Deliverable 4.1: Progress Report 1

Overview

		Water Quality		Toxicology		Stormwater	
Water Year	Event	Basic	Full	Zfish	Coho	Collection Date	Treatment Dates
1	1	x	x	*	x		
	2	x				12/12/19	12/13/19-12/14/19
	3	x				12/16/18	12/17/19-12/18/19
	4	x				1/8/20	1/9/20-1/10/20
	5	x				1/17/20	1/17/20-1/18/20
	6	x	x**	*		1/27/20	1/28/20-1/29/20
2	7	x				2/6/20	2/6/20-2/7/20
	8	x				2/10/20	2/10/20-2/11/20
	9	x				2/17/20	2/17/20-2/18/20
	10	x				2/24/20	2/24/20-2/25/20

*Zebrafish assays not yet been completed for Events 1 and 6.

**Results from Spectra Laboratories have not yet been received for Event 6. Only PAH results (from ARI Laboratories) are presented here.

Report Summary

1. Urban Stormwater Runoff Collection
   - Stormwater runoff was collected from downspouts draining SR 16 West in Tacoma, WA in polyethylene tanks.
   - Runoff was collected and transported to the WSU-Puyallup campus in stainless steel tanks.
   - The temperature in the environmental growth chamber where experimental columns are located was set to approximate temperatures expected from in-ground installations of bioretention cells during the accelerated water year.

2. Event 1
   - Influent stormwater to the experimental bioretention columns was most concentrated in zinc among the measured metals.
   - Effluent stormwater from the bioretention columns was most concentrated in copper among the measured metals.
   - A net export of nitrates, orthophosphate, and DOC from the bioretention columns was observed for all treatment depths.
   - PAHs in influent stormwater were predominantly low molecular weight, dominated by carbazole, with a summed total concentration (TPAH) of 0.474 ppb.
   - PAHs in bioretention-treated effluent waters were predominantly low molecular weight PAHs, dominated by naphthalene.
   - Mean TPAH removal efficiency ranged from 86-93.1%.

- Untreated highway runoff was acutely lethal to juvenile coho salmon (87.5% mortality).
- Treatment of runoff through the bioretention soil medium completely prevented mortality for all depths of bioretention.

3. Events 2-5
    - Stormwater for events 2-5 was collected between December 12, 2019 and January 17, 2020.

4. Event 6
    - PAHs in stormwater runoff were predominantly high molecular weight, dominated by pyrene with TPAH concentration = 0.789 ppb.
    - PAHs in bioretention-treated effluent waters were predominantly low molecular weight.
    - Mean TPAH removal efficiency ranged from 93.2-95.1%.

5. Saturated Hydraulic Conductivity (K_{sat})
    - K_{sat} values (determined after Event 6) were generally less than the baseline K_{sat} determined prior to stormwater dosing in July 2019.

1. Urban Stormwater Runoff Collection

Stormwater runoff was collected from SR 16 West just west of the I-5/SR 16 interchange in Tacoma, WA (Figure 1A). Runoff was collected in two 500-gallon polyethylene collection tanks from downspouts draining the same section of highway (Figure 1B). The collection tanks remain permanently at the site.

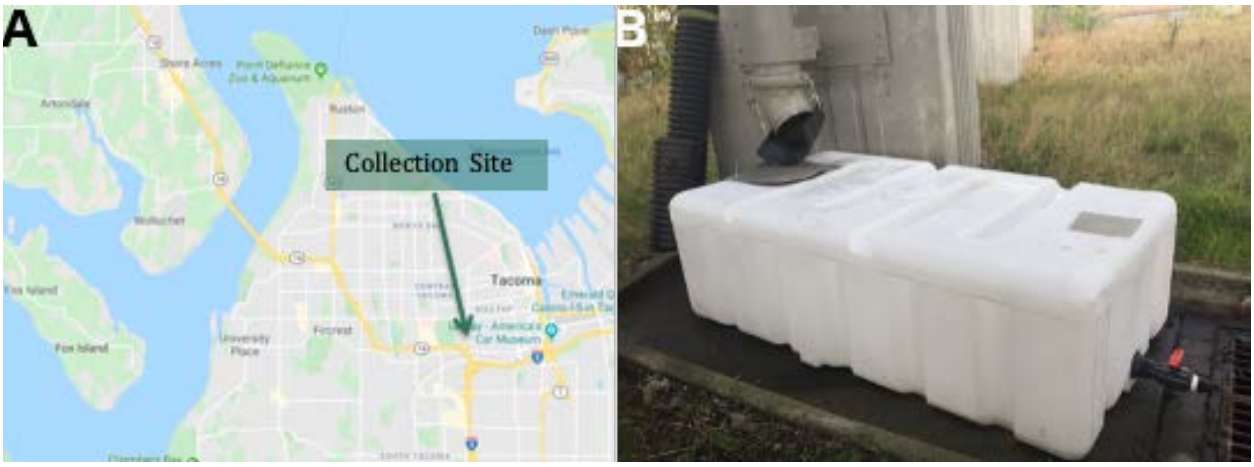
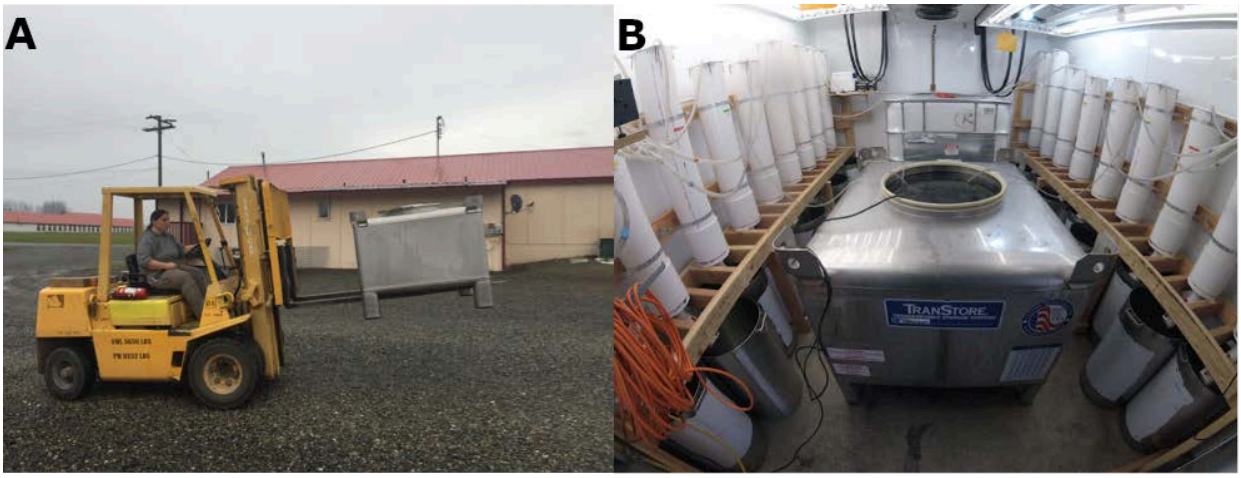


Figure 1. A) Approximate collection site just past the I-5/ SR16 interchange. B) 500-gallon polyethylene collection tank used to collect urban stormwater runoff.

Runoff accumulated in the collection tanks was collected and tranported to the WSU-Puyallup campus using stainless steel 250-gallon tanks affixed to a truck bed and/or trailer (Figure 2). Before stormwater was transferred from the collection tank to the transportation tank, the water was recirculated within the collection tank for 10 minutes to evenly disperse particulates that may have settled to the base of the tank. Once the stormwater was well-mixed and homogenous, it was pumped from the collection tank to the transportation tank using a gas-powered pump and food-grade hosing. A 400-micron mesh sock placed over the receiving end of the hose filtered out larger particles that have the potential to clog the tubing system used to deliver stormwater runoff to the experimental columns. Filtering stormwater at this size does not exclude particles most commonly generated on roads from tires and other traffic-related sources. Roadway particles (which include contributions from sources including tires, fuel, brakes, pavement, and atmospheric deposition) collected in a study by Kreider et al. (2010) were unimodally distributed from 4 µm to 280 µm.

Figure 2. A) Stainless steel transportation tanks at the WSU-Puyallup campus ready to transfer stormwater from the collection site. B) Stormwater runoff pumped from the polyethylene collection tanks to the stainless steel transportation tanks using a gas-powered pump and food-grade hosing.

At the WSU-P campus, transportation tanks were transferred by forklift and pallet jack to the environmental growth chamber where the experimental columns are located (Figure 3). A 300-gallon polyethylene tank filled with clean water (used to dose the clean water control experimental columns) was also placed in the chamber. Clean water was municipal water treated by reverse osmosis and re-constitution with salts for rearing fish at WSU-Puyallup. A submersible pump placed in the stormwater transportation tank recirculated the water during dosing. The transportation and clean water tanks were placed inside the environmental chamber so that the temperatures of the influent waters matched that of the ambient chamber temperature.



F**igure 3.** A) Transportation tanks filled with stormwater runoff are moved into the temperature-controlled chamber. B) The clean water tank (rear) and stormwater transportation tank (front) in the temperature-controlled chamber with the experimental columns.

Temperature and relative humidity (RH) are controlled in the environmental growth chamber with a programmable electronic system. Temperature was set to approximate

temperatures expected from in-ground installations of bioretention cells at an average 8-inch soil depth of the simulated month in Puyallup, WA. RH was similarly set to match the average air RH of the simulated month. Simulated months were accelerated to last 5 days each, so that the length of one water year (WY) is approximately two months (Figure 4). Water Year 1 (WY1) was comprised of the first six collected events; three during December 2019 and three during January 2020 (Figure 4). Water Year 2 began in February 2020.

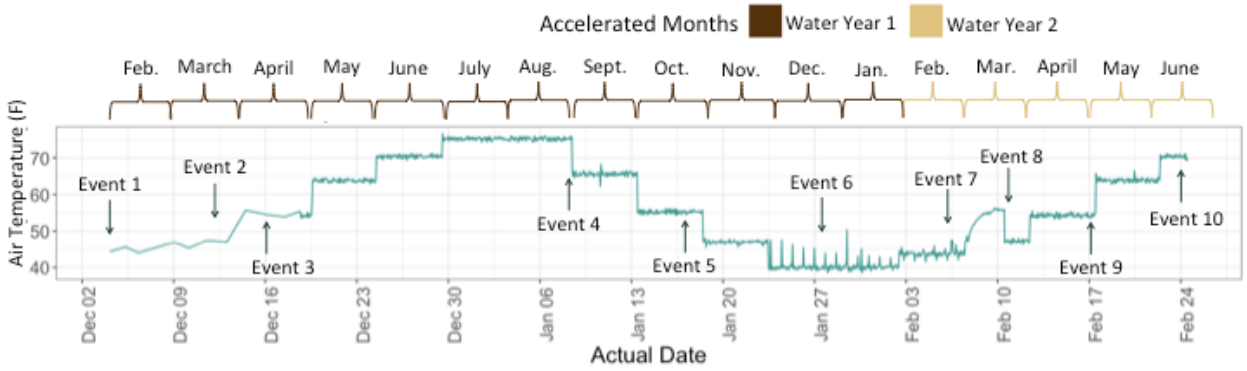


Figure 4. Timeline of temperatures (F) in the environmental chamber during the first 10 events.

2. Event 1: Chemistry and Toxicology

Experimental columns were dosed for approximately 24-hours beginning at 8 a.m. on December 5, 2019. Each experimental column received 55.6 L of water at 32.5 – 36.6 mL min^{-1} (target rate: 38.3 mL min^{-1}). Effluent was collected in stainless steel containers below the valve draining each column. The containers were stirred with stainless steel paddles prior to sub-sampling for water chemistry and toxicology. Stainless steel pitchers were then used to collect grab samples from each of the containers. Effluent temperatures from each column were measured prior to sample collection.

Chemistry Results

Metals

Total metals in the influent stormwater were concentrated in the order of Zn>Cu>Ni>Pb (Table 1). Arsenic and cadmium were not detected in influent stormwater. In effluent stormwater, total metals were detected at concentrations of Cu>Zn>Ni>As>Pb regardless of the bioretention depth being tested. The dominance of Cu in effluent, as well as the detection of As, can be attributed to the bioretention media as a source of these two metals, as was evident from the concentrations measured in effluent from the clean water control columns (18” CWC).

To determine whether there was a difference in effluent total metals concentrations between the five BSM depths, a nonparametric approach to multivariate inference was utilized (Woodrow et al. 2017). The effect of treatment was significant, according to a Wilks' Lambda type test (Test Statistic = 3.06, p-value = 0.007); with higher metals in effluent from the 18-inch depth than the 9-inch and 6-inch depths, according to a subset algorithm that identifies significant response factors while controlling the familywise error rate (Woodrow et al. 2017).

Differences among the five treatment depths for concentrations of each metal in effluent were then assessed by a Kruskal-Wallis test, followed by a post-hoc Dunn's test. Statistically significant differences existed for concentrations of total copper ($\chi^2(5) = 1.8$, p=0.038), total zinc ($\chi^2(5) = 14.6$, p=0.012), and total nickel ($\chi^2(5) = 13.1, \mathrm{p} = 0.023$), with significantly higher concentrations in effluent from the 18-inch depth compared with the 6-inch depth. Leaching of these metals from the BSM is the likely cause of higher concentrations for the treatments with more depth of BSM.

Dissolved metals in the influent stormwater were concentrated in the order of Zn>Cu>Ni (Table 1). Dissolved arsenic, cadmium, and lead were not detected in the influent stormwater. In effluent, dissolved metals were detected at concentrations of Cu>Zn>Ni>Pb>As regardless of depth. The effect of treatment (varying depths) was not significant, according to a Wilks' Lambda type test (Test Statistic = 0.975, p-value = 0.531).

For total metals with higher concentration in the influent than the effluent, removal efficiency (%) was calculated as: $\frac{\mathrm{Influent-Effluent}}{\mathrm{Influent}}$ x 100, where influent is the metal concentration in stormwater and effluent is the concentration of metal in effluents from each of the five treatment depths. For total metals with higher concentrations in the effluent than in influent, the net concentration was calculated by subtracting the influent from the effluent concentration.

Mean removal efficiencies for total copper were 44-53%, 87-97% for lead, and 89-92% for zinc (Figure 5A). For the 6" depth, there was 20% removal of nickel, but this declined to the point that there was net export of nickel at the 18" BSM depth. All depths showed a net export of arsenic, with net concentrations increasing from 0.91 µg/L in effluent from the 6" depth to 1.5 µg/L at the 18" depth.

Mean removal efficiencies (standard error) for dissolved copper were 12-45%, 61-76% for zinc, and 7-74% for nickel. For the 15" depth, there was net export of dissolved lead of 3 µg/L. There was also a net export of dissolved arsenic for the 6", 15", and 18" depth (net concentrations = 0.4-0.6 µg/L).

Table 1. Mean (standard error) of total metals in influent waters (clean water and stormwater runoff; SW) and triplicate effluent waters from each of the five treatment depths plus the clean water control (CWC). One-half of the value of the detection limit (DL) was substituted for the value of non-detects in calculating means unless all replicates were below the detection limit (BDL).

Metal	DL	Influent Water (µg/L)		Effluent Water (µg/L)					
		Clean	SW	6"	9"	12"	15"	18"	18" CWC
Arsenic	0.05	BDL	BDL	0.93 (0.09)	0.9 (0.2)	1.3 (0.2)	1.5 (0.4)	1.5 (0.1)	1.7 (0.5)
Cadmium	0.05	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL
Copper	0.2	4.8	25.5	12.0 (0.5)^{a}	12.93 (0.07)^{ab}	13.0 (0.3)^{ab}	12.2 (0.5)^{ab}	14.3 (0.7)^{b}	12.2 (0.4)
Lead	0.079	BDL	1.5	0.2 (0.2)	BDL	BDL	BDL	BDL	BDL
Nickel	0.2	BDL	2.9	2.3 (0.2)^{a}	2.8 (0.2)^{ab}*	3.1 (0.1)^{ab}**	3.1 (0.2)^{ab}	4.2 (0.4)^{b}	3.4 (0.2)
Zinc	0.19	1.2	77.6	6.6 (0.5)^{a}	7.2 (0.3)^{ab}	8.1 (0.2)^{ab}	7.9 (0.2)^{ab}	8.57 (0.09)^{b}	2.3 (0.3)
Dissolved Arsenic	0.05	BDL	BDL	0.4 (0.2)	BDL	BDL	1.0 (0.6)	0.6 (0.3)	0.8 (0.6)
Dissolved Cadmium	0.05	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL
Dissolved Copper	0.05	BDL	12.6	9.8 (0.1)	10 (1)	9.1 (0.9)	7 (3)	11.0 (0.6)	9.0 (0.1)
Dissolved Lead	0.079	0.8	BDL	BDL	BDL	BDL	3 (3)	BDL	BDL
Dissolved Nickel	0.2	BDL	2.9	1.9 (0.3)	0.8 (0.2)*	0.8 (0.7)**	1.7 (0.6)	2.7 (0.5)	3.0 (0.4)
Dissolved Zinc	0.19	0.6	18.3	6 (1)	4 (1)	5 (1)	7.2 (0.3)	6.3 (0.7)	1.8 (0.3)

BDL = Below Detection Limit (DL)

Note: Treatments with different superscript group labels (a, b, c) are significantly different at □ = 0.05 (Kruskal-Wallis with post-hoc Dunn Test).

*Average of two replicates. The third replicate was excluded as an outlier because its value was two orders of magnitude greater and did not agree with the low inter-replicate variability in total Ni for these samples.

** Average of two replicates. The third replicate was excluded as an outlier because its value was two orders of magnitude greater.

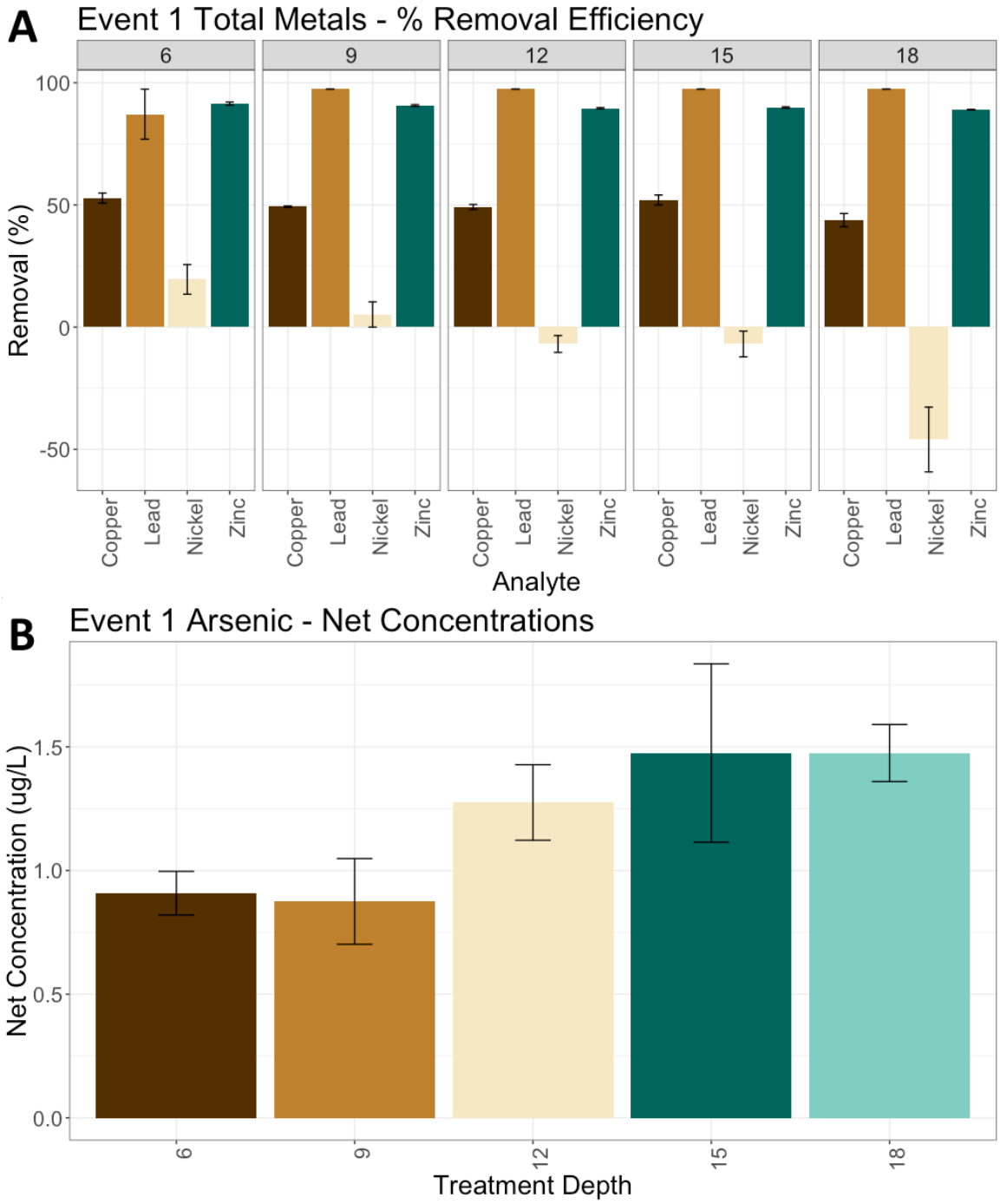


Figure 5. A) Mean (standard error) total metals removal efficiency for each of the five treatment depths for the measured metals with higher concentrations in the influent than in the effluent. B) Mean (standard error) net concentration of total arsenic, which was more highly concentrated in the effluent than in influent for all treatment depths. Cadmium was not included in the plots because it was not detected in the stormwater influent sample or in any effluent samples. One-half of the value of the detection limit (DL) was substituted for the value of non-detects in calculating mean removal efficiencies for total metals.

PAHs

TPAH was calculated by summing the concentrations of each of the PAH congeners detected above the method detection limit (MDL). TPAH in influent stormwater was 0.474 µg/L (Table 2). In effluent samples, TPAH ranged from 0.033-0.070 µg/L (Table 2). No significant differences were observed in effluent TPAHs among the five treatment depths, according to a Kruskal-Wallis test ($\chi^2(4)=6.657$, p = 0.155). Removal efficiencies for TPAHs were 86-93% (Table 2). PAHs in stormwater runoff for Event 1 were predominantly low molecular weight, dominated by carbazole. In bioretention-treated effluent waters, PAHs were similarly predominantly low molecular weight but were dominated by naphthalene. A complete table and figure of the PAH congeners can be found in Appendix A.

Table 2. Mean (standard error) of TPAHs in influent waters (clean water and stormwater runoff; SW) and triplicate effluent waters from each of the five treatment depths plus the clean water control (CWC), and removal efficiencies (%) for the five treatment depths. PAH congeners below the method detection limit (MDL) were assigned a value of zero.

Treatment	TPAH (µg/L)	Removal Efficiency (%)
SW influent	0.474	n.a.
6	0.07 (0.02)	86 (4)
9	0.037 (0.004)	92.1 (0.8)
12	0.033 (0.002)	93.1 (0.4)
15	0.037 (0.004)	92.1 (0.9)
18	0.0380 (0.0006)	92.0 (0.9)
Clean water influent	0.018	n.a.
18 CWC	0.0250 (0.0006)	n.a.

Nutrient & Conventional Water Chemistry

A net export of nitrates (nitrate + nitrite) and orthophosphate was observed for all treatment depths (Table 3; Figure 6). The average effluent concentrations of these nutrients generally increased with increasing BSM depth. However, nitrates concentrations were not significantly different between the five BSM depths (Kruskall-Wallis: $\chi^2 = 5.9$, p = 0.207), nor were orthophosphate concentrations ($\chi^2 = 8.43$, p =0.077).

Differences among the five treatment depths for concentrations of each conventional parameter in effluent were assessed by a Kruskal-Wallis test, followed by a post-hoc Dunn's test. Turbidity generally increased with treatment depth ($\chi^2 = 9.63$, p = 0.047) and was significantly greater in effluent from the 18" columns compared to the 6" columns (p = 0.0259).

Additionally, pH varied significantly among treatment depths ($\chi^2 = 11$, $p = 0.024$) and was significantly greater in effluent from the 6”columns than from the 15”columns ($p = 0.0346$).

Table 3. Mean (standard error) of nutrients and conventional parameters in influent waters (clean water and stormwater runoff; SW) and triplicate effluent waters from each of the five treatment depths plus the clean water control (CWC).

Measurement	Detection Limit	Influent Water		Effluent Water					
		Lab	SW	6”	9”	12”	15”	18”	18” CWC
Nitrates (mg/L)	0.003	0.17	0.67	2.2 (0.3)	2.6 (0.4)	3.5 (0.7)	5 (2)	4.6 (0.5)	2.6 (0.4)
Orthophosphate (mg/L)	0.01	0.04	0.04	0.14 (0.02)	0.22 (0.04)	0.27 (0.02)	0.29 (0.03)	0.30 (0.02)	0.41 (0.05)
DOC (mg/L)**	0.08	0.5	11.5	16.8 (0.3)	19 (1)	18.1 (0.9)	19.3 (0.8)	22 (3)	11.1 (0.5)
Temperature (°F)		-	-	41.4 (0.3)	40.8 (0.3)	41.2 (0.4)	40.3 (0.6)	41.5 (0.3)	41.3 (0.4)
pH	n.a.	7.68	7.76	7.51 (0.01)^{a}	7.489 (0.009)^{ab}	7.45 (0.02)^{ab}	7.43 (0.02)^{b}	7.462 (0.009)^{ab}	7.47 (0.01)
Conductivity ($\mu S/cm$)		1561	1478	1503 (7)	1507 (6)	1529 (8)	1539 (10)	1540 (4)	1645 (5)
Turbidity (NTU)	n.a.	0.25	17.9	3.6 (0.3)^{a}	8 (2)^{ab}	9.1 (0.7)^{ab}	8 (1)^{ab}	12 (1)^{b}	14 (1)
Alkalinity (as $CaCO_3$)	0.3	32.9	62.2	53.5 (0.8)	56 (1)	54.3 (0.4)	61 (2)	60 (2)	39.6 (0.8)
TSS (mg/L)	0.5	0.5	27.4	1.5 (0.2)	1.3 (0.1)	1.7 (0.4)	2.8 (0.5)	3.5 (0.5)	3.3 (0.2)
Fecal Coliform (CFU/100 mL)	n.a.	5000	10	453 (271)	177 (147)	10 (0)	27 (12)	BDL*	693 (693)*
Dissolved Calcium (mg/L)	3.4	7.89	86.3	60 (2)	54 (2)	52 (2)	54 (4)	49 (1)	32.6 (0.3)
Dissolved Magnesium (mg/L)	1.9	20.9	3.1	12.3 (0.9)	13.8 (0.9)	14.0 (0.9)	13.9 (0.5)	14.5 (0.6)	10.9 (0.1)
Dissolved Sodium (mg/L)	27	239	195	198 (1)	202 (2)	196 (2)	199 (2)	195 (2)	230.0 (0.6)

Note: Treatments with different superscript group labels (a, b, c) show significance at □ = 0.05 (Kruskal-Wallis with post-hoc Dunn Test).
BDL = Below Detection Limit
NTU = Nephelometric Turbidity Units
*A large amount of non-fecal coliform growth on the plate for two of three treatment replicates, which the lab flagged as contamination.
**Dissolved organic carbon was analyzed outside of holding time due to an instrument issue.

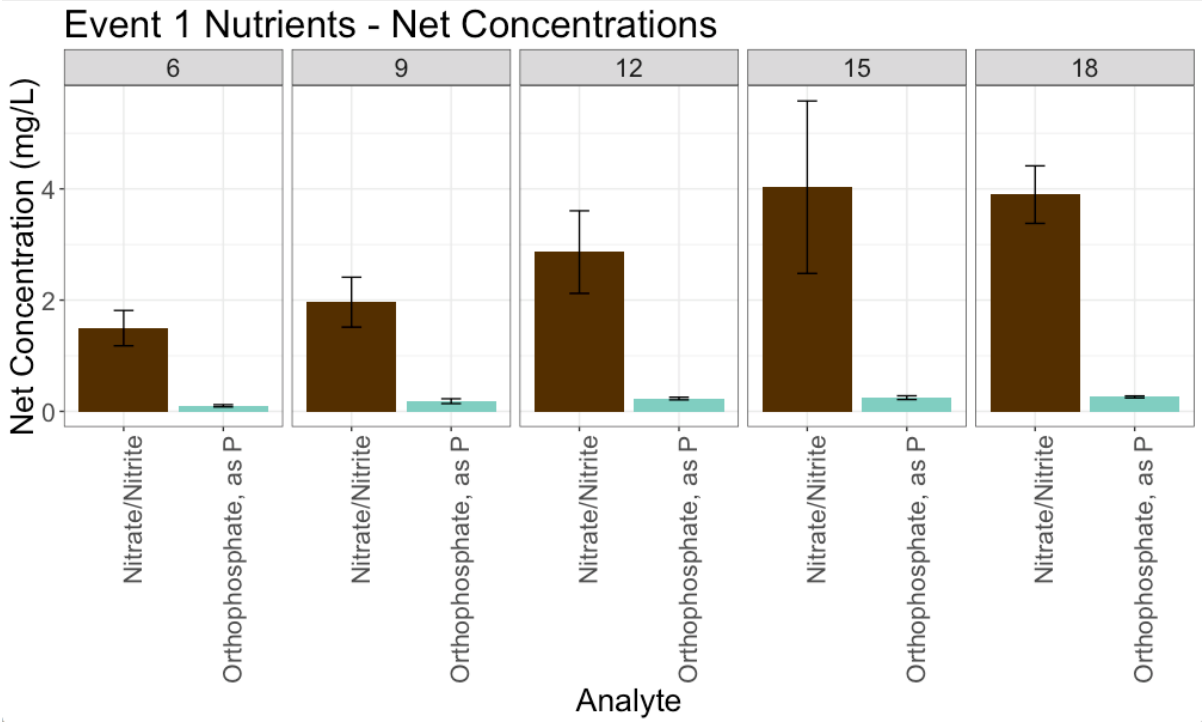


Figure 6. Mean (standard error) net concentration (effluent minus influent) for nutrients among the five treatment depths.

Toxicity Testing – Salmon

Influent and effluent waters were tested for acute toxicity to juvenile coho salmon in 24-h static exposures. The effluent used in the juvenile coho salmon exposures was transferred from the stainless steel sump of each column into glass carboys for transport to the fish lab at WSU-P (Figure 7A). A volume of 30 L of treated runoff was collected from each column and pooled across triplicates of each treatment depth so that 10 L of effluent from each column went into each of three replicate exposure tanks for each treatment depth and the clean water control effluent.

Triplicate 35-L glass aquaria per treatment were filled with influent or effluent water and maintained at 13 ℃ in recirculating water baths. The placement of aquaria in the water baths was randomized. An airstone was placed in each aquarium to maintain dissolved oxygen levels above 6 mg/L. Water quality parameters (temperature, dissolved oxygen, pH and conductivity) were monitored for each aquarium. When exposure waters had reached 13 ℃, juvenile coho ($\bar{x}$ (SD): length = 104.5(8) mm) were placed (n=8) in each aquarium. The pH, dissolved oxygen (DO), and conductivity measurements at the outset of the exposure were within a normal range for maintaining healthy juvenile coho (pH = 7.90–8.11, DO = 11.1-13.3 mg L^{-1}, conductivity = 1090-1203 $\mu S/cm$). Water quality parameters and fish behavior were measured every 3-4 h during the day. Unexpected changes in fish behavior (e.g. rising to the surface or loss of equilibrium; Figure 7B) were recorded. Mortality was the endpoint used to assess toxicity. Surviving coho were euthanized with MS-222 after 24 h and individual lengths

were measured and recorded. Replicate survival relative to controls was analyzed by one-way ANOVA with a Dunnett post hoc.

Untreated highway runoff was acutely lethal to juvenile coho salmon. By 14 h of exposure, 75% mortality was observed in the influent stormwater. At the end of the exposure (24 h), 87.5% mortality was observed (Figure 8). Treatment of runoff through any of the bioretention soil columns completely prevented mortality (Figure 8).

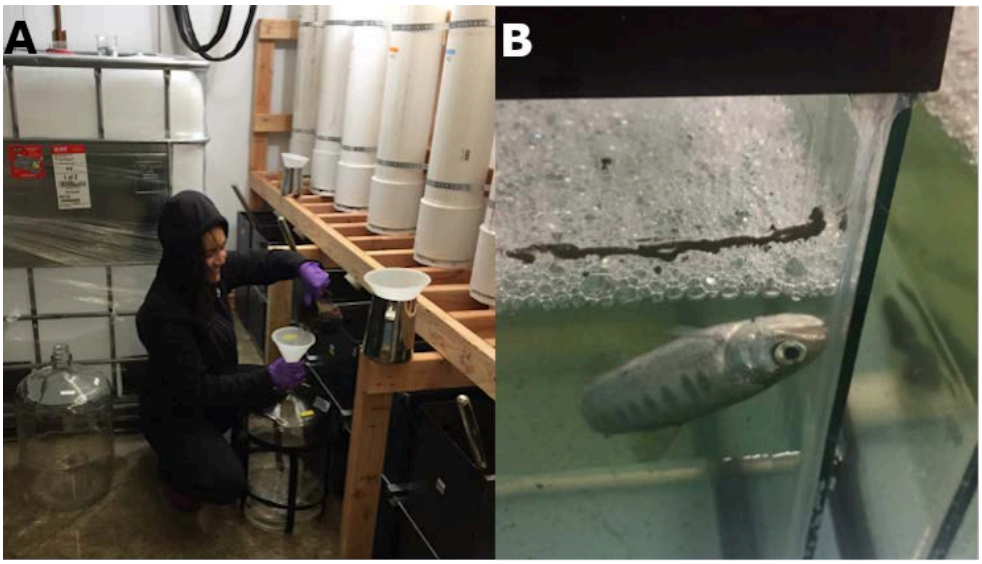


Figure 7. A) Effluent from the bioretention columns to be used for toxicity testing using juvenile coho salmon is collected into glass carboys. B) Juvenile coho salmon exposed to 100% urban stormwater runoff suffering from loss of equilibrium.

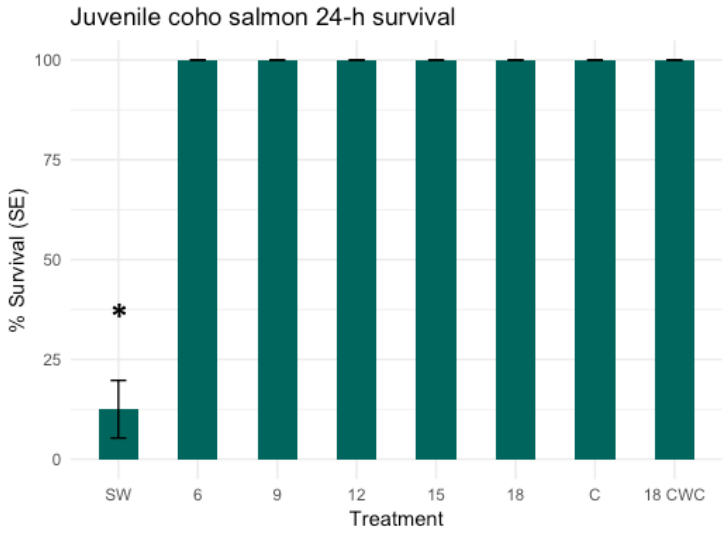


Figure 8. Survival of juvenile coho salmon following 24-h exposure to urban stormwater runoff influent (SW) and bioretention-treated runoff pooled across triplicates of each bioretention treatment depth (6”, 9”, 12”, 15”, 18”, and 18” clean water control (CWC)). An asterisk indicates that an exposure significantly affected survival relative to controls.

3. Summary of Events 2-5

Events 2-5 were used to age the experimental columns. Influent water (stormwater runoff and clean lab water) and effluent waters (filtered through experimental columns) were not sub-sampled for chemistry or toxicology during these events. Temperature, pH, conductivity, and turbidity of influent and effluents waters were recorded (Table 4). Differences among the five treatment depths for concentrations of each conventional parameter in effluent were assessed by a Kruskal-Wallis test, followed by a post-hoc Dunn's test. There were significant differences in conductivity between the 6- and 18-inch depths during Events 3 and 4. There were also significant differences in pH between the 6-inch and both 15- and 18-inch depths, and in temperature between the 6- and 15-inch depths during Event 5.

Table 4. Mean (standard error) of conventional parameters in influent waters (clean water and stormwater runoff; SW) and triplicate effluent waters from each of the five treatment depths plus the clean water control (CWC).

Measurement	Influent Water		Effluent Water					
	Clean water	SW	6"	9"	12"	15"	18"	18" CWC
Event 2								
Temperature (°F)	-	-	44.0 (0.0)	43.2 (0.2)	43.3 (0.1)	42.83 (0.03)	42.0 (0.2)	42.0 (0.4)
pH	7.66	7.74	7.726 (0.008)	7.745 (0.002)	7.723 (0.009)	7.71 (0.02)	7.74 (0.02)	7.560 (0.009)
Conductivity ($\mu S/cm$)	1423	97	130.7 (0.7)	139 (4)	145 (10)	165 (2)	174 (12)	1536 (5)
Turbidity (NTU)	0.19	28.4	43 (5)	65 (17)	52 (2)	57 (5)	61 (4)	14 (2)
Event 3								
Temperature (°F)	-	-	51.0 (0.1)	51.27 (0.07)	51.67 (0.07)	51.9 (0.2)	51.8 (0.2)	51.9 (0.1)
pH	7.65	7.94	7.80 $(0.01)^{a}$	7.80 $(0.01)^{a}$	7.76 $(0.01)^{ab}$	7.696 $(0.002)^{ab}$	7.68 $(0.03)^{ab}$	7.57 $(0.02)^{b}$
Conductivity ($\mu S/cm$)	1516	150	150.8 $(0.8)^{a}$	152.4 $(0.9)^{ab}$	156 $(1)^{ab}$	158.5 $(0.5)^{ab}$	166 $(2)^{b}$	1535 (5)
Turbidity (NTU)	0.58	31.5	23 (4)	33 (7)	32 (3)	33.0 (0.7)	38 (4)	15 (2)
Event 4								
Temperature (°F)	-	45.5	63.1 (0.1)	62.5 (0.1)	63.1 (0.3)	62.4 (0.4)	63.5 (0.4)	63.9 (0.2)
pH	7.71	7.86	7.72 (0.02)	7.71 (0.02)	7.68 (0.01)	7.64 (0.02)	7.63 (0.05)	7.53 (0.02)
Conductivity ($\mu S/cm$)	1506	105.7	117 $(2)^{a}$	121 $(2)^{ab}$	128 $(2)^{ab}$	131 $(1)^{ab}$	149 $(4)^{b}$	1553 (2)
Turbidity	0.2	154	22	27	34	29	42	25

(NTU)			(4)	(3)	(2)	(3)	(4)	(4)
Event 5								
Temperature (°F)	50.3	37.9	48.0 (0.2)[a]	46.7 (0.3)[ab]	46.3 (0.1)[ab]	45.1 (0.2)[b]	47.0 (0.8)[ab]	47.6 (0.4)
pH	7.57	7.56	7.24 (0.02)[a]	7.13 (0.01)[ab]	7.08 (0.05)[ab]	6.95 (0.06)[b]	6.99 (0.03)[b]	7.48 (0.02)
Conductivity ($\mu S/cm$)	1510	2290	2260 (6)	2250 (10)	2240 (0.0)	2220 (10)	2213 (3)	1548 (2)
Turbidity (NTU)	0.2	89.7	2.0 (0.7)	2.2 (0.7)	2.1 (0.3)	2.0 (0.5)	2.5 (0.4)	12 (1)

Note: Treatments with different superscript group labels (a, b, c) show significance at $\alpha = 0.05$ (Kruskal-Wallis with post-hoc Dunn Test).
BDL = Below Detection Limit
NTU = Nephelometric Turbidity Units

4. Event 6 – Chemistry

Stormwater runoff was collected and placed in the environmental chamber on January 27, 2020. Experimental columns were each dosed with 55.6 L of water for approximately 24-hours, beginning at 8:30am on January 28, 2020.

PAHs

TPAH in stormwater influent was greater for Event 6 (TPAH = 0.789) than for Event 1 (TPAH = 0.474). Effluents for Event 6 contained similar levels of PAHs (TPAH= 0.038-0.053 μg/L) (Table 6). There were no statistically significant differences in effluent TPAHs between the five different depths, according to a Kruskal-Wallis with a post-hoc Dunn's test ($\chi^2 = 6.43$, df = 4, p = 0.170). TPAHs were removed at rates of 93-95% across the treatment depths. PAHs in stormwater runoff for Event 6 were predominantly high molecular weight, dominated by pyrene. In contrast, PAHs in bioretention-treated effluent waters were predominantly low molecular weight PAHs. A complete table of PAH congeners can be found in Appendix A.

Table 6. Mean (standard error) of TPAHs in influent waters (clean water and stormwater runoff; SW) and triplicate effluent waters from each of the five treatment depths plus the clean water control (CWC), and removal efficiencies for the five treatment depths. PAH congeners below the method detection limit (MDL) were assigned a value of zero.

Treatment	TPAH (μg/L)	Removal Efficiency (%)
SW influent	0.789	n.a.
6	0.045 (0.003)	94.3 (0.4)
9	0.046 (0.005)	94.2 (0.6)
12	0.038 (0.004)	95.1 (0.6)
15	0.048 (0.006)	93.9 (0.8)
18	0.053 (0.001)	93.2 (0.2)
Clean water influent	0.029	n.a.
18 CWC	0.028 (0.002)	n.a.

Conventionals

Differences among the five treatment depths for conventional parameters in effluent were assessed by a Kruskal-Wallis test with a post-hoc Dunn's test (Table 5). There were statistically significant differences in effluent conductivity ($\chi^2 = 13.5$, $p = 0.009$) and pH ($\chi^2 = 13.1$, $p = 0.011$) between the five treatment depths. Conductivity was significantly greater in the 18" column compared to the 6" column ($p = 0.010$), and pH was significantly greater in the 15" and 18" columns compared the 6"column ($p= 0.046$; $p=0.010$).

Table 5. Mean (standard error) of conventional parameters in influent waters (clean water and stormwater runoff; SW) and triplicate effluent waters from each of the five treatment depths plus the clean water control (CWC).

Measurement	Influent Waters		Bioretention Treated Effluent Water					
	Clean water	**SW**	**6"**	**9"**	**12"**	**15"**	**18"**	**18" CWC**
Temperature (°F)	46.7	39.3	39.9 (0.2)	40.8 (0.3)	39.5 (0.8)	39.3 (0.2)	39.6 (0.9)	39.9 (0.4)
pH	7.57	7.73	8.137 (0.009)^{a}	7.99 (0.03)^{ab}	7.87 (0.01)^{ab}	7.86 (0.01)^{ab}	7.778 (0.008)^{b}	7.48 (0.01)
Conductivity ($\mu S/cm$)	1559	76.7	750 (12)^{a}	942 (41)^{ab}	1146 (22)^{ab}	1264 (5)^{b}	1490 (8)^{b}	1540 (4)
Turbidity (NTU)	0.09	58.9	27 (3)	28 (1)	30.4 (0.9)	32 (1)	34 (2)	5.2 (0.3)

Note: Treatments with different superscript group labels (a, b, c) show significance at □ = 0.05 (Kruskal-Wallis with post-hoc Dunn Test).
NTU = Nephelometric Turbidity Units

5. Saturated Hydraulic Conductivity

Saturated hydraulic conductivity (K_{sat}) of the each bioretention treatment was measured following Event 6 using the falling head method (Klute and Dirksen 1986). Average K_{sat} values for each depth were less than the baseline K_{sat} determined prior to stormwater dosing in July 2019 (Pre-WY1; Table 7). K_{sat} values are expected to decrease as settling occurs with treatment time, or as suspended solids from stormwater fill the interstitial space in the bioretention media. There were no statistically significant differences in K_{sat} values among the treatments either pre-WY1 (χ^2 =6.03, p=0.303) or post-WY1 (χ^2 = 7.34, p = 0.197).

Table 7. Average (standard error) of saturated hydraulic conductivity (K_{sat}) measurements for each treatment depth prior to stormwater dosing in July 2019 (pre-WY1) and in February 2020 at the end of the first water year (post-WY1).

	Ksat (cm/hr)	
Treatment	**Pre-WY1**	**Post-WY1**
6”	423 (130)	391 (50)
9”	515 (220)	427 (252)
12”	525 (150)	280 (94)
15”	610 (31)	327 (139)
18”	391 (27)	200 (70)
18” CWC	431 (94)	164 (4)
Average	*489 (33)	304 (38)

*The K_{sat} values of the columns pre-WY1 were significantly greater than post-WY1, according to a paired-sample Wilcoxon test (V=165, p <0.001).

Summary of Events 7-10

Significant differences in conductivity between the 6- and 18-inch depths were observed during Events 7, 8, 9, and 10, as determined by a Kruskal–Wallis test with a post-hoc Dunn's test (Table 8). Significant differences in conductivity were also observed between the 15- and 6-inch depths during Events 7 and 10.

Table 8. Mean (standard error) of conventional parameters in influent waters (clean water and stormwater runoff; SW) and triplicate effluent waters from each of the five treatment depths plus the clean water control (CWC).

Measurement	Influent Water		Effluent Water					
	Clean water	SW	6"	9"	12"	15"	18"	18" CWC
Event 7								
Temperature (°F)	-	41.5	42.3 (0.2)	41.6 (0.6)	40.4 (0.3)	40.8 (0.1)	41.0 (0.2)	42.2 (0.5)
pH	7.48	7.69	8.16 (0.04)	8.09 (0.02)	8.03 (0.01)	8.08 (0.02)	8.02 (0.02)	7.44 (0.02)
Conductivity ($\mu S/cm$)	1521	76.8	123.9 (0.4)^{a}	140 (3)^{ab}	156 (2)^{ab}	164 (1)^{ab}	183 (1)^{b}	1533 (3)
Turbidity (NTU)	0.42	50.2	22 (4)	29 (3)	22 (4)	22 (2)	26 (1)	6.4 (0.6)
Event 8								
Temperature (°F)	-	40.6	50.4 (0.1)	49.8 (0.1)	50.37 (0.07)	50.1 (0.2)	49.6 (0.3)	50.6 (0.2)
pH	7.72	7.74	8.09 (0.01)	8.091 (0.006)	8.080 (0.005)	8.08 (0.03)	8.042 (0.006)	7.46 (0.04)
Conductivity ($\mu S/cm$)	1526	136.7	138 (2)^{a}	146.5 (0.8)^{ab}	152.5 (0.9)^{ab}	157 (2)^{ab}	167 (1)^{b}	1538 (2)
Turbidity (NTU)	0.04	151.5	30 (4)	38 (2)	31 (3)	33 (2)	38 (4)	7.3 (0.4)
Event 9								
Temperature (°F)	58.2	44.6	60.3 (0.1)	60.07 (0.07)	60.27 (0.07)	60.1 (0.4)	60.4 (0.00)	60.6 (0.2)
pH	7.62	7.95	7.86 (0.02)^{a}	7.97 (0.03)^{ab}	8.09 (0.01)^{ab}	8.12 (0.03)^{b}	8.07 (0.02)^{ab}	7.56 (0.01)
Conductivity ($\mu S/cm$)	1554	201	206 (2)^{a}	218 (2)^{ab}	229.7 (0.7)^{ab}	233 (1)^{ab}	245 (1)^{b}	1543 (3)
Turbidity (NTU)	0.1	47.7	29 (3)	30 (3)	27 (4)	31 (3)	33 (2)	12 (1)
Event 10								
Temperature (°F)	-	54.8	65.67 (0.07)	65.6 (0.00)	65.4 (0.5)	64.43 (0.03)	65.8 (0.2)	66.23 (0.03)
pH	7.75	7.63	7.76 (0.03)	7.79 (0.02)	7.89 (0.03)	7.925 (0.004)	7.91 (0.02)	7.51 (0.02)

Conductivity ($\mu S/cm$)	1522	93.8	105 (1)[a]	113.1 (0.7)[ab]	127 (1)[ab]	135 (2)[b]	155 (3)[b]	1548 (3)
Turbidity (NTU)	0.01	59.2	29.0 (0.6)	35 (2)	32 (3)	35 (3)	40 (1)	15 (1)

Note: Treatments with different superscript group labels (a, b, c) show significance at □ = 0.05 (Kruskal-Wallis with post-hoc Dunn Test).
NTU = Nephelometric Turbidity Units

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Appendix A.

Table A1.1. Mean (standard error) of total PAHs in influent waters (clean water and stormwater runoff; SW) and triplicate effluent waters from each of the five treatment depths plus the clean water control (CWC) during Event 1. One-half of the value of the detection limit (DL) was substituted for the value of non-detects in calculating means unless all replicates were below the detection limit (BDL).

Compound	DL (µg/ L)	Influent Waters (µg/ L)		Bioretention-Treated Effluent Waters (µg/ L)					
		Clean water	SW	6"	9"	12"	15"	18"	18" CWC
Total PAHs		0.035	0.48	0.077 (0.009)	0.051 (0.002)	0.047 (0.001)	0.051 (0.002)	0.052 (0.002)	0.0405 (0.0007)
Benzo(ghi)perylene	0.001	BDL	0.008	BDL	BDL	BDL	BDL	BDL	BDL
Indeno(1,2,3-cd)pyrene	0.001	BDL	0.003	BDL	BDL	BDL	BDL	BDL	BDL
Dibenzo(a,h)anthracene	0.001	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL
Perylene	0.006	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL
Benzo(a)pyrene	0.002	BDL	0.003	BDL	BDL	BDL	BDL	BDL	BDL
Benzo(j)fluoranthene	0.002	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL
Benzo(k)fluoranthene	0.003	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL
Benzo(b)fluoranthene	0.0005	BDL	0.004	BDL	BDL	BDL	BDL	BDL	BDL
Chrysene	0.0009	BDL	0.01	0.0013 (0.0003)	0.001 (0.000)	0.001 (0.000)	0.0017 (0.0003)	0.0017 (0.0003)	0.001 (0.000)
Benzo(a)anthracene	0.0008	BDL	0.005	0.0006 (0.0002)	BDL	BDL	BDL	BDL	BDL
Pyrene	0.001	BDL	0.038	0.0023 (0.0003)	0.0017 (0.0003)	0.0013 (0.0003)	0.002 (0.000)	0.002 (0.000)	0.0007 (0.0002)

Fluoranthene	0.002	BDL	0.047	0.0017 (0.0007)	BDL	BDL	BDL	0.0013 (0.0003)	BDL
Carbazole	0.001	BDL	0.143	0.017 (0.008)	0.005 (0.001)	0.0043 (0.0003)	0.006 (0.002)	0.005 (0.002)	0.0067 (0.0003)
Anthracene	0.001	BDL	0.005	0.0010 (0.0005)	BDL	BDL	BDL	BDL	BDL
Phenanthrene	0.001	BDL	0.1	0.007 (0.003)	0.0037 (0.0003)	0.0037 (0.0003)	0.0033 (0.0003)	0.0033 (0.0003)	0.0023 (0.0003)
Fluorene	0.002	BDL	0.012	0.0033 (0.0009)	BDL	BDL	BDL	BDL	BDL
Dibenzofuran	0.002	BDL	0.011	0.0037 (0.0007)	0.0027 (0.0003)	0.0013 (0.0003)	0.002 (0.000)	0.002 (0.000)	BDL
Acenaphthene	0.003	BDL	0.006	0.0023 (0.0008)	BDL	BDL	BDL	BDL	BDL
Acenaphthylene	0.002	BDL	0.003	BDL	BDL	BDL	BDL	BDL	BDL
1-Methylnaphthalene	0.001	0.002	0.011	0.0073 (0.0003)	0.0070 (0.0006)	0.0067 (0.0003)	0.0067 (0.0003)	0.007 (0.000)	0.0027 (0.0003)
2-Methylnaphthalene	0.001	0.005	0.015	0.0053 (0.0009)	0.0047 (0.0003)	0.0047 (0.0007)	0.0047 (0.0007)	0.0053 (0.0003)	0.0033 (0.0003)
Naphthalene	0.001	0.011	0.04	0.0127 (0.0007)	0.0113 (0.0007)	0.0103 (0.0009)	0.011 (0.002)	0.011 (0.001)	0.0087 (0.0003)
2-Chloronaphthalene	0.001	BDL	0.002	0.0013 (0.0008)	BDL	BDL	BDL	BDL	BDL
Sum Low Molecular Weight (LMW)*		0.0245	0.356	0.063 (0.009)	0.039 (0.002)	0.036 (0.001)	0.038 (0.002)	0.038 (0.002)	0.0292 (0.0007)
Sum High Molecular Weight (HMW)**		0.0106	0.124	0.0083 (0.0008)	0.0123 (0.0003)	0.0119 (0.0003)	0.0133 (0.0003)	0.0137 (0.0005)	0.0113 (0.0002)

*Compounds composed of fewer than four rings

**Compounds composed of four or more rings

BDL = Below Detection Limit

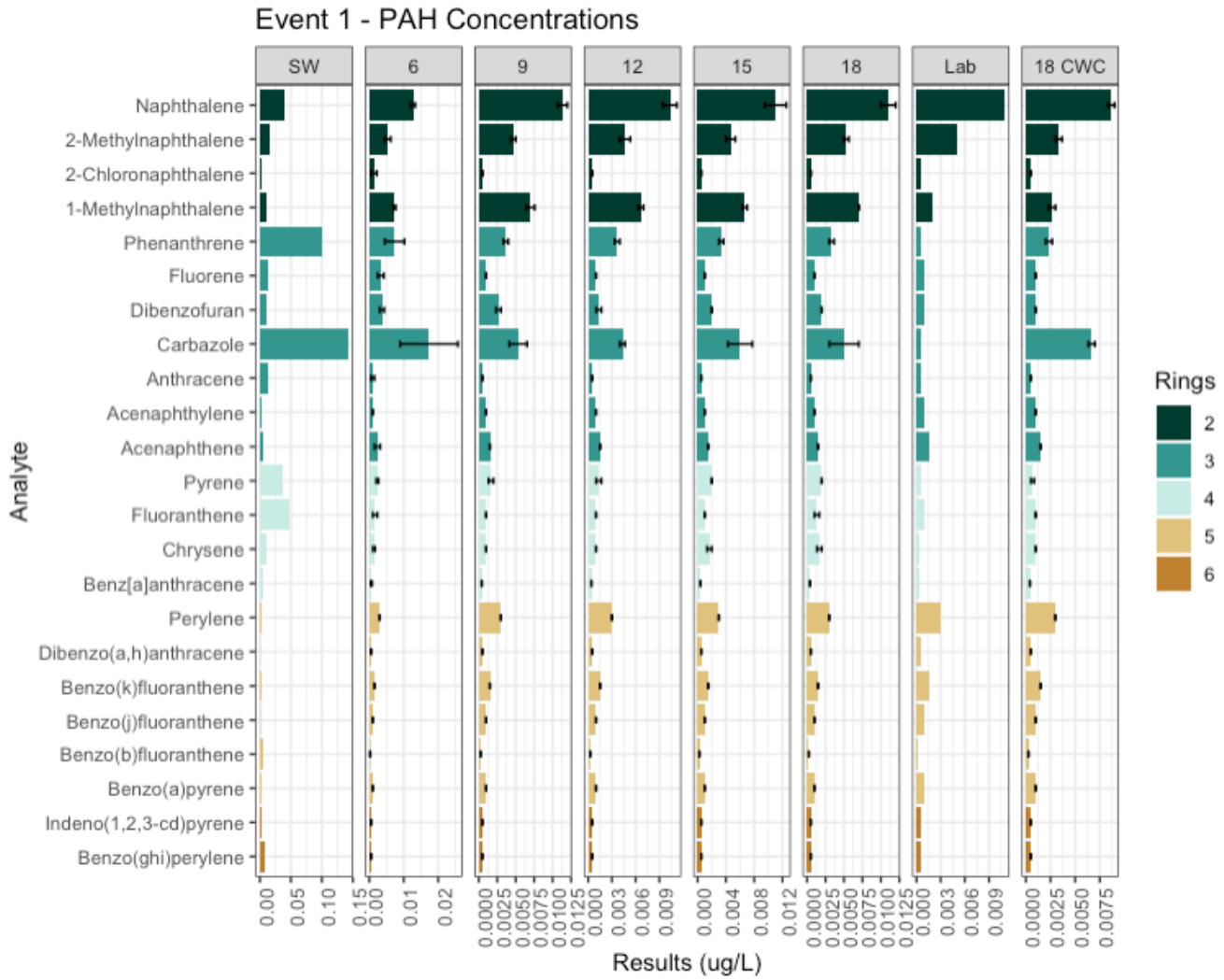

Figure A1.1. PAH concentrations in influent clean water (Lab), influent stormwater (SW), and triplicate samples of effluent from each of the five treatment depths (6”, 9”, 12”, 15”, 18”) plus the clean water control (18” CWC) during Event 1. Error bars are one standard error of the mean. One-half of the value of the detection limit (DL) was substituted for the value of non-detects.

Table A1.2. Mean (standard error) of total PAHs in influent waters (clean water and stormwater runoff; SW) and triplicate effluent waters from each of the five treatment depths plus the clean water control (CWC) during Event 6. One-half of the value of the detection limit (DL) was substituted for the value of non-detects in calculating means unless all replicates were below the detection limit (BDL).

Compound	DL (µg/ L)	Influent Waters (µg/ L)		Bioretention-Treated Effluent Waters (µg/ L)					
		Clean water	SW	6"	9"	12"	15"	18"	18" CWC
Total PAHs		0.046	0.79	0.061 (0.002)	0.061 (0.002)	0.054 (0.002)	0.063 (0.007)	0.052 (0.007)	0.046 (0.002)
Benzo(ghi)perylene	0.001	BDL	0.057	0.003 (0.001)	0.004 (0.00)	0.0033 (0.0003)	0.0033 (0.0003)	0.0023 (0.0007)	BDL
Indeno(1,2,3-cd)pyrene	0.001	BDL	0.021	0.0013 (0.0008)	0.0017 (0.0003)	0.0010 (0.0005)	0.0010 (0.0005)	BDL	BDL
Dibenzo(a,h)anthracene	0.001	BDL	0.003	BDL	BDL	BDL	BDL	0.0007 (0.0002)	0.0008 (0.0002)
Perylene	0.006	BDL	0.01	0.0035 (0.00)	0.0035 (0.00)	0.0035 (0.00)	0.0032 (0.0002)	0.0032 (0.0002)	0.0033 (0.0007)
Benzo(a)pyrene	0.003	BDL	0.019	BDL	0.0020 (0.0005)	BDL	BDL	BDL	BDL
Benzo(j)fluoranthene	0.002	BDL	0.010	BDL	BDL	BDL	BDL	BDL	BDL
Benzo(k)fluoranthene	0.004	BDL	0.014	BDL	BDL	BDL	BDL	BDL	BDL
Benzo(b)fluoranthene	0.0005	BDL	0.031	0.002 (0.00)	0.002 (0.00)	0.0012 (0.0009)	0.002 (0.00)	0.0023 (0.0003)	BDL
Chrysene	0.001	BDL	0.043	0.003 (0.00)	0.002 (0.00)	0.0027 (0.0003)	0.003 (0.00)	0.003 (0.00)	BDL
Benzo(a)anthracene	0.0008	BDL	0.022	0.0011 (0.0005)	BDL	BDL	0.0009 (0.0005)	0.002 (0.00)	BDL
Pyrene	0.001	BDL	0.131	0.0047 (0.0003)	0.0037 (0.0003)	0.0037 (0.0003)	0.0033 (0.0003)	0.003 (0.00)	BDL

Fluoranthene	0.002	BDL	0.095	0.004 (0.00)	0.0033 (0.0003)	0.003 (0.00)	0.003 (0.00)	0.003 (0.00)	BDL
Carbazole	0.001	0.004	0.04	BDL	BDL	BDL	BDL	BDL	BDL
Anthracene	0.001	BDL	0.01	BDL	BDL	BDL	BDL	BDL	BDL
Phenanthrene	0.001	0.004	0.084	0.004 (0.00)	0.003 (0.001)	0.0037 (0.0007)	0.0037 (0.0003)	0.004 (0.00)	0.0027 (0.0007)
Fluorene	0.002	BDL	0.008	BDL	BDL	BDL	BDL	BDL	BDL
Dibenzofuran	0.002	BDL	0.008	BDL	BDL	BDL	BDL	BDL	BDL
Acenaphthene	0.003	BDL	0.004	BDL	BDL	BDL	BDL	BDL	BDL
Acenaphthylene	0.002	BDL	0.008	BDL	BDL	BDL	BDL	BDL	BDL
1-Methylnaphthalene	0.001	0.006	0.021	0.007 (0.001)	0.011 (0.001)	0.009 (0.001)	0.016 (0.007)	0.018 (0.002)	0.012 (0.002)
2-Methylnaphthalene	0.001	0.004	0.036	0.0037 (0.0003)	0.0037 (0.0003)	0.0033 (0.0003)	0.0033 (0.0003)	0.0050 (0.0006)	0.0037 (0.0003)
Naphthalene	0.001	0.011	0.114	0.0117 (0.0007)	0.0103 (0.0007)	0.0083 (0.0007)	0.009 (0.001)	0.0107 (0.0003)	0.009 (0.001)
2-Chloronaphthalene	0.001	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL
Sum Low Molecular Weight (LMW)*		0.0345	0.3335	0.033 (0.002)	0.035 (0.002)	0.030 (0.002)	0.038 (0.007)	0.044 (0.002)	0.034 (0.002)
Sum High Molecular Weight (HMW)**		0.0117	0.456	0.028 (0.002)	0.0261 (0.0007)	0.024 (0.001)	0.0244 (0.0009)	0.0245 (0.0008)	0.0128 (0.0002)

*Compounds composed of fewer than four rings

**Compounds composed of four or more rings

BDL = Below Detection Limit

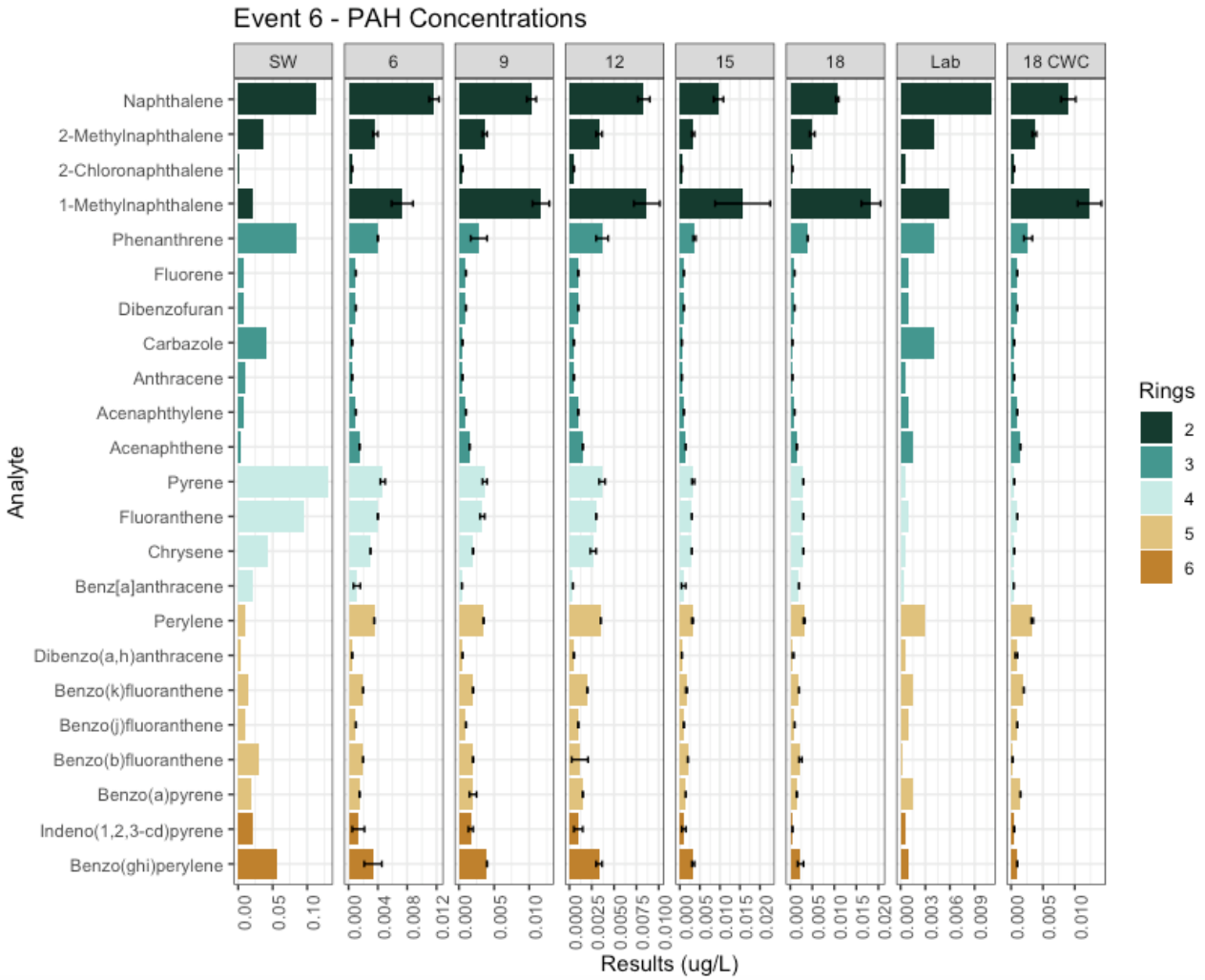

Figure A1.2. PAH concentrations in influent clean water (Lab), influent stormwater (SW), and triplicate samples of effluent from each of the five treatment depths (6”, 9”, 12”, 15”, 18”) plus the clean water control (18” CWC) during Event 6. Error bars are one standard error of the means. One-half of the value of the detection limit (DL) was substituted for the value of non-detects.