring 2023, additional time was needed to capture the three storm events required by the QAPP (King County 2023). While enough juvenile coho salmon were obtained for the original project schedule, the extended project timeline required additional reference tests because the juvenile coho salmon aged and led to the cohort being depleted prior to the third storm's toxicity testing.
- Additional coho salmon were obtained for this third event. This deviation is described in the QAPP Appendix – D, section 1: Corrective actions to meet QAPP requirements.