RSMP Project: Testing the effectiveness of bioretention at reducing the toxicity of urban stormwater to coho salmon

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Deliverable 1: Bioretention cell construction and preparation

In October 2013 the research team constructed a portable bioretention treatment system for pilot work treating runoff for adult salmon exposures at Grover's Creek Salmon Hatchery (Poulsbo, WA). Four new 55-gallon polyethylene drums were fitted with a slotted underdrain. The underdrain was constructed from a 2" PVC pipe capped on one end and the other end attached to a bulkhead fitting near the base of the drum (Figures 1, 3). Slots in the underdrain were cut following guidance in Section 6.1.2 of the 2012 Low Impact Development Technical Guidance Manual for Puget Sound (Publication No. PSP 2012-3). On the exterior of each drum, a 2" PVC ball valve was attached to the bulkhead fitting.

In September 2014, the bioretention system was emptied of treatment media used in 2013. The new drainage layer (12") was a Seattle Type 26 mixed gravel aggregate obtained from CalPortland in DuPont, WA (product #8495). The bioretention soil media (BSM) was a mixture of 60% sand and 40% Cedar Grove compost mixed in 2011 and stored at Washington State University in Puyallup (WSU-P). The BSM was tamped down every 6" to reduce settling during conditioning to a total depth of 24". The BSM was topped with 2" of bark mulch created by Barri Hermann at WSU-P (Crop & Soil Sciences). One sample of BSM was taken from the center of each drum during construction for analysis of metals. Chilled samples were taken to ARI Laboratories in Tukwila, WA for analysis.

After transporting the bioretention system to Grover's Creek Salmon Hatchery, the bioretention media were conditioned in preparation for use in the coho study. Over two days, a total of 660 L of well water was passed through each bioretention cell at a rate of 2 L/min, equivalent to 2 months of summer rainfall on a contributing area 20x that of the treatment area (i.e., the treatment area is 5% of the contributing area – within recommended guidelines for the use of bioretention for treatment of runoff). Influent and effluent well water samples were collected on October 15, 2014 and transported on ice to Am-Test Laboratories in Kirkland, WA for analysis of metals and conventional water chemistry. Samples for PAH analysis were preserved with 10% methylene chloride and transported on ice to NOAA-Northwest Fisheries Science Center (NWFSC) for analysis.

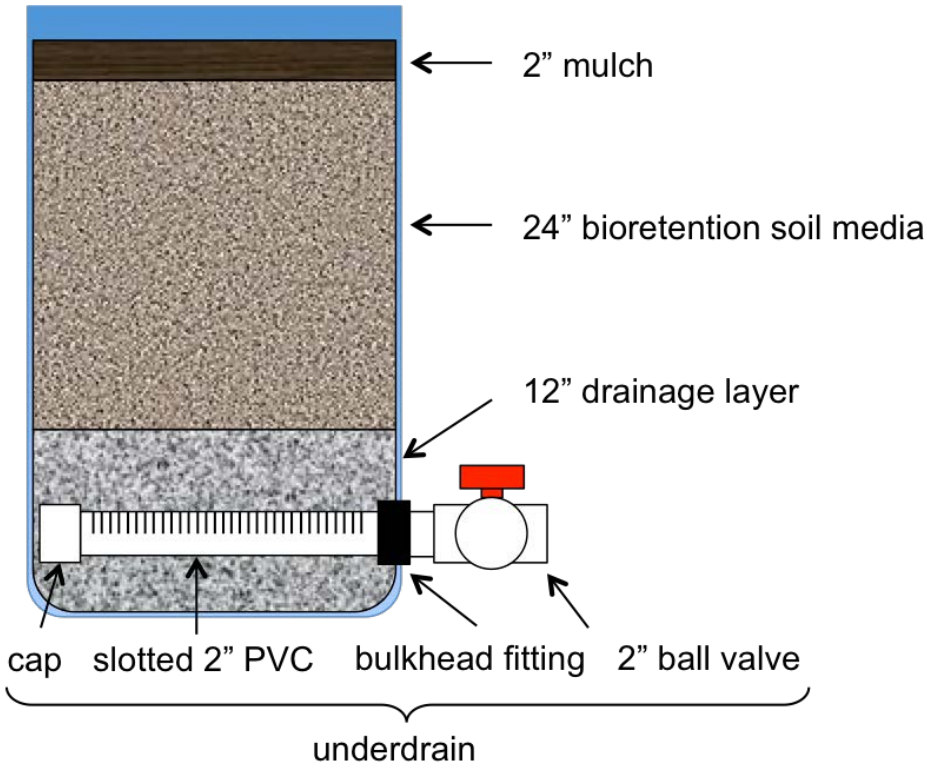

Figure 1. Diagram of bioretention unit using 55-gallon drum.

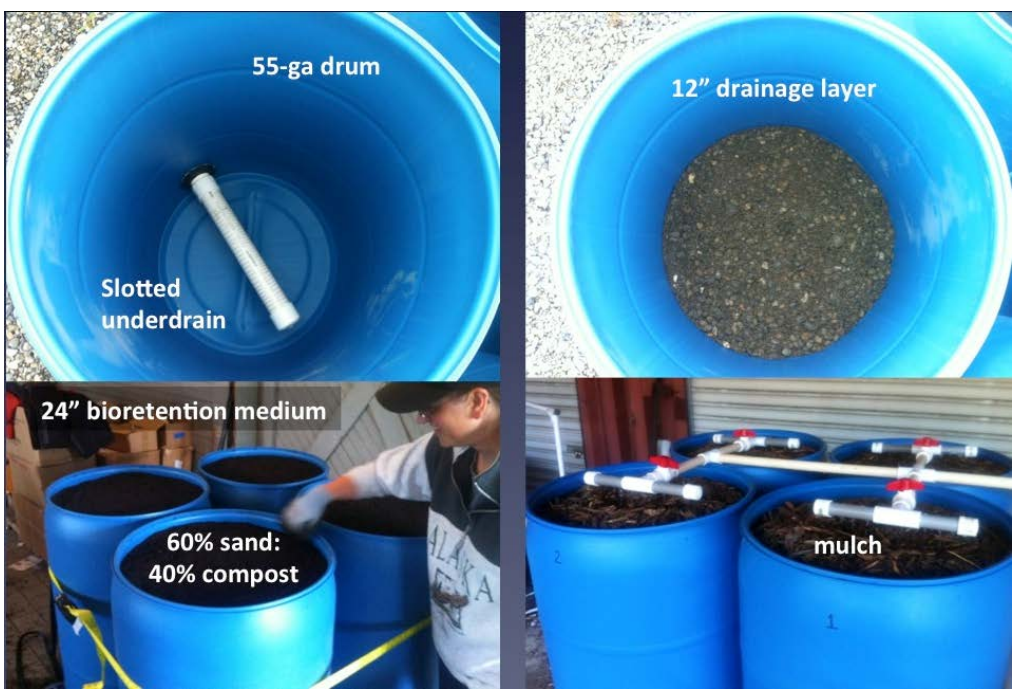

Figure 2. Images of the construction of the bioretention treatment system.

Figure 3. Image of the exterior of one treatment unit and effluent from conditioning.

Deliverable 2.1: Metal concentrations of BSM used in bioretention cells

The most abundant metal in the BSM was Zn. Metal abundance was in the order Zn>Ni>Cr=Cu>Pb>As>Cd. Silver (Ag) was below the 0.2 mg/kg limit of quantitation in all BSM samples.

Table 1. Metal concentrations in the bioretention soil medium from each bioretention cell.

mg/kg dry	LOQ[a]	Cell 1	Cell 2	Cell 3	Cell 4	Mean	SE
As	0.2	2	2	1.9	2.2	2.0	0.1
Cd	0.09	0.11	0.12	0.1	0.1	0.11	0.0
Cr	2	28	28	26	25	27	1
Cu	0.5	21.4	21.1	21.5	22.3	21.6	0.3
Pb	0.09	6.97	7.17	6.6	7.5	7.06	0.19
Ni	0.5	35.9	38.2	38	33.8	36.5	1.0
Ag	0.2	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	0.0
Zn	4	50	51	49	52	51	1

[a] LOQ = limit of quantitation

Deliverable 2.2: Water chemistry of effluent from conditioned cells

Following conditioning of bioretention cells, well water leached significant ($p < 0.01$) concentrations of most water chemistry parameters from the cells including bacteria, solids, organic matter, nitrogen, total P, and metals. There was a slight but significant loss of minerals (Ca, Mg), and total Ag from well water to the bioretention cells. The only parameter not significantly changed by passing through the bioretention cells was dissolved P ($p = 0.77$). Neither total nor dissolved Cd was above the detection limit for well water either before or after filtration through the conditioned bioretention cells.

Dissolved metals in the effluent from bioretention cell conditioning were in the order Zn>Cu>As>Ni>Cr, with undetectable levels of Cd, Pb, and Ag. Based on the concentrations native to the BSM (Table 1), we conclude that As in the BSM was relatively mobile and Cr was relatively immobile.

Table 2. Water chemistry of effluent from conditioned cells on Oct 15, 2014. Values are the mean and standard error of the mean of triplicate samples.

Category	Parameter	D.L.	Units	Well Water	Filtered Well Water
Microbiological	Fecal Coliform	5	CFU/100 mL	< D.L.	307 (63)
	E. coli	5	CFU/100 mL	< D.L.	287 (56)
Conventionals	pH	0.1	-	7.7 (0.1)	7.3 (0.0)
	TSS	1	mg/L	29 (1)	18 (6)
	SSC	0.2	mg/L	< D.L.	25.3 (0.3)
Demand	TOC	0.5	mg/L	0.5 (0)	32.7 (0.3)
	COD	10	mg/L	< D.L.	89 (6)
	DOC	0.5	mg/L	< D.L.	30.3 (1.2)
Minerals	Alkalinity	1	mg CaCO3/L	85 (1)	110 (0)
	Hardness	0.05	mg CaCO3/L	74.00 (1.00)	56.33 (0.33)
	Ca	0.05	mg/L	18.33 (0.33)	14.00 (0.00)
	Mg	0.01	mg/L	6.83 (0.03)	5.17 (0.07)
Nutrients	Ammonia	0.01	mg/L	0.29 (0.02)	1.47 (0.00)
	Total N	0.1	mg/L	0.5 (0.0)	4.7 (0.9)
	Nitrate	0.025	mg/L	< D.L.	2.893 (0.009)
	Ortho-P	0.005	mg/L	0.223 (0.006)	0.205 (0.059)
	Total P	0.005	mg/L	0.251 (0.002)	0.571 (0.014)
Total Metals	As	0.02	ug/L	0.25 (0.00)	7.18 (0.15)
	Cd	0.025	ug/L	< D.L.	< D.L.
	Cr	0.05	ug/L	0.21 (0.00)	2.57 (0.03)
	Cu	0.1	ug/L	1.6 (0.1)	15.0 (0.4)
	Pb	0.05	ug/L	0.06 (0.01)	0.7 (0.07)
	Ni	0.05	ug/L	0.49 (0.01)	8.58 (1.66)

	Ag	0.05	ug/L	0.15 (0.01)	< D.L.
	Zn	0.05	ug/L	4.70 (0.58)	36.53 (3.25)
Dissolved Metals	As	0.02	ug/L	0.14 (0.01)	6.72 (0.04)
	Cd	0.025	ug/L	< D.L.	< D.L.
	Cr	0.05	ug/L	< D.L.	2.07 (0.09)
	Cu	0.1	ug/L	0.8 (0.1)	12.6 (0.1)
	Pb	0.05	ug/L	0.05 (0.00)	< D.L.
	Ni	0.05	ug/L	0.11 (0.00)	4.82 (0.21)
	Ag	0.05	ug/L	< D.L.	< D.L.
	Zn	0.05	ug/L	1.55 (0.04)	23.37 (1.66)

Table 3. Polycyclic aromatic hydrocarbons (PAHs) in unfiltered well water and well water filtered with bioretention during cell conditioning.

Polycyclic aromatic hydrocarbons	Well Water (ug/L)	Filtered Well Water (ug/L)
Naphthalene	0.05 (0.00)*	0.05 (0.00)
C1-alkyl-napthalene	0.04 (0.00)	0.04 (0.00)
C2-alkyl-naphthalene	0.07 (0.00)	0.07 (0.00)
C3-alkyl-napthalene	0.13 (0.00)	0.13 (0.00)
C4-alkyl-napthalene	0.12 (0.02)	0.11 (0.01)
Acenaphthylene	0.01 (0.00)	0.01 (0.00)
Acenaphthene	0.01 (0.00)	0.01 (0.00)
Fluorene	0.03 (0.00)	0.02 (0.00)
C1-alkyl-fluorene	0.03 (0.01)	0.03 (0.00)
C2-alkyl-fluorene	0.06 (0.02)	0.04 (0.01)
C3-alkyl-fluorene	0.13 (0.00)	0.05 (0.02)
Dibenzothiophene	0.01 (0.00)	0.01 (0.00)
C1-alkyl-dibenzothiophene	0.06 (0.02)	0.04 (0.01)
C2-alkyl-dibenzothiophene	0.13 (0.00)	0.04 (0.02)
C3-alkyl-dibenzothiophene	0.06 (0.00)	0.03 (0.01
C4-alkyl-dibenzothiophene	0.01 (0.00)	0.01 (0.00)
Anthracene	0.01 (0.00)	0.01 (0.00)
Phenanthrene	0.14 (0.02)	0.10 (0.01)
C1-alkyl-phenanthrene	0.27 (0.11)	0.16 (0.07)
C2-alkyl-phenanthrene	0.25 (0.11)	0.12 (0.07)
C3-alkyl-phenanthrene	0.08 (0.04)	0.07 (0.04)
C4-alkyl-phenanthrene	0.03 (0.00)	< D.L.**
Pyrene	0.08 (0.03)	0.03 (0.02)
Fluoranthene	0.10 (0.04)	0.06 (0.03)
C1-alkyl-fluoranthene	0.01 (0.00)	0.02 (0.00)
C2-alkyl-fluoranthene	< D.L.	< D.L.
C3-alkyl-fluoranthene	< D.L.	< D.L.

C4-alkyl-fluoranthene	< D.L.	< D.L.
Chrysene	0.01 (0.00)	< D.L.
C1-alkyl-chrysene	< D.L.	< D.L.
C2-alkyl-chrysene	< D.L.	< D.L.
C3-alkyl-chrysene	< D.L.	< D.L.
C4-alkyl-phenanthrene	< D.L.	< D.L.
Benzo[*a*]anthracene	< D.L.	< D.L.
Benzo[*b*]fluoranthene	0.01 (0.00)	< D.L.
Benzo[*k*]fluoranthene	< D.L.	< D.L.
Benzo[*e*]pyrene	< D.L.	< D.L.
Benzo[*a*]pyrene	< D.L.	< D.L.
Perylene	< D.L.	< D.L.
Indeno[*1,2,3-cd*]pyrene	< D.L.	< D.L.
Dibenz[*a,h*]anthracene (and [*a,c*])	< D.L.	< D.L.
Benzo[*ghi*]perylene	< D.L.	< D.L.
Total PAHs	1.77 (0.56)	1.24 (0.34)

* Mean and standard error of triplicates
** D.L. = detection limit was 0.01 ug/L

Deliverable 3.1: Effects of treated effluent on adult coho salmon

During the 2013 spawning season (Sep-Dec), we tested the ability of bioretention to prevent pre-spawn mortality in adult coho at the end of the run (November) for highway runoff during one 4-h and one 24-h exposure for two separate storms. During the 2014 spawning season (Oct-Dec), we completed three exposures, focusing on the early part of the run (October). All exposures were 24 h duration with an observation period at 4 h. Additionally, in 2014 an exposure was run comparing well water exposure with well water passed through the bioretention cells.

Healthy adult coho returning to the Suquamish Tribal Hatchery on Grovers Creek were randomly selected and placed in individual PVC holding tubes. Only fish exhibiting normal behavior and with no obvious signs of trauma, disease, or poor condition were included. Four fish per treatment were placed in 440L of experimental water. Each holding tube was equipped with a hose to pump water flow (4L/min) across the fish's head and each treatment tank was aerated to maintain dissolved oxygen at optimum levels for adult coho health during exposures.

In both years, all of the coho exposed to the unfiltered runoff were dead at the end of the exposure period, whereas all of the coho exposed to the filtered runoff or to well water were still alive at the end of the exposure period. All fish exposed to well water or filtered well water were alive and behaving normally at 24 h. During 2014, nearly all (11/12) coho exposed to unfiltered runoff were dead within 4 h of

exposure. By the end of the 24 h trials, not only was there 0% mortality in the filtered runoff exposure, we did not observe any of the overt symptoms of 'pre-spawn mortality' that were observed in coho exposed to unfiltered runoff prior to death.

Table 4. Mortality of adult coho exposed to well water, or highway runoff that was unfiltered or filtered through the bioretention cells during 2013 or 2014. N = 4 spawners were used in each treatment for each trial.

Exposure Trial		Adult Coho Mortality		
Date	Duration (h)	Well Water	Unfiltered Runoff	Filtered Runoff
11/8/2013	4	0% (0/4)	100% (4/4)	0% (0/4)
11/18/2013	24	0% (0/4)	100% (4/4)	0% (0/4)
10/20/2014	24	0% (0/4)	100% (4/4)	0% (0/4)
10/22/2014	24	0% (0/4)	100% (4/4)	0% (0/4)
10/27/2014	24	0% (0/4)	100% (4/4)	0% (0/4)

Deliverable 3.2: Effects of treated effluent on coho embryo development

On November 13, 2014, eggs and milt were removed from ripe adult coho spawners returning to Grovers Creek Salmon Hatchery. Fertilization took place in paper cups. Approximately 90 eggs were placed in each cup and each cup fertilized by one male. Fertilized eggs were poured into mesh-bottomed cups (Figure 4) that were placed in the trays of heath stacks (Figure 4) used for the experimental rearing. Each tray held nine cups of eggs, with seven vertical trays per stack. Each stack was a separate exposure (Table 5). Each stack was supplied with flow-through 'control' water that was a mixture of well water and stream water. Water was distributed to the top tray of each stack and flowed down through each tray and out a common trough. On exposure days, water was switched from flow-through control water to recirculating water from a 114-L aluminum sump from which treatment water for each stack was pumped to the top tray using a submersible pump (Lifeguard Aquatics Quiet One 3000, 2-3 gal/min). The outflow from each stack returned to the sump for each stack. After 24-h of recirculating exposure waters, all stacks were switched back to flow-through control water. Temperature was measured in the top tray of each stack at 15-min intervals throughout development. Temperature across stacks averaged 9.9 °C, with short-term deviations ranging from 6.9 to 12.2 °C. Stacks were treated 1/week with formalin (Parasite-S, 37% formalin, 15 min at 167 ppm formalin) to prevent fungal buildup per standard hatchery protocol. Hatchery personnel stopped formalin treatment after 12/22/2014 - two weeks before the termination of the experiment.

Figure 4. Embryo cups on a tray in the heath stack (left). Heath stack showing sump filled with 100% unfiltered runoff (right).

Seven exposures were conducted during the experiment (Table 6) - one during gastrulation and the rest during organogenesis (Figure 5). Lack of precipitation during early development prevented more exposures during cleavage and gastrulation. Three cups were sampled from each treatment on each sampling date: one cup from each of trays 1-3 for each stack. Although a higher number of replicates would have been preferred, 15 replicates (3 x 5 treatments) were the most our crew of 6-8 could process in a full field day. Sampling included dechorionating and inspecting 10 embryos from each cup. Each embryo was examined for developmental stage, and digitally photographed using a SMZ-800 stereomicroscope. Images were later analyzed (ImageJ software) for embryo length, eye size, and cardiovascular abnormalities.

Table 5. Treatments used in the episodic exposure study

Stack	Treatment	Trays/Sampling
1	Filtered 100%	3
2	Unfiltered 10%	3
3	Control	2-3
4	Unfiltered 50%	3
5	Unfiltered 100%	3

Table 6. Precipitation and developmental details for each sampling and exposure date during coho embryo development.

Date	Sampling/ Exposure	DPF	Degree Days (°C)	Development Period
11/19/2014	Sampling	6	62	Cleavage
11/24/2014	Exposure	11	112	Gastrulation

12/1/2014	Sampling	18	182	Organogenesis
12/5/2014	Exposure	22	222	Organogenesis
12/8/2014	Sampling	25	250	Organogenesis
12/9/2014	Exposure	26	259	Organogenesis
12/12/2014	Exposure	29	291	Organogenesis
12/15/2014	Sampling	32	320	Organogenesis
12/17/2014	Exposure	34	348	Organogenesis
12/19/2014	Exposure	36	367	Organogenesis
12/22/2014	Sampling	39	388	Organogenesis
12/23/2014	Exposure	40	398	Organogenesis
12/29/2014	Sampling	46	455	Hatch
1/5/2015	Sampling	53	525	Hatch

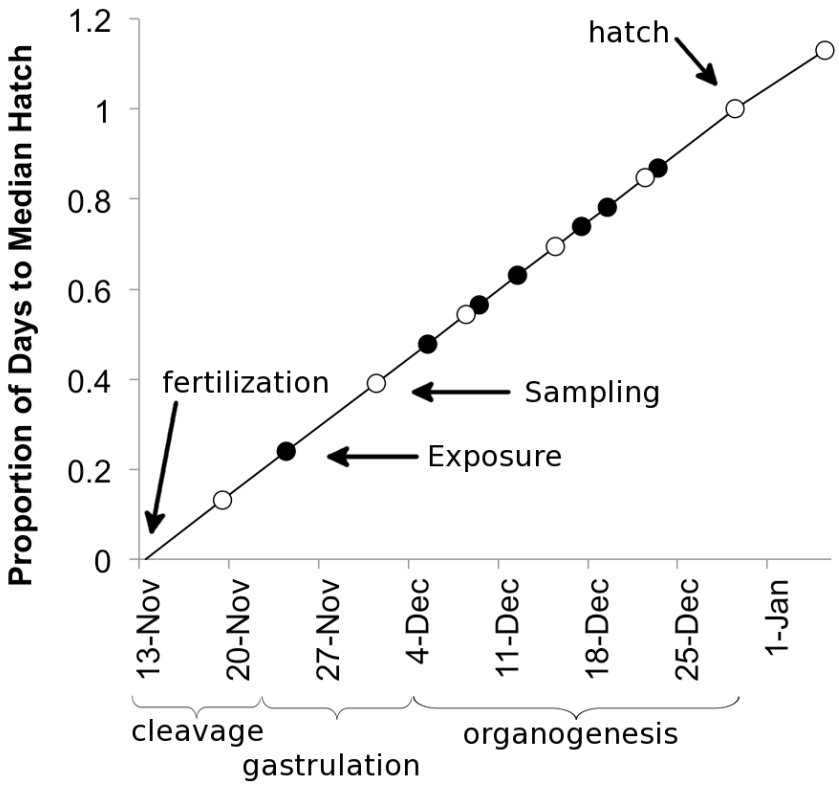

Figure 5. Developmental sequence of coho embryos during the study, including exposure (closed circles) and sampling events (open circles).

Fertilization success was generally high (>90%) across treatments, although individual cups sometimes contained high numbers of unfertilized embryos (4 cups had 24-51% unfertilized). This was likely due to low fertility of individual males – a disadvantage of the single-male cup approach. Embryo development followed expected timelines based on measured degree-days (Table 6).

Survival and hatch rates across treatments showed a similar trend with highest rates in filtered runoff, followed by 10% runoff, 50% runoff, control, and the lowest survival and hatch rates in the 100% runoff.

By the end of the experiment, embryos exposed to the 7 episodes of 100% unfiltered runoff were significantly smaller than controls (Figure 6), whereas embryos exposed to the 7 episodes of filtered runoff were not different than controls. Although eye size followed the same trend, there were no significant differences among treatments (Figure 7). Proportion of embryos with cardiovascular abnormalities similarly suggested more abnormalities in the 100% unfiltered treatment and a reduced effect in the filtered runoff treatment (Figure 8). However, the loss of one replicate in the 100% unfiltered treatment likely affected analytical power and thus for analysis purposes all groups had triplicates except 100% unfiltered which had duplicates. Cardiovascular abnormalities included hemorrhage spots in the head, trunk, or tail. The score of abnormalities (sum of individual counts) increased with the concentration of runoff, but those differences were also not statistically significant.

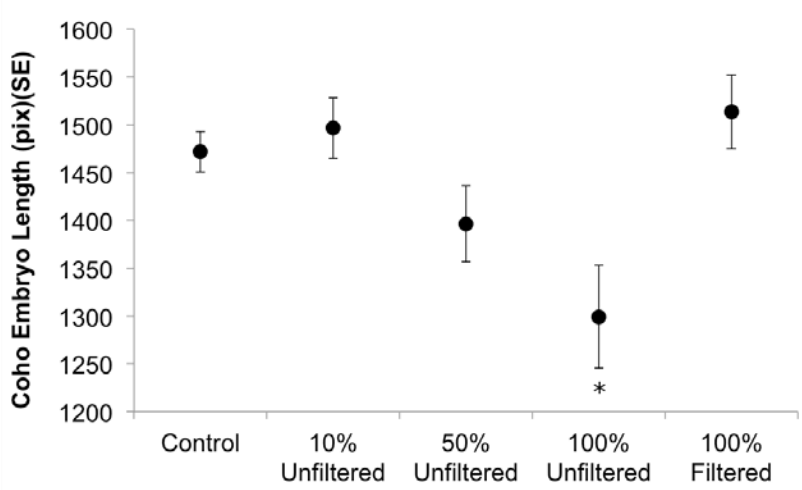

Figure 6. Embryo length following 7 episodic exposures to runoff. Embryos in the 100% unfiltered treatment were significantly smaller than controls ($F(4,14) = 4.992$, $p = 0.021$, Dunnett post-hoc $p = 0.042$).

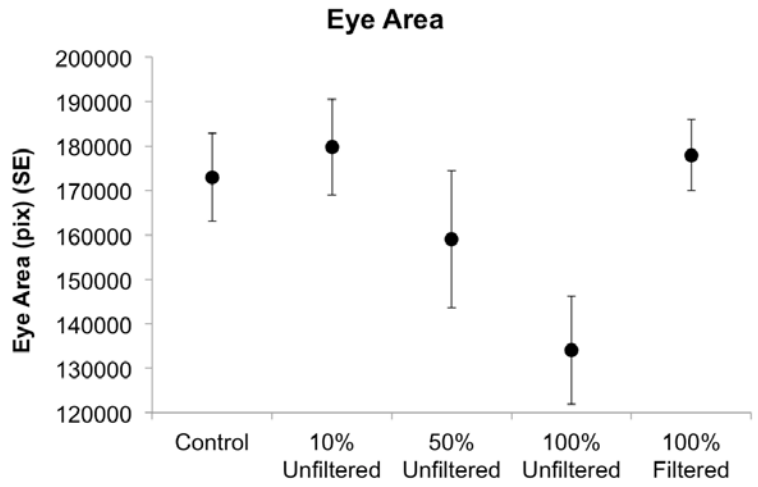

Figure 7. Eye area of embryos following 7 episodic exposures to runoff. There were no significant differences among treatments ($F(4,14) = 2.197$, $p = 0.150$).

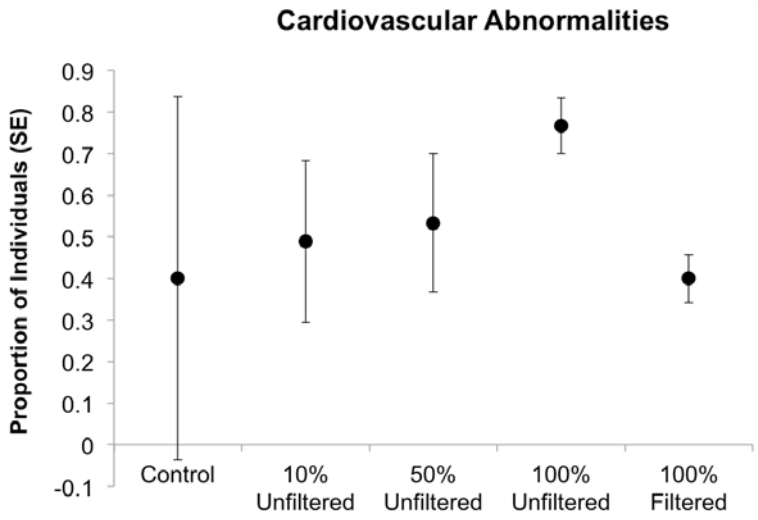

Figure 8. Proportion of embryos with cardiac abnormalities following 7 episodic exposures to runoff. Differences among treatments were not statistically significant ($F(4,14) = 2.235$, $p = 0.145$).

Deliverable 3.3: Chemistry of treated effluent

Bioretention treatment significantly altered runoff chemistry. Nutrient concentrations in highway runoff were typically low (medians: Total N = 4.3 mg/L; Total P = 0.224 mg/L). Runoff water picked up nutrients as it passed through the bioretention cells, resulting in higher concentrations of nitrates and phosphates leaving than entering the bioretention cells (Table 7; Figure 9).

On a mass base of total metals, the bioretention cells had a net export of As (9.3 mg), Ni (0.4 mg), and Ag (0.5 mg), but a net retention of the other metals; 0.2 mg Cd, 12.9 mg Cr, 121.7 mg Cu, 43.0 mg Pb, and 625.1 mg Zn (Table 7).

PAH concentrations in influent runoff ranged from 5-31 µg/L, with a median concentration of 10 µg/L. In the bioretention-filtered effluent, PAH concentrations ranged from 0.1-1.6 µg/L with a median concentration of 0.8 µg/L; similar to control waters (Figure 10). The variability in PAH values was low, with a COV of <10% (mean COV = 3%). Therefore, error bars are not shown for the PAH values in Figure 10. In terms of pollutant mass, 19.4 mg of measured PAHs entered the bioretention cells during the 10 experimental filtrations whereas only 1.7 mg were released – a total reduction of 91%.

Table 7. Median (and range) of water chemistry values for influent untreated highway runoff and effluent treated runoff from the bioretention cells and net mass retained by bioretention soil media (BSM) across the 10 experimental storm events.

Category	Parameter	D.L.	Units	Untreated Runoff	Bioretention-Treated	Net Mass Retained	% Mass Retained
						10^6 CFU	
Microbiology	Fecal Coliform	5	CFU/100 mL	910 (150-3100)	567 (73-1200)	96	7
	E. coli	5	CFU/100 mL	730 (0-2200)	297 (0-1213)	831	47
						Net kg	
Conventional	pH	0.1	-	6.7 (5.6-7.3)	6.9 (6.5-7.1)	n.a.	n.a.
	TSS	1	mg/L	150 (93-500)	44 (28-52)	255	75
	SSC	0.2	mg/L	172 (93-510)	47 (41-701)	275	74
Demand	TOC	0.5	mg/L	31 (4-50)	40 (24-61)	(-38.3)	(-89)
	COD	10	mg/L	190 (24-1000)	103 (83-190)	70	24
	DOC	0.5	mg/L	5 (1-37)	34 (21-65)	(-59)	(-435)
Minerals	Alkalinity	1	mg CaCO3/L	31 (10-58)	79 (37-100)	(-102)	(-202)
	Hardness	0.05	mg CaCO3/L	39 (12-340)	68 (7-840)	(-180)	(-194)
	Ca	0.05	mg/L	12 (4-120)	17 (2-200)	(-38)	(-128)
	Mg	0.01	mg/L	3 (1-35)	5 (1-20)	(-8.2)	43
Nutrients	Ammonia	0.01	mg/L	0.70 (0-4.55)	0.75 (0.04-1.56)	(-0.2)	(-12)
	Total N	0.1	mg/L	4.3 (1.2-70.1)	5.2 (2.5-54.6)	(-1.3)	(-10)
	Nitrate	0.025	mg/L	0.35 (0.06-1.50)	11.45 (2.16-34.20)	(-15)	(-2141)
	Ortho-P	0.005	mg/L	0.007 (0-0.884)	0.767 (0.011-1.287)	(-1.1)	(-262)
	Total P	0.005	mg/L	0.224 (0.094-0.438)	1.300 (0.278-2.063)	(-2.3)	(-594)
						Net mg	
Total Metals	As	0.02	ug/L	3.49 (1.23-15.00)	8.34 (2.73-10.90)	(-9.3)	(-152)
	Cd	0.025	ug/L	0.43 (0.14-2.64)	0.14 (0.09-3.59)	0.2	26
	Cr	0.05	ug/L	11.6 (7.6-23.0)	4.8 (3.0-6.5)	13	57
	Cu	0.1	ug/L	115.5 (46.5-184.0)	26.6 (18.4-60.5)	122	69
	Pb	0.05	ug/L	24.9 (12.7-67.1)	3.1 (1.40-5.4)	43	88
	Ni	0.05	ug/L	11.18 (3.90-20.00)	8.46 (5.54-18.60)	(-0.4)	(-2)
	Ag	0.05	ug/L	0.11 (0.05-0.52)	0.12 (0.06-0.23)	(-0.5)	(-110)
	Zn	0.05	ug/L	375.3 (140.7-1320.0)	46.0 (25.7-191.0)	625	85
Dissolved Metals	As	0.02	ug/L	1.4 (0.4-7.4)	7.1 (3.2-10.4)	(-11)	(-355)
	Cd	0.025	ug/L	0.14 (0.04-7.35)	0.12 (0.06-0.23)	0.2	41
	Cr	0.05	ug/L	3.82 (2.16-6.17)	2.86 (1.42-9.36)	(-0.3)	(-4)
	Cu	0.1	ug/L	20.1 (5.1-115.0)	25.8 (13.3-38.7)	0.5	1
	Pb	0.05	ug/L	3.53 (0.14-24.80)	1.91 (0.34-3.28)	3.9	64
	Ni	0.05	ug/L	3.25 (0.49-9.48)	6.35 (3.50-15.40)	(-7.1)	(-154)
	Ag	0.05	ug/L	0.05 (0-0.07)	0.09 (0.08-0.95)	(-0.4)	(-1471)
	Zn	0.05	ug/L	148.0 (44.0-876.0)	32.5 (17.8-57.2)	261	86
Total	PAHs	<0.01	ug/L	9.81 (4.93-30.76)	0.76 (0.13-1.62)	18	91

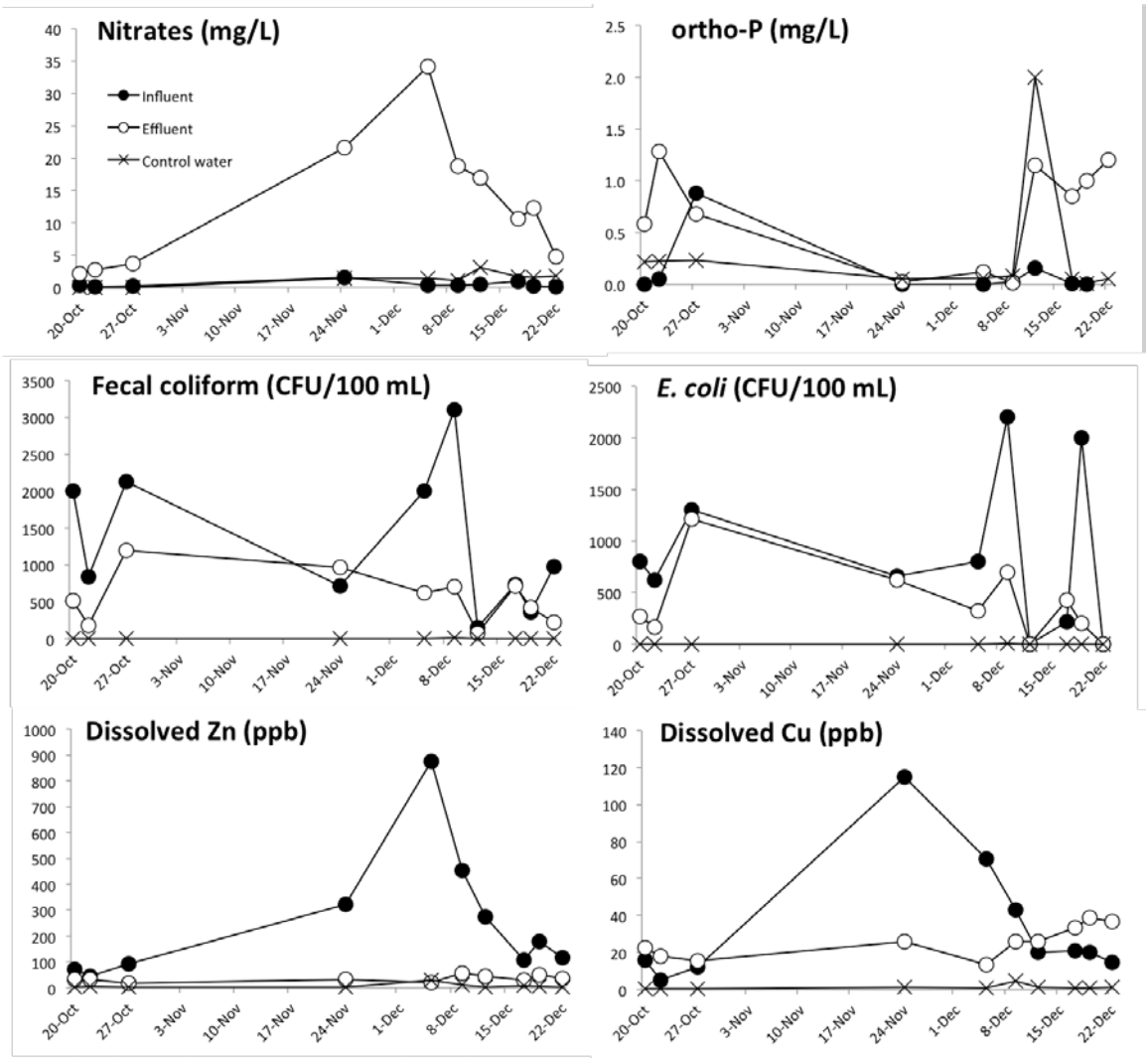

Figure 9. Nutrients, bacteria, and dissolved metals in unfiltered highway runoff, runoff filtered with bioretention, and control well water across 10 storm events during 2014.

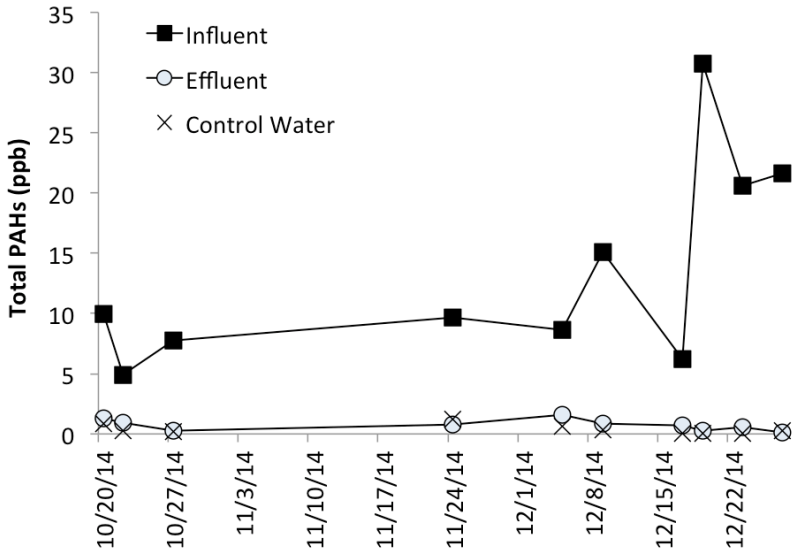

Figure 10. The sum of 42 parent and alkylated homologue PAHs in unfiltered highway runoff, runoff filtered by bioretention, or control well water for 10 storm events during 2014.