Deliverable 2: Report on chemistry and toxicology of bioretention soil media (BSM) components

Report Summary

Bioretention components analysis

- Compost contains higher concentrations of metals and PAHs than sand
- All compost samples exceeded zinc Ecological Soil Screening Levels (Eco-SSL) for avian wildlife
- The mean value of high molecular weight PAHs in compost exceeded the mammalian Eco-SSL

Bioretention leachate analysis

- Compost and mulch leached more metals than the other BSM components
- Low molecular weight PAHs leached more from the bioretention components than high molecular weight PAHs
- Mulch leachate contained the highest concentrations of PAHs (TPAH = 0.986 μg/L)

Toxicity Testing

- Zebrafish exposed to mulch leachate experienced 100% mortality
- Despite concerns of toxic effects from mulch leachate, the mulch layer in bioretention is not expected to pose the same risk at the field scale

1. Bioretention Components Analysis

Individual bioretention soil media components were analyzed prior to placement in the experimental columns to characterize the chemical composition of each of the components. Triplicate samples of sand and compost were analyzed for chemical composition, including metals (Cu, Zn, Cd, Pb, As, Ni, Mg, Ca, Na), nutrients (ammonia, total nitrogen, nitrate-nitrite, total phosphorous), organic matter, total organic carbon, total solids, and PAHs (See Table 6 of the Quality Assurance Project Plan for analytical chemistry methods). Given the low surface-to-volume ratio for mulch and gravel, these components were not analyzed.

Total metal concentrations in bioretention soil media (BSM) are presented in Table 1. Total metal concentrations in BSM were compared to EPA's Ecological Soil Screening Levels (Eco-SSLs). Eco-SSLs are threshold contaminant concentrations in soil intended to protect terrestrial ecosystems. These values are intended for use during the screening stage of ecological risk assessment to identify contaminants of potential concern that require further evaluation (EPA 2018). Eco-SSL values for arsenic, cadmium, copper, lead, nickel, and zinc are presented for plants, soil invertebrates, avian wildlife, and mammalian wildlife (Table 2). All sand and compost samples were below Eco-SSLs for arsenic, cadmium, copper, and lead.

Table 1. Concentrations of total metals in triplicate samples of sand and compost. Values presented are mean (standard deviation).

Compound	Detection Limit (mg/kg)	Result (mg/kg dry weight)	
		Compost	Sand
Total Arsenic	0.4	BDL	BDL
Total Cadmium	0.03	0.19 (0.02)	BDL
Total Copper	0.03	21 (3)	16.6 (0.5)
Total Lead	0.03	6.8 (0.6)	1.13 (0.06)
Total Nickel	0.5	3.9 (0.5)	40 (4)
Total Zinc	0.2	54 (4)	29.1 (0.8)

BDL = Below Detection Limit

Table 2. EPA Ecological Soil Screening Levels for metals in soil.

Metals Eco-SSLs (mg/kg dry weight)				
Analyte	Plants	Soil Invertebrates	Wildlife	
			Avian	Mammalian
Arsenic	18	NA	43	46
Cadmium	32	140	0.77	0.36
Copper	70	80	28	49
Lead	120	1,700	11	56
Nickel	38	280	210	130
Zinc	160	120	46	79

NA= no SSL available

All compost samples were below the Eco-SSLs for nickel. For sand, the concentrations of two of the triplicate samples exceeded the nickel Eco-SSL value for terrestrial plants. However, concentrations were less than the maximum reported background concentrations of nickel in western U.S. soils (U.S. EPA, 2007e).

All sand samples were below zinc Eco-SSLs. However, all compost samples exceeded zinc Eco-SSLs for avian wildlife, but were similar to the median background concentrations of zinc in western U.S. soils (U.S. EPA, 2007f).

Compared with previous bioretention studies by Washington State University, the current bioretention media components have less concentrated metals — mostly due to lower metal concentrations in the compost (Figure 1).

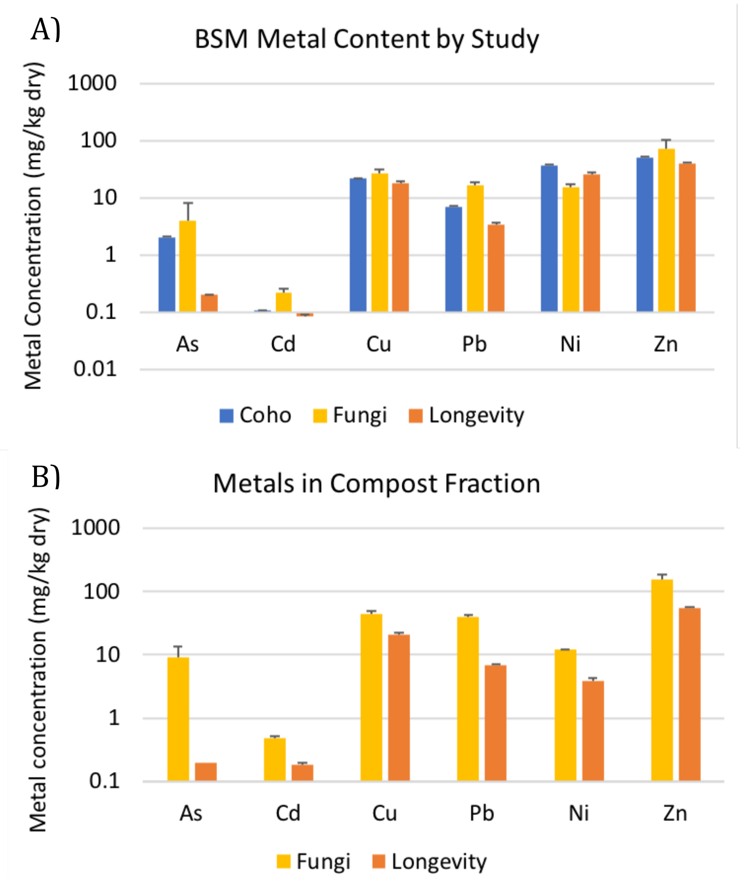

Figure 1. Metal concentration in A) the BSM and B) the compost fraction of various SAM studies (Coho, Fungi, and Longevity) conducted by the McIntyre lab at WSU. 'Longevity' represents the current study. Concentrations in BSM were measured in the 'Coho' study and presumed based on concentrations measured in sand and compost for the 'Fungi' study and the current 'Longevity' study based on 60:40 sand:compost. Error bars are one standard error of the mean.

Polycyclic aromatic hydrocarbon (PAH) concentrations in sand and compost samples (Table 3) were well below PAH Eco-SSLs for soil invertebrates and the low molecular weight (HMW) PAH Eco-SSL for mammals (Table 4). The mean value of high molecular weight PAHs in compost (1.6 ppm) exceeded the mammalian Eco-SSL (1.1 ppm) but was less than the mean of these same PAHs for background urban soils in the U.S. (2.5 ppm; Mauro et al. 2008). The distribution of PAHs in compost were also similar to background urban soils with the most abundant PAHs being fluoranthene > pyrene > phenanthrene.

Table 3. PAH concentrations in triplicate samples of compost and sand. Values presented are mean (standard deviation).

Compound	Detection Limit (µg/kg)	Result (µg/kg dry weight)	
		Compost	Sand
Benzo(ghi)perylene	1.01	45 (5)	BDL
Indeno(1,2,3-cd)pyrene	1.05	35 (6)	BDL
Dibenzo(a,h)anthracene	0.85	8 (2)	BDL
Benzo(a)pyrene	0.58	66 (12)	BDL
Benzo(j)fluoranthene	0.65	50 (10)	BDL
Benzo(k)fluoranthene	0.72	51 (10)	BDL
Benzo(b)fluoranthene	1.30	107 (20)	0.9 (0.4)
Chrysene	1.00	179 (40)	1.10 (0.06)
Benz[a]anthracene	0.78	126 (20)	0.5 (0.3)
Pyrene	0.60	353 (20)	1.7 (0.7)
Fluoranthene	0.45	559 (80)	1.6 (0.9)
Anthracene	0.83	47 (2)	BDL
Phenanthrene	0.68	264 (30)	1.1 (0.9)
Fluorene	0.60	27 (7)	BDL
Dibenzofuran	1.31	30 (4)	BDL
Acenaphthene	0.54	20 (5)	0.8 (0.5)
Acenaphthylene	1.03	7 (2)	BDL
1-Methylnaphthalene	0.38	8.1 (0.1)	0.3 (0.2)
2-Methylnaphthalene	1.05	12 (2)	BDL
Naphthalene	1.21	15 (4)	BDL
Sum Low Molecular Weight (LMW)*		430	5.2
Sum High Molecular Weight (HMW)**		1580	8.2
Sum Total PAHs		2010	13.4

*Compounds composed of fewer than four rings
**Compounds composed of four or more rings

Table 4. EPA Ecological Soil Screening Levels for PAHs in soil.

PAH Eco-SSLs (mg/kg dry weight)				
Analyte	Plants	Soil Invertebrates	Wildlife	
			Avian	Mammalian
Low Molecular Weight (LMW)*	NA	29	NA	100
High Molecular Weight (HMW)**	NA	18	NA	1.1

NA = No SSL available
*Compounds composed of fewer than four rings
**Compounds composed of four or more rings

As expected, nutrient and organic matter concentrations were greater in compost than in sand samples (Table 5).

Table 5. Nutrient concentrations, organic matter, total organic carbon, and total solids in triplicate samples of sand and compost. Values presented are mean (standard deviation).

Compound	Reporting Limit (mg/kg)	Result (mg/kg dry weight)	
		Compost	Sand
Nitrate/Nitrite	0.1	9 (5)	0.3 (0.1)
Ammonia	4	85 (40)	16 (10)
Total Phosphorous	0.1	45 (40)	1.6 (0.5)
Total Nitrogen	10,000	17,715 (2000)	BDL
Organic Matter (%)	0.5	57 (2)	1.17 (0.06)
Total Organic Carbon	1000	247,000 (20,000)	<20,000*
Total Solids (%)	0.5	42.5 (0.8)	95.4 (0.2)

*Lower reporting limits could not be achieved due to sample matrix.

2. Bioretention Leachate Analysis

Triplicates of each of the bioretention soil media (BSM) components (sand, compost, gravel, and mulch) were leached according to EPA method 1312 (EPA 1994) to determine the leaching potential of these components. Briefly, 5-L polypropylene beakers were filled with 3L reverse osmosis (RO) water and 150g of one of four bioretention media components. Prior to the addition of the BSM component, the pH of the extraction fluid (RO water) was adjusted with sulfuric acid/nitric acid (60/40 weight percent mixture) to a pH of 5.00 ± 0.05. Beakers were placed on an orbital shaker (rpm=9.2) and agitated for 18 hours. Following the 18-hour extraction, the liquid and solid phases were separated by filtering through acid-washed 0.7µm glass fiber filters (Whatman, Cat No 1825-047). Prior to filtration, the leachate was centrifuged at 14,000 rpm for 20 minutes to promote the settling of larger particles in the leachate and aid filtration. The collected leachate was then assessed for PAHs at Analytical Resources, Inc. (ARI) and for metals (total and dissolved), fecal coliform, total suspended solids, dissolved organic carbon, pH, ortho-phosphate, and nitrite+nitrate at Spectra Laboratories. Alkalinity was determined by Standard Method 2320 B (Titration Method) at the WSU-P Aquatic Toxicology Laboratory.

Metals

Overall, compost and mulch leached more metals than the other BSM components. Zinc was the dominant metal that leached from all of the BSM components in the order of mulch > compost > gravel > sand (Table 6 & 7). Zinc appeared much more mobile than the other metals as both total and dissolved Zn appeared at much higher concentrations in the compost leachate and sand leachate relative to their presence in the solid media (Figure 2 & 3). This is supported by research indicating that zinc activity in soil is high and concentrations of zinc in solution are comparable to total concentrations of zinc in soil (Rutkowska et al. 2014). However, metal concentrations in soil samples do not always serve as a proxy for the metals most prone to leaching from these samples. Sodium, magnesium, and calcium were much more concentrated in the mulch and compost leachates than in the gravel or sand leachates (Table 7).

Table 6. Summary of total metal concentrations in leachate of bioretention components. Values presented are mean (standard deviation).

Compound	Detection Limit (µg/ L)	Leachate (µg/ L)			
		Compost	**Sand**	**Gravel**	**Mulch**
Arsenic	0.05	11 (1)	0.3 (0.3)	0.4 (0.4)	1.6 (0.2)
Cadmium	0.05	BDL	BDL	BDL	BDL
Copper	0.05	23 (20)	0.6 (0.3)	0.4 (0.5)	5.4 (0.4)
Lead	0.079	3 (3)	BDL	BDL	0.5 (0.1)
Nickel	0.2	12 (5)	1.5 (0.3)	0.7 (0.6)	2.4 (0.5)
Zinc	0.19	161 (30)	55 (10)	96 (20)	199 (50)

BDL = Below Detection Limit

Table 7. Summary of dissolved metal concentrations in leachate of bioretention components. Values presented are mean (standard deviation).

Compound	Detection Limit (µg/ L)	Leachate (µg/ L)			
		Compost	**Sand**	**Gravel**	**Mulch**
Arsenic	0.05	11 (1)	0.2 (0.2)	BDL	1.4 (0.2)
Cadmium	0.05	BDL	BDL	BDL	BDL
Copper	0.05	11 (2)	0.3 (0.4)	0.2 (0.3)	3 (3)
Lead	0.079	0.8 (0.2)	BDL	BDL	0.6 (0.4)
Nickel	0.2	8.1 (0.5)	1.2 (0.3)	0.4 (0.5)	2.1 (0.3)
Zinc	0.19	140 (40)	51 (10)	89 (10)	181 (40)
Sodium	27	29500 (2000)	800 (90)	876 (100)	4250 (700)
Magnesium	1.9	881 (50)	199 (4)	140 (8)	1443 (200)
Calcium	3.4	3010 (100)	118 (20)	358 (90)	3870 (500)

BDL = Below Detection Limit

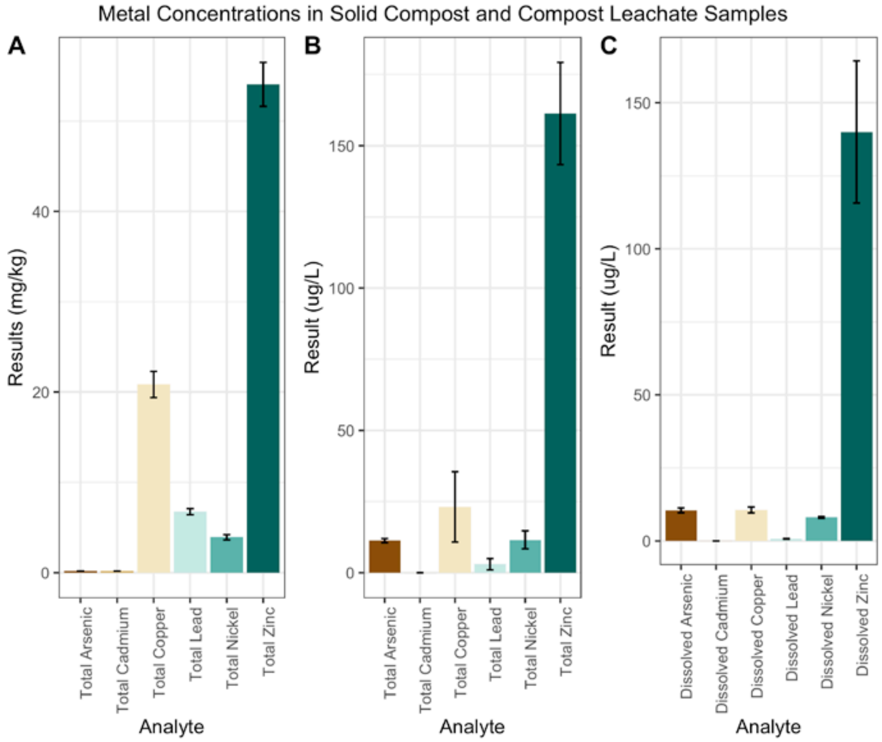

Figure 2. Comparison of A) total metal concentrations in solid compost (dry weight basis), B) total metal concentrations in compost leachate, and C) dissolved metal concentrations in compost leachate.

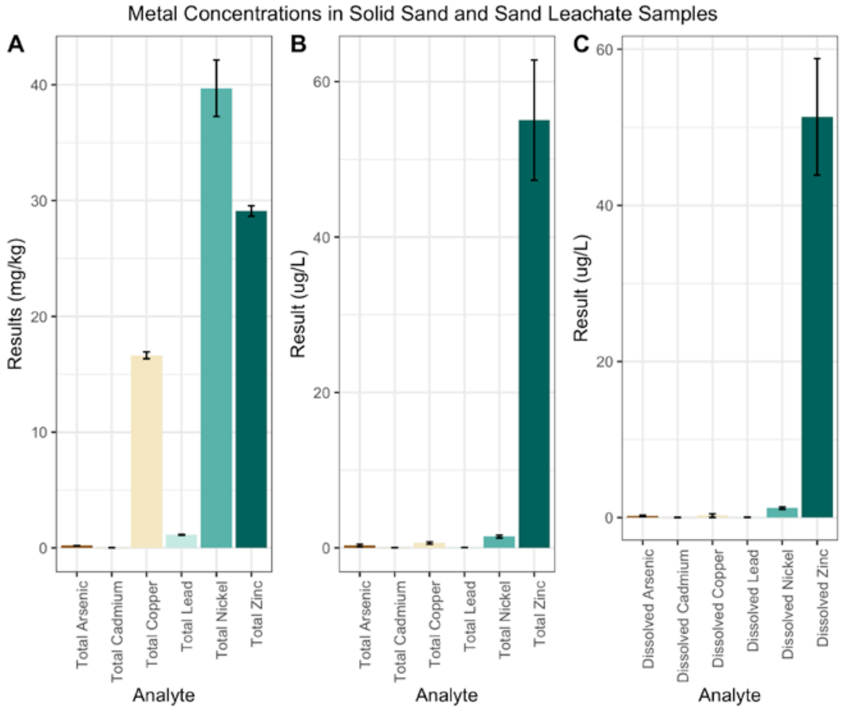

Figure 3. Comparison of A) total metal concentrations in solid sand (dry weight basis), B) total metal concentrations in sand leachate, and C) dissolved metal concentrations in sand leachate.

PAHs

PAHs that leached from the bioretention components were predominantly low molecular weight, dominated by phenanthrene (Table 8). Mulch leachate contained the highest concentrations of PAHs (TPAH = 0.986 µg/L) whereas in leachates of compost, sand, and gravel TPAH was 0.05-0.11 µg/L. Among the PAHs in the mulch leachate, phenanthrene concentrations were greater even than concentrations that may be seen in road runoff (Spromberg et al. 2016; McIntyre et al. 2014).

Comparing the PAH concentrations in the solid compost and sand with that in the leachate (Figure 4 & 5), we see that lower molecular weight PAHs were more likely to leach into water than the higher molecular weight PAHs. Whereas 4-ring fluoranthene and then pyrene dominated in the solid samples, 3-ring phenanthrene and then 2-ring naphthalene overtook these as the most dominant PAHs in the leachates of compost and sand.

Table 8. Summary of total PAHs in leachate from each bioretention component. Values presented are mean (standard deviation).

Compound	Detection Limit (µg/ L)	Leachate (µg/ L)			
		Compost	Sand	Gravel	Mulch
Total PAHs		0.111	0.054	0.106	0.986
Benzo(ghi)perylene	0.002	0.001 (<)	BDL	BDL	0.005 (0.001)
Indeno(1,2,3-cd)pyrene	0.001	BDL	BDL	BDL	0.004 (0.002)
Dibenzo(a,h)anthracene	0.002	BDL	BDL	BDL	0.004 (0.003)
Perylene	0.008	BDL	BDL	BDL	BDL
Benzo(a)pyrene	0.004	BDL	BDL	BDL	BDL
Benzo(j)fluoranthene	0.003	BDL	BDL	BDL	BDL
Benzo(k)fluoranthene	0.005	BDL	BDL	BDL	BDL
Benzo(b)fluoranthene	0.0007	0.002 (<)	BDL	BDL	BDL
Chrysene	0.001	0.005 (<)	BDL	BDL	BDL
Benzo(a)anthracene	0.001	0.004 (<)	BDL	0.005 (0.008)	BDL
Pyrene	0.002	0.005 (0.002)	0.001 (<)	0.008 (0.01)	0.017 (0.004)
Fluoranthene	0.002	0.015 (0.002)	BDL	0.003 (0.003)	0.006 (0.002)
Carbazole	0.002	0.003 (<)	BDL	BDL	BDL
Anthracene	0.002	0.001 (<)	BDL	0.006 (0.007)	BDL
Phenanthrene	0.002	0.023 (0.003)	0.011 (0.002)	0.022 (0.006)	0.62 (0.09)
Fluorene	0.002	0.008 (0.002)	0.003 (<)	0.011 (0.006)	0.12 (0.01)
Dibenzofuran	0.002	0.007 (0.002)	0.003 (<)	0.004 (0.004)	0.039 (0.002)
Acenaphthene	0.004	BDL	BDL	0.006 (0.007)	0.009 (<)

Acenaphthylene	0.003	BDL	BDL	BDL	BDL
1-Methylnaphthalene	0.001	0.001 (0.001)	0.003 (<)	0.003 (<)	0.05 (0.01)
2-Methylnaphthalene	0.001	0.005 (0.001)	0.004 (<)	0.006 (0.002)	0.003 (0.002)
Naphthalene	0.002	0.014 (0.003)	0.010 (0.001)	0.015 (0.005)	0.100 (0.003)
2-Chloronaphthalene	0.001	BDL	BDL	BDL	BDL
Sum Low Molecular Weight (LMW)*		0.067	0.039	0.077	0.940
Sum High Molecular Weight (HMW)**		0.044	0.015	0.029	0.046

*Compounds composed of fewer than four rings
**Compounds composed of four or more rings
< indicates a value less than 0.001
BDL = Below Detection Limit

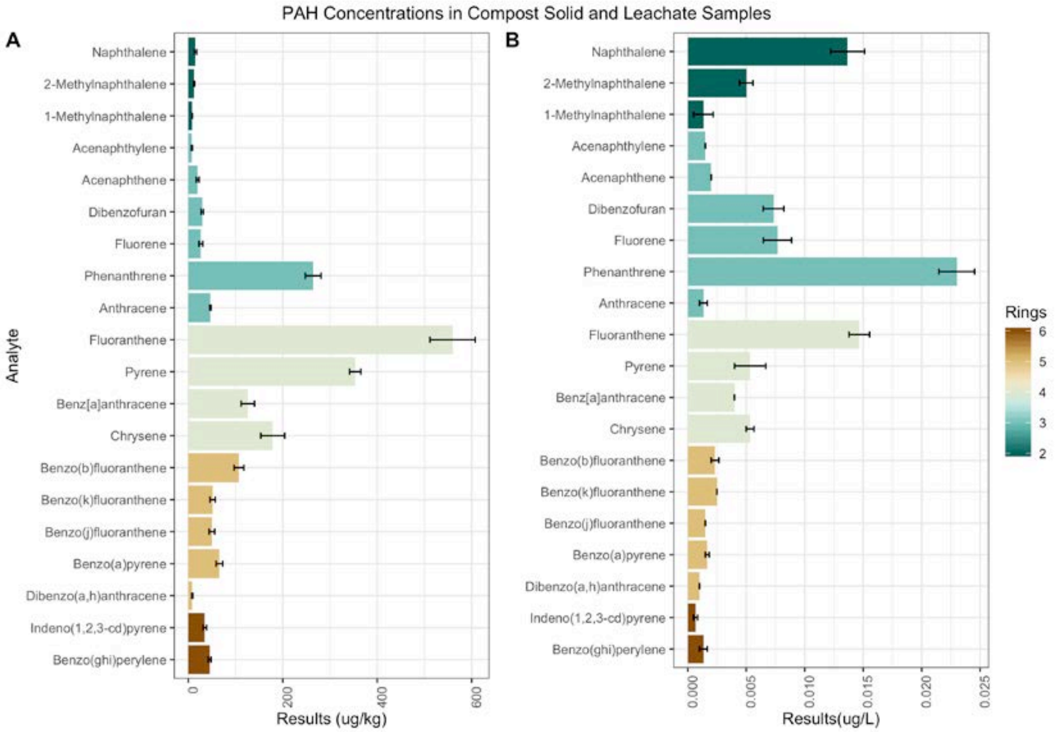

Figure 4. Comparison of relative PAH concentrations in A) solid compost (dry weight basis) vs. B) compost leachate samples. Error bars are one standard error of the mean.

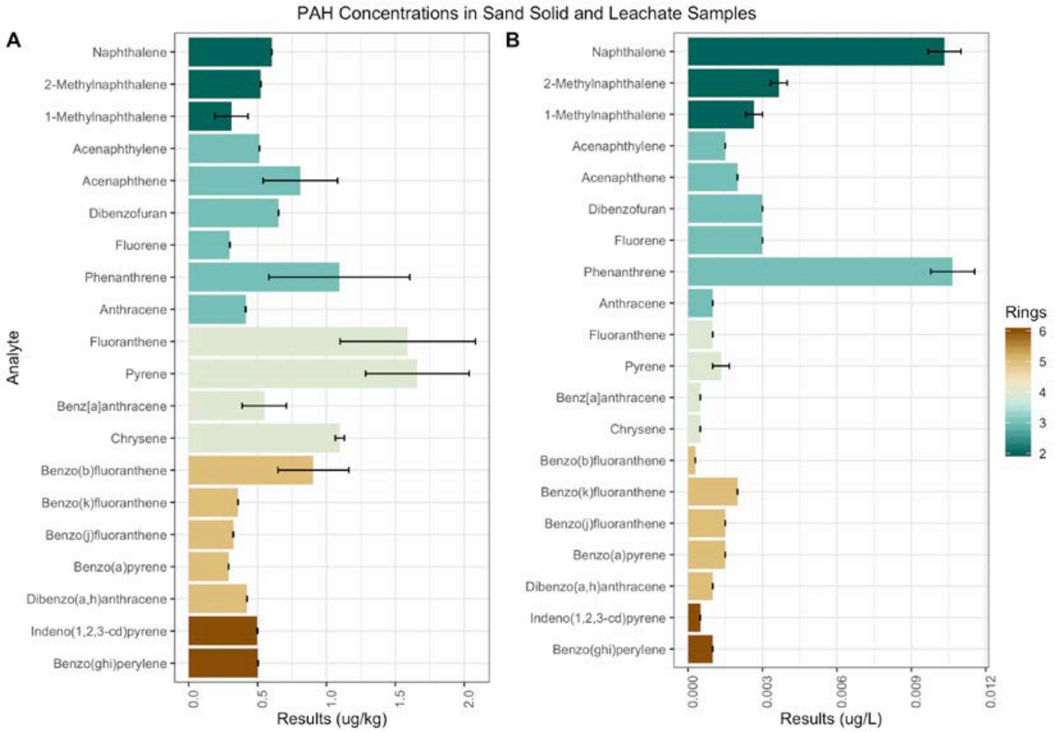

Figure 5. Comparison of relative PAH concentrations in A) solid sand (dry weight basis) vs. B) sand leachate samples. Error bars are one standard error of the mean.

Nutrients & Conventionals

Compost leachate had the highest concentration of nitrates, alkalinity, and hardness, whereas mulch leachate contained the highest concentration of orthophosphate and dissolved organic carbon (DOC; Table 9). Both compost and mulch were sources of total suspended solids (TSS; Table 9).

Table 9. Summary of nutrient and conventional parameters in leachate of bioretention components. Values presented are mean (standard deviation).

Compound	Detection limit (mg/L)	Leachate (mg/L)			
		Compost	**Sand**	**Gravel**	**Mulch**
Nitrate/Nitrite	0.003	79 (60)	1.0 (0.2)	5 (3)	0.3 (0.1)
Orthophosphate	0.01	1.5 (0.1)	BDL	0.02 (0.02)	9.8 (0.1)
DOC	0.08	32.3 (0.3)	BDL	BDL	147 (9)
Alkalinity (as $CaCO_3$)	n.a.	39 (13)	-0.8 (0.6)	1 (1)	3.88 (0.06)
Hardness (as $CaCO_3$)	calculated	11.1 (0.6)	1.11 (0.07)	1.5 (0.2)	0.015 (0.002)
TSS	0.5	1 (1)	0.4 (0.2)	0.6 (0.3)	1.3 (0.3)

BDL = Below Detection Limit

3. Toxicity Testing

Following leaching of the bioretention soil media components for water chemistry analysis, each component was then leached with zebrafish (*Danio rerio*) embryo rearing medium (RO water reconstituted with salts). The leachate was assessed for acute toxicity using embryos of wild type (AB) zebrafish at WSU-P.

Exposure began approximately 2.5 hours post fertilization (hpf). For each of the four leachates (compost, sand, gravel, mulch) plus the embryo medium control, 32 embryos were placed in a glass-lined 96-well microplate containing 300 µL of test solution. A water change was performed at approximately 24 hpf and zebrafish were imaged at approximately 48 hpf. Images were analyzed for morphometrics including embryo length, eye area, and heart-related metrics including pericardial area (PCA) and periventral area (PVA). A Kruskal-Wallis test was performed to compare medians of the treatment groups for each morphometric (Figure 6). For metrics with a significant difference among treatment groups, a post-hoc Dunn's test was performed to determine which treatment groups differed from the control.

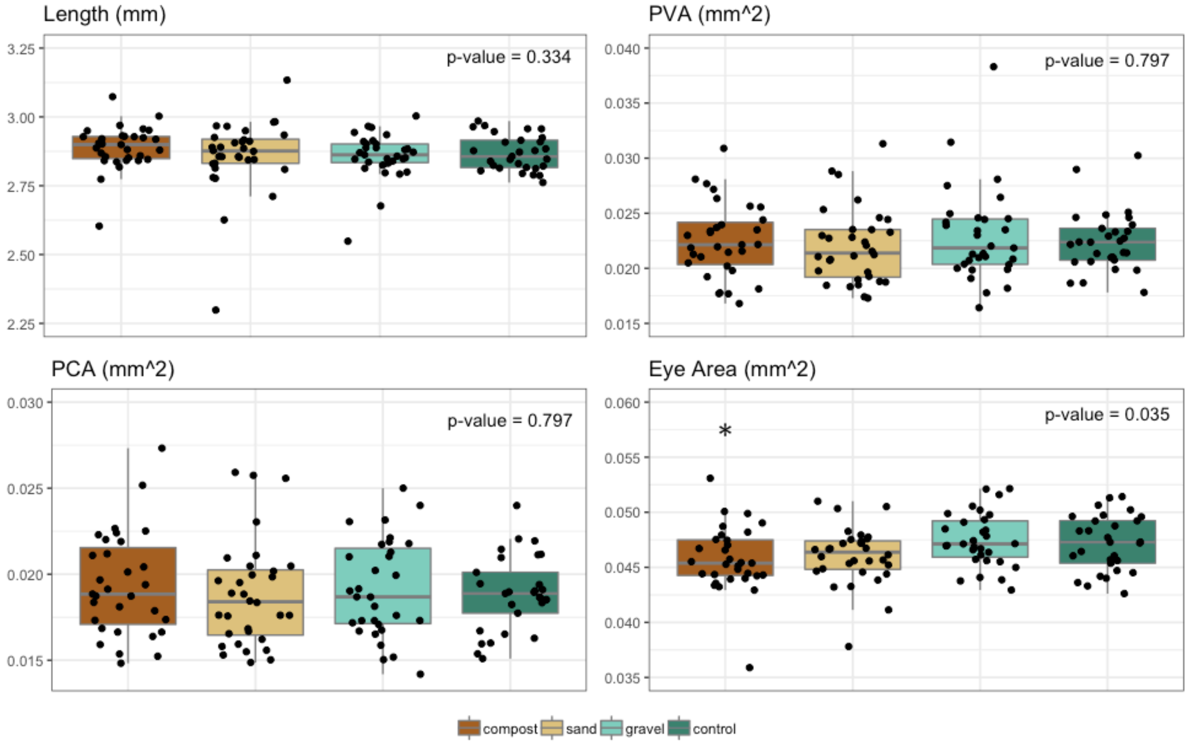

Figure 6. *Danio rerio* morphometrics for 48 h exposure to fish rearing water (control) and leachates of BSM components. The mulch leachate caused 100% mortality. PVA = periventral area and PCA = pericardial area. * denotes significant difference from control.

Fish exposed to mulch leachate experienced 100% mortality within 24 h of exposure. Survival was 100% for sand and compost, 97% for gravel, and 100% for the embryo rearing medium control (Table 10). Three outliers (two control and one compost treatment) were removed from the dataset due to extreme developmental abnormalities. There were no significant differences in length, PCA, or PVA among treatment groups. However, there were statistically significant differences in eye area among treatment groups ($\chi^2(3) = 8.6$, p=0.035). The Dunn's post-hoc test revealed a slight but significant reduction in eye area (4 %) for embryos reared in compost leachate (p=0.035), although this may not be biologically relevant.

The lack of acute lethal or sublethal effects for leachates from compost, sand, and gravel was expected. Clean water effluent from previous conditioning of experimental bioretention columns containing these three components was similarly not acutely toxic to zebrafish embryos (McIntyre et al. 2016; SAM project: "Field Test of Plants and Fungi on Bioretention Performance Over Time", Deliverable 4.2).

The acute mortality caused by the mulch leachate, while surprising, was not completely unexpected. The mulch used for this study was arborist mulch, comprised of chipped branches and trees from arborist activities. Wood chips can leach highly toxic compounds into water, resulting in acute mortality in fish and other aquatic organisms (Taylor et al. 1996, 2003, Rex et al. 2016). Resin acids are considered some of the most abundant sources of toxic compounds naturally present in wood (Ali & Sreekrishnan 2001). Additionally, several studies have indicated the toxic potential of phenanthrene and its alkyl homologs (especially retene) to fish (Brinkworth et al. 2003; Hawkins et al. 2003; Mu et al. 2014). Brinkworth et al. (2003), for example, observed hemorrhaging, yolk-sac edema, and mortality in early life-stage rainbow trout exposed to retene. Phenanthrene was by far the most abundant PAH measured in mulch leachate, and was several orders of magnitude higher in the mulch leachate than in the other bioretention components (Table 8). Among the innovations explored by the pulping and forestry industries to address toxicity of runoff from wood include soil infiltration (Hedmark & Scholz 2008) and pre-treatment of wood chips with fungi (Wang et al. 1995; Dorado et al. 2000; Hedmark & Scholz 2008), both of which tend to be involved in bioretention applications.

Although potentially concerning, toxic compounds contributed from the mulch layer are not expected to pose a problem during stormwater treatment for four reasons: 1) We expect much less contact between water and mulch during stormwater treatments than during the leach test. This is because water will drip over the mulch layer during stormwater treatment whereas mulch was completely submerged during the leachate test. 2) The concentration of compounds leaching from the mulch will be less. The amount of mulch used in each bioretention column is approximately 140 g, over which 56 L of water will pass during each stormwater treatment. This is in contrast to 150 g of mulch that was leached into 3 L of water in the leach test. 3) Compounds leaching from the mulch should decrease over time as these compounds are depleted by leaching and also by fungal and bacterial degradation. 4) Potentially toxic compounds leaching from the mulch as it passes over will likely be captured and treated by the BSM along with organic contaminants in the stormwater itself.

An interesting side-study could be to test for how long the mulch leaches compounds acutely toxic to fish.

Table 10. Summary of sublethal effects of bioretention component leachates on zebrafish development at 48 hpf. Values presented are mean (standard deviation).

Treatment	Mortality Rate	PCA (mm^2)	PVA (mm^2)	Eye Area (mm^2)	Length (mm)
Control	0%	0.019 (0.002)	0.022 (0.003)	0.047 (0.003)	2.87 (0.06)
Mulch	100%	na	na	na	na
Gravel	3%	0.019 (0.003)	0.023 (0.004)	0.047 (0.002)	2.86 (0.08)
Sand	0%	0.019 (0.003)	0.022 (0.003)	0.046 (0.003)	2.90 (0.10)
Compost	0%	0.019 (0.003)	0.023 (0.003)	0.045 (0.003)*	2.89 (0.08)

* Significantly different from control ($p < 0.05$)

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