Effect of Roof Material on Water Quality for Rainwater Harvesting Systems – Additional Physical, Chemical, and Microbiological Data

Report

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List of Abbreviations

AASHTO	American Association of State Highway and Transportation Officials
Al	aluminum
As	arsenic
AWWA	American Water Works Association
bp	base pairs
°C	degrees Celsius
Cd	cadmium
CFU	colony forming units
Cl^-	chloride ion
Cr	chromium
Cu	copper
DBP	disinfection by-product
DNA	deoxyribonucleic acid
DOC	dissolved organic carbon
^oF	degrees Fahrenheit
FC	fecal coliform
Fe	iron
ft	foot
HAA	haloacetic acid
ICMB	Institute for Cellular and Molecular Biology
in	inch
LB	Lysogeny Broth
mA	milliamp
MCL	maximum contaminant level
mg/L	milligram per liter
mL	milliliter
mL/min	milliliters per minute
mM	millimolar
MPa	megapascal
Na^+	sodium ion
ng	nanogram
nm	nanometer
NO_2^-	nitrite
NO_2^--N	nitrite-nitrogen
NO_3^-	nitrate
NO_3^--N	nitrate-nitrogen
NSF	National Science Foundation
NTU	nephelometric turbidity units
Pb	lead
PCR	polymerase chain reaction
PVC	polyvinyl chloride
PVDF	polyvinylidene fluoride
rRNA	ribosomal ribonucleic acid
Se	selenium
SI	Sørenson index

SWI	Shannon-Weaver index
TC	total coliform
TCEQ	Texas Commission on Environmental Quality
TDS	total dissolved solids
TE	tris(hydroxymethyl)aminomethane-ethylenediaminetetraacetic acid
THM	trihalomethane
T-RFLP	terminal restriction fragment length polymorphism
TSS	total suspended solids
TWDB	Texas Water Development Board
U	unit
μg/L	microgram per liter
μL	microliter
μm	micrometer
μM	micromolar
μS/cm	microSiemens per centimeter
USEPA	United States Environmental Protection Agency
UV	ultraviolet
Zn	zinc

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1 Executive summary

Harvested rainwater is an excellent source of water for potable and nonpotable applications in many parts of the United States. The quality of harvested rainwater can be affected by a variety of factors including ambient conditions, season, and roofing material. The main objective of this research was to provide information to the rainwater harvesting community in Texas regarding the impact of roofing material on harvested rainwater quality.

This research was a follow-up study to Mendez et al. (2010), and we collected additional data regarding the impact of roofing material on the quality of harvested rainwater from pilot-scale roofs (asphalt fiberglass shingle, Galvalume®, concrete tile, cool, and green) and a full-scale Kynar®-coated Galvalume® roof. The harvested rainwater was collected from multiple rain events and analyzed for the following parameters: pH, conductivity, turbidity, total suspended solids (TSS), total coliform (TC), fecal coliform (FC), nitrate, nitrite, dissolved organic carbon (DOC), and selected metals. We examined the diversity of the microbial communities from rainwater harvested from the pilot-scale roofs by terminal restriction fragment length polymorphism (T-RFLP) and 16S ribosomal ribonucleic acid (rRNA) gene sequencing. We conducted lab-scale studies with new and artificially aged coupons of asphalt fiberglass shingle, Galvalume® metal, and concrete tile to examine how the release of contaminants might change as these roofing materials age.

Our work shows that harvested rainwater quality generally improves with roof flushing, indicating the importance of an effective first-flush diverter. However, the rainwater harvested after the first-flush from all of the pilot-scale roofs did contain some contaminants at levels above United States Environmental Protection Agency (USEPA) drinking water standards (i.e., turbidity, TC, FC, iron[1], and aluminum); the harvested rainwater after the first-flush from the full-scale Kynar®-coated Galvalume® roof exceeded the turbidity, TC, and FC standards. The quality of rainwater harvested for potable use at a private residence is not regulated by the Texas Commission on Environmental Quality (TCEQ), and, thus, the USEPA drinking water standards do not have to be met. However, to best protect the public health, we recommend the use of a first-flush diverter and additional treatment prior to potable use of harvested rainwater.

Although metal roofs are commonly recommended for rainwater harvesting applications, our data show that concrete tile and cool roofs also are good candidate roofing materials for rainwater harvesting applications. The rainwater harvested from the Galvalume® roof had lower concentrations of fecal indicator bacteria as compared to the other roofing materials. This suggests that the Galvalume® roof might have an advantage over other roofing materials in terms of producing rainwater with lower concentrations of human pathogens (i.e., microorganisms that cause disease in humans).

If chlorine is the disinfectant of choice, the use of green and asphalt fiberglass shingle roofs for rainwater harvesting applications must be carefully weighed. The green roof consistently yielded harvested rainwater with the highest DOC concentrations, which could lead to high concentrations of disinfection by-products (DBPs) after chlorination. Exposure to certain DBPs is known to be harmful to human health. The asphalt fiberglass shingle roof also produced high DOC concentrations in harvested rainwater when the roof was new, but the DOC concentrations were similar to the Galvalume®, Kynar®-coated Galvalume®, concrete tile, and cool roofs after

[1] Only the green pilot-scale roof did not violate the iron standard.

the roof had been in the field for about one year; more data are needed to study this phenomenon. At present, we recommend that rainwater harvested from a green roof and probably an asphalt fiberglass shingle roof not be disinfected with chlorine.

In addition to the potential decline in DOC from rainwater harvested from aging asphalt fiberglass shingle roofs (noted above), we also observed increases in conductivity, concentration of particulate matter, and iron and zinc release from artificially aged asphalt fiberglass shingles. Thus, the potential exists for changes in harvested rainwater quality as the roofing material ages.

The quality of commercial growing media must be carefully examined if green roofs were to be used in potable rainwater harvesting applications. Our research shows measurable concentrations of arsenic and lead in the rainwater harvested from the green roof, with the arsenic concentration in the rainwater harvested after the first-flush approaching the USEPA drinking water standard (10 μg/L).

Rainwater harvested from all of the pilot-scale roofing materials showed diverse microbial communities. The community in the rainwater harvested from the green roof showed the highest microbial diversity, and the communities in the rainwater harvested from each of the pilot-scale roofs were more similar to each other than they were to that of ambient rain. The rainwater harvested from each pilot-scale roof contained genera associated with human pathogens (e.g., *Staphylococcus* and *Bacillus*) and genera associated with soil (e.g., *Acidovorax* and *Rhizobium*). Many of the genera found in the harvested rainwater were associated with gram-positive bacteria, indicating that sufficient disinfection practices must be in place to inactivate these generally more recalcitrant microorganisms.

2 Introduction

The main objective of this research is to provide recommendations to the rainwater harvesting community in Texas regarding the selection of roofing material for rainwater harvesting for potable use and to support these recommendations with scientific data. In the initial phase of this project (Tasks 1-4 in Mendez et al. [2010]), we assessed the quality of harvested rainwater using five pilot-scale roofs (asphalt fiberglass shingle, Galvalume® metal, concrete tile, cool, and green) and three full-scale roofs (two asphalt fiberglass shingle and one Galvalume® metal). The roof runoff was tested for pH, conductivity, turbidity, TSS, TC, FC, nitrate, nitrite, DOC, selected synthetic organic compounds, and selected metals. Data from three rain events were collected for the pilot- and full-scale roofs. Generally, the first-flush contained the highest concentrations of contaminants as compared to the subsequent collection tanks, indicating that the quality of harvested rainwater improves with roof flushing. However, the rainwater harvested after the first-flush did contain some contaminants at concentrations above primary USEPA drinking water standards (including turbidity, TC, and FC) and secondary USEPA drinking water standards (including iron and aluminum). The quality of rainwater harvested for potable use at a private residence is not regulated by the TCEQ, and, thus, the USEPA drinking water standards do not have to be met. However, to best protect the public health, we recommend that harvested rainwater be treated prior to potable use.

In the current study (Tasks 5 through 7, which are presented in this report), we collected additional data regarding the impact of roofing material on the quality of harvested rainwater. In Task 5, the five pilot-scale roofs were sampled for three additional rain events, and a full-scale Galvalume® metal roof coated with Kynar® was sampled for two rain events. In Task 6, the overall microbial community diversity of the rainwater harvested from the pilot-scale roofs was assessed by T-RFLP and sequencing of the 16S rRNA gene. In Task 7, new and artificially aged coupons of asphalt fiberglass shingle, Galvalume® metal, and concrete tile were used in lab-scale studies to examine the potential for changes in harvested rainwater quality as the roofing materials age.

3 Task 5. Additional sampling of pilot- and full-scale roofs

Mendez et al. (2010) provide a description of the five pilot-scale roofs located at the Lady Bird Johnson Wildflower Center, the ambient sampler, and the harvested rainwater sampler. The same ambient sampler and harvested rainwater sampler (including the first-flush, first tank, and second tank) were used in the current study to collect data from the pilot-scale roofs at the Lady Bird Johnson Wildflower Center and the full-scale roof. Figure 3-1A shows the harvested rainwater sampling device, which collected rainwater to sequentially fill the first-flush tank, the first tank, and the second tank.

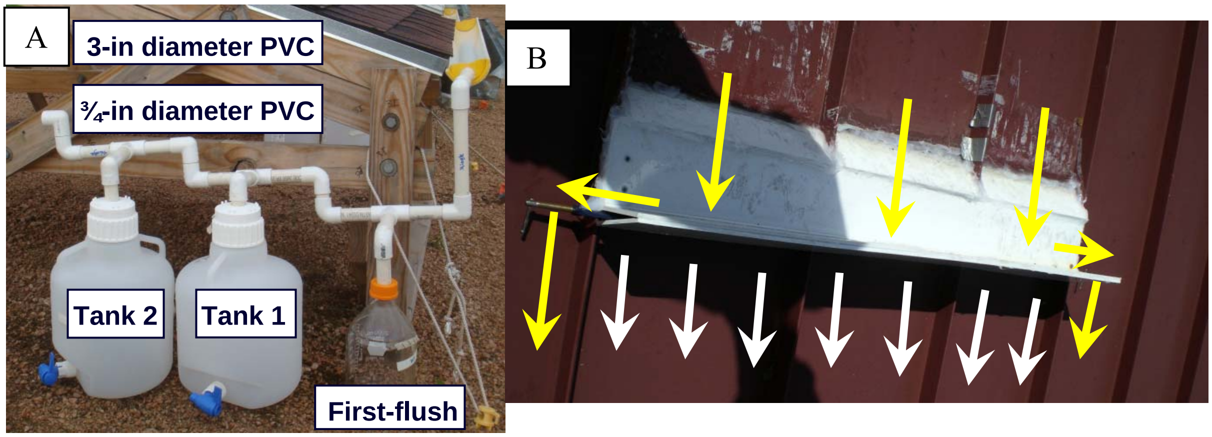

Figure 3-1. Sampling systems. A. Three-tank sampler set up at each roofing site. B. Diverter system in place on Kynar®-coated Galvalume® roof. Yellow arrows show water diverted from the targeted sampling area, and white arrows show water flowing inside the targeted sampling area.

The full-scale site (University of Texas at Austin Child Development Center, Comal location) has a 7-year-old Kynar®-coated Galvalume® roof with a 20˚ slope, an 8-foot (ft) run from the peak of the roof to the gutters, and no overhanging vegetation. Kynar® is a polyvinylidene fluoride (PVDF) resin-based coating that is often applied to Galvalume® or to galvanized steel; it is used as a roof coating for a variety of reasons including its resistance to corrosion, impact, abrasion, ultraviolet (UV) light, and particle accumulation. A rainwater catchment area with plan dimensions of 4 ft (width) by 7.5 ft (length) was targeted to provide a collection area similar to those of the pilot-scale roofs (30.1 square feet (ft^2) for the full-scale roof, 30.4 ft^2 for the asphalt fiberglass shingle, Galvalume® metal, and concrete tile pilot-scale roofs, and 36.6 ft^2 for the cool and green pilot-scale roofs). The corrugated Kynar®-coated Galvalume® roof was composed of linked sheets, producing lanes that were approximately 16 inches (in) wide. The lanes were then fused together by folding the material of two lanes into a flange. To isolate the sampling area, a diverter system composed of polyvinyl chloride (PVC) was installed at the top of the targeted sampling area on the Kynar®-coated Galvalume® roof (Figure 3-1B). Rainwater that fell above the targeted sampling area (yellow arrows in Figure 3-1B) was diverted away from the sampling device, and rainwater that fell in the targeted sampling area (white arrows in Figure 3-1B) was collected in the sampling device.

The locations of the pilot-scale and full-scale roofs that were sampled for this study are shown in Figure 3-2. The five pilot-scale roofs were sampled for three rain events on June 30, 2010, July 8, 2010, and September 22, 2010. The full-scale Kynar®-coated Galvalume® roof was sampled for two rain events on September 7, 2010 and September 24, 2010. The ambient rain, first-flush, first tank, and second tank were analyzed for two of the rain events (June 30, 2010 and September 7, 2010). Insufficient rain fell in the other three rain events (July 8, 2010, September 22, 2010, and September 24, 2010), so only the water from the first-flush and first tanks could be analyzed. The rain events are summarized in Table 3-1.

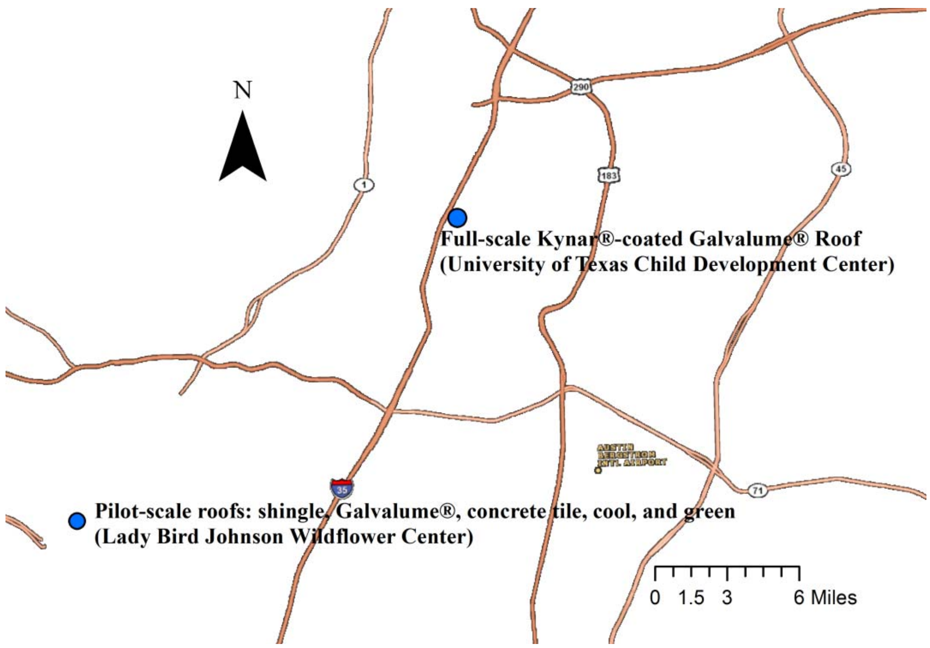

Figure 3-2. Map showing locations of the sampled roofs.

Table 3-1. Description of rain events for pilot-scale roof studies.

Date	Rainfall (in)	Temperature (°F)	Number of preceding dry days
6/30/2010	>5	74-85	11
7/8/2010	0.8	74-84	4
9/7/2010	1.1	72-77	0
9/22/2010	0.7	74-87	0
9/24/2010	0.4	76-90	1

Note: Rainfall is in inches (in) and temperature is in degrees Fahrenheit (°F).

The ambient rain, first-flush, and first and second tanks were analyzed in triplicate for pH, conductivity, turbidity, TSS, DOC, metals (total metals = dissolved + particulate), TC, and FC. Nitrate (NO_3^-) and nitrite (NO_2^-) were measured once for each sample. Samples were preserved and analyzed as described in Mendez et al. (2010), with the exception of DOC. In the current study, DOC was measured with an Aurora Model 1030 Total Organic Carbon Analyzer (OI Analytical, College Station, Texas).

The data from 3 pilot-scale rain events previously collected in 2009 by Mendez et al. (2010) and the data from 3 rain events collected in 2010 for the current study have been combined and tabulated in this section, such that the minimum, median, and maximum values for each parameter are shown for the six rain events. Since the full-scale Kynar®-coated Galvalume® roof was sampled for only two rain events, the data are summarized with the range (minimum and maximum values) for each parameter. Since many rainwater harvesting applications divert the first-flush from use, the discussion of water quality in this report generally focuses on the quality after the first-flush tank (i.e., first and second tanks of the sampler). **For reference purposes, we compare the quality of the harvested rainwater to the USEPA drinking water standards. However, this does not imply that the quality of rainwater harvested for potable use at a private residence is regulated by the TCEQ nor that the USEPA drinking water standards have to be met; the comparison simply provides a framework for evaluating the quality of harvested rainwater.**

Table 3-2 summarizes the median, minimum, and maximum pH for the pilot-scale roofs. Table 3-3 summarizes the range of pH for the full-scale Kynar®-coated Galvalume® roof. The pH of rainwater is approximately 5.7 (Texas Water Development Board [TWDB], 2005), and our ambient rain samples from the pilot-scale study had pH values from 5.5 to 6.7 in 2009, comparable to the range of 5.7 to 6.3 observed in 2010. For all rain events, the pilot-scale roofs showed that the pH of the harvested rainwater was higher than that of ambient rainfall, ranging from 6.0 to 8.2 in 2009, comparable to the range of 5.8 to 8.7 observed in 2010. For the full-scale study, the ambient rain had pH values ranging from 6.5 to 7.3. According to the 2010 data, the harvested rainwater after the first-flush from the Galvalume® roof showed higher pH values than the ambient rain, but the Kynar®-coated Galvalume® roof showed lower pH values than the ambient rain. For all rain events, the rainwater harvested after the first-flush from the tile roof consistently yielded higher pH values than all other roofs. Chemical reactions between the rainwater and roofing material components (e.g., limestone in the asphalt fiberglass shingles or concrete) can lead to an increase in harvested rainwater pH, allowing the rainwater harvested after the first-flush to meet the USEPA (2009) secondary drinking water standard for pH (6.5-8.5). However, some roofing materials (e.g., Galvalume®, concrete tile, green, and Kynar®-coated Galvalume®) occasionally produced rainwater after the first-flush with pH values that were outside of the range specified by the standard. In those cases, the user might consider an appropriate treatment for pH (e.g., using a concrete-lined storage tank, adding limestone to the storage tank, adjusting the pH by chemical addition). All of the pH values in the harvested rainwater after the first-flush are comparable to other studies of harvested rainwater including Yaziz et al. (1989), who reported pH values of 5.9 to 6.9, and Simmons et al. (2001), who reported pH values of 5.2 to 11.4.

Table 3-2. pH in harvested rainwater from pilot-scale roofs. Median (minimum-maximum) values for 6 rain events collected in 2009 (Mendez et al., 2010) and 2010 (current study) are shown.

Roof type	First-flush	Tank 1	Tank 2
Shingle	6.5 (6.3-7.1)	6.7 (6.5-7.1)	6.7 (6.6-6.9)
Galvalume®	6.6 (6.3-7.6)	6.6 (5.8-6.8)	6.0 (5.8-6.8)
Tile	7.6 (7.1-8.2)	7.9 (7.5-8.7)	7.6 (6.5-7.7)
Cool	6.7 (6.3-8.1)	6.7 (6.5-8.0)	7.0 (6.7-7.2)
Green	7.2 (6.3-7.6)	7.2 (6.4-7.6)	7.2 (6.3-7.5)
Ambient rain	6.1 (5.5-6.7)		

Table 3-3. pH in harvested rainwater from full-scale Kynar®-coated Galvalume® roof. The range of values for 2 rain events collected in 2010 is shown.

Roof type	First-flush	Tank 1	Tank 2 [a]
Kynar®-coated Galvalume®	5.8-6.8	5.7-7.0	5.4
Ambient rain	6.5-7.3		

[a] Only the September 7, 2010 event had enough rainfall to reach Tank 2. Thus, only one data point is shown for Tank 2.

Conductivity is a measure of the ability of a solution to conduct electricity; a solution with a higher concentration of charged constituents (e.g., Na^+, Cl^-) will have a higher conductivity. A conductivity standard is not specified by the USEPA for drinking water, but conductivity is correlated to total dissolved solids (TDS), for which a USEPA secondary standard exists (500 mg/L). Waters with high concentrations of TDS can have a disagreeable taste or color. Table 3-4 summarizes the median, minimum, and maximum conductivity for the pilot-scale roofs. Table 3-5 summarizes the range of conductivity for the full-scale Kynar®-coated Galvalume® roof. In general, the conductivity of the harvested rainwater decreased from the first-flush through the first and second tanks. For all rain events except June 30, 2010, rainwater harvested after the first-flush from the Galvalume® roof yielded lower conductivity values as compared to the other roofing materials with values ranging from 9 to 56 microSiemens per centimeter (µS/cm) in 2009, comparable to the range of 5 to 39 µS/cm observed in 2010. For all rain events, the rainwater harvested after the first-flush from the green roof yielded higher conductivity values as compared to the other roofing materials with values ranging from 118 to 336 µS/cm in 2009 and 45 to 92 µS/cm in 2010. Conductivity values in the ambient rain of the pilot-scale study ranged from 18 to 61 µS/cm in 2009 and 9 to 17 µS/cm in 2010; conductivity values in the ambient rain of the full-scale study ranged from 5 to 13 µS/cm. These values are comparable to those measured by Yaziz et al. (1989) in ambient rain, ranging from 6 µS/cm to 33 µS/cm. Conductivity in the rainwater harvested after the first-flush for the full-scale Kynar®-coated Galvalume® roof ranged from 5 to 10 µS/cm. The 2010 data showed that the rainwater harvested after the first-flush from the Galvalume® roof had slightly higher conductivity values than did the Kynar®-coated-Galvalume® roof. Using a conductivity-TDS correlation from the literature (Singh and Kalra, 1975), the estimated TDS of rainwater harvested after the first-flush for all of the roofs in this study met the secondary standard for TDS.

Table 3-4. Conductivity (µS/cm) in harvested rainwater from pilot-scale roofs. Median (minimum-maximum) values for 6 rain events collected in 2009 (Mendez et al., 2010) and 2010 (current study) are shown.

Roof type	First-flush	Tank 1	Tank 2
Shingle	103 (24-344)	16 (9-57)	26 (8-47)
Galvalume®	34 (6-167)	16 (5-56)	23 (9-39)
Tile	58 (12-413)	30 (12-180)	29 (18-139)
Cool	57 (18-184)	22 (7-59)	18 (11-53)
Green	180 (53-343)	98 (45-336)	187 (92-319)
Ambient rain	18 (9-61)		

Table 3-5. Conductivity (µS/cm) in harvested rainwater from full-scale Kynar®-coated Galvalume® roof. The range of values for 2 rain events collected in 2010 is shown.

Roof type	First-flush	Tank 1	Tank 2 [a]
Kynar®-coated Galvalume®	11-29	10 [b]	5
Ambient rain	5-13		

[a] Only the September 7, 2010 event had enough rainfall to reach Tank 2. Thus, only one data point is shown for Tank 2.
[b] The same conductivity was measured for both rain events.

Turbidity is a measure of the cloudiness of a water and is measured in standard nephelometric turbidity units (NTU); turbidity is undesirable because the particulate matter might harbor chemical pollutants or microorganisms that are harmful if ingested. Table 3-6 summarizes the median, minimum, and maximum turbidity for the pilot-scale roofs. Table 3-7 summarizes the range of turbidity for the full-scale Kynar®-coated Galvalume® roof. In general for the pilot- and full-scale roofs, turbidity decreased from the first-flush to the subsequent tank(s), with final values of turbidity that were on the same order as that of ambient rain. For the pilot-scale study, turbidity readings in the first-flush through the second tank ranged from 2 NTU to 105 NTU in 2009 and 2 to 11 NTU in 2010. These are comparable to the 4 to 94 NTU reported in harvested rainwater from Yaziz et al. (1989). For all rain events in 2009, rainwater harvested after the first-flush from the Galvalume®, tile, and cool roofs yielded higher turbidity values as compared to other roofing materials, up to 36 NTU. Also in 2009, the lowest turbidity values were found in rainwater harvested after the first-flush from the green roof, ranging from 3 NTU to 11 NTU. This result was not consistent in the 2010 rain events, which showed that rainwater harvested after the first-flush from the Galvalume® roof yielded the lowest turbidity values as compared to all other roofs, with values ranging from 2 to 3 NTU. According to the 2010 data, the harvested rainwater after the first-flush from the Galvalume® roof showed slightly higher turbidity values than did the Kynar®-coated Galvalume® roof, but the turbidity in the ambient rain at the pilot-scale site was higher than that at the full-scale site. All roofing materials yielded higher turbidity values than the 1 NTU maximum recommended for potable use of harvested rainwater (TWDB, 2006); all turbidity values also were higher than the USEPA primary drinking water standard (USEPA, 2009), which states that for systems using conventional or direct filtration, turbidity must never be above 1 NTU and 95% of samples in one month must be less than or equal to 0.3 NTU. Thus, we recommend treatment for turbidity (e.g., filtration) in rainwater harvested for potable use.

Table 3-6. Turbidity (NTU) in harvested rainwater from pilot-scale roofs. Median (minimum-maximum) values for 6 rain events collected in 2009 (Mendez et al., 2010) and 2010 (current study) are shown.

Roof type	First-flush	Tank 1	Tank 2
Shingle	14 (4-41)	9 (3-24)	9 (3-14)
Galvalume®	31 (2-102)	7 (2-30)	7 (2-9)
Tile	26 (5-64)	16 (2-36)	4 (2-9)
Cool	37 (9-105)	7 (2-26)	5 (2-13)
Green	4 (3-15)	3 (3-11)	4 (3-4)
Ambient rain	4 (3-8)		

Table 3-7. Turbidity (NTU) in harvested rainwater from full-scale Kynar®-coated Galvalume® roof. The range of values for 2 rain events collected in 2010 is shown.

Roof type	First-flush	Tank 1	Tank 2 [a]
Kynar®-coated Galvalume®	3-4	1-2	1
Ambient rain	1-2		

[a] Only the September 7, 2010 event had enough rainfall to reach Tank 2. Thus, only one data point is shown for Tank 2.

TSS is a measure of the particulate matter present in a water; TSS is undesirable because the particulate matter might harbor chemical pollutants or microorganisms that are harmful if ingested. Table 3-8 summarizes the median, minimum, and maximum TSS for the pilot-scale roofs. Table 3-9 summarizes the range of TSS for the full-scale Kynar®-coated Galvalume® roof. In comparison to the TSS values in 2009, similar trends were seen for TSS in 2010. Most of the data showed that TSS decreased from the first-flush to the subsequent tank(s), with final values of TSS that were on the same order as that of ambient rain. However, for the June 30, 2010 rain event, there was an increasing trend in TSS from the first-flush to the first tank for the shingle, Galvalume®, and cool roofs; this is most likely due to the high precipitation of more than 5 in, which might have abruptly flushed particles into the sampling system. Yaziz et al. (1989) reported 53 to 276 milligrams per liter (mg/L) TSS in harvested rainwater; these are similar to the rainwater harvested after the first-flush in the pilot-scale study with values of 1 to 118 mg/L in 2009, comparable to the range of 0 to 128 mg/L observed in 2010. TSS in the rainwater harvested after the first-flush for the full-scale Kynar®-coated Galvalume® roof ranged from 4 to 7 mg/L. According to the 2010 data, the rainwater harvested after the first-flush from the Galvalume® roof showed higher TSS than did the Kynar®-coated Galvalume® roof, but the TSS in the ambient rain at the pilot-scale site was higher than that at the full-scale site.

Table 3-8. **TSS (mg/L) in harvested rainwater from pilot-scale roofs. Median (minimum-maximum) values for 6 rain events collected in 2009 (Mendez et al., 2010) and 2010 (current study) are shown.**

Roof type	First-flush	Tank 1	Tank 2
Shingle	29 (6-123)	30 (6-128)	38 (12-53)
Galvalume®	96 (4-260)	58 (2-87)	33 (20-75)
Tile	95 (3-164)	23 (1-80)	19 (0-37)
Cool	114 (6-238)	76 (6-118)	33 (4-46)
Green	18 (3-84)	12 (3-53)	10 (1-49)
Ambient rain	17 (0-46)		

Table 3-9. **TSS (mg/L) in harvested rainwater from full-scale Kynar®-coated Galvalume® roof. The range of values for 2 rain events collected in 2010 is shown.**

Roof type	First-flush	Tank 1	Tank 2 [a]
Kynar®-coated Galvalume®	8-9	4-5	7
Ambient rain	0-1		

[a] Only the September 7, 2010 event had enough rainfall to reach Tank 2. Thus, only one data point is shown for Tank 2.

Nitrate is an inorganic ion that can be reduced to nitrite in the human body, and nitrite can cause blue baby syndrome in infants. Table 3-10 summarizes the median, minimum, and maximum nitrate concentrations for the pilot-scale roofs. Table 3-11 summarizes the range of nitrate concentrations for the full-scale Kynar®-coated Galvalume® roof. For each rain event, nitrate concentrations decreased from the first-flush to the subsequent tank(s), with final values of nitrate that were similar to that of ambient rain. Nitrate concentrations in the rainwater harvested after the first-flush for the pilot-scale roofs ranged from 0 to 3.3 mg/L NO_3^--N in 2009, comparable to the range of 0.3 to 2.1 mg/L NO_3^--N observed in 2010; nitrate concentrations in the rainwater harvested after the first-flush for the full-scale Kynar®-coated Galvalume® roof ranged from 0 to 1.2 mg/L NO_3^--N. According to the 2010 data, the harvested rainwater after the first-flush from the Galvalume® roof showed nitrate concentrations that were comparable to those from the Kynar®-coated Galvalume® roof. All nitrate concentrations are well below the USEPA drinking water maximum contaminant level (MCL) of 10 mg/L NO_3^--N. Thus no treatment for nitrate is needed for potable use of harvested rainwater from the tested roofing locations. However, nitrate concentrations in harvested rainwater can vary based on geographical location, and we recommend that those employing harvested rainwater for potable use should occasionally check the nitrate concentration in their harvested rainwater.

Table 3-10. Nitrate (mg/L NO_3^--N) in harvested rainwater from pilot-scale roofs. Median (minimum-maximum) values for 6 rain events collected in 2009 (Mendez et al., 2010) and 2010 (current study) are shown.

Roof type	First-flush	Tank 1	Tank 2
Shingle	3.7 (0.9-5.4)	1.5 (0.1-2.0)	0.9 (0.0-1.4)
Galvalume®	1.6 (0.0-3.7)	1.6 (0.0-2.0)	1.2 (0.0-1.8)
Tile	2.9 (1.0-3.7)	1.1 (0.2-2.2)	1.3 (0.0-1.4)
Cool	1.7 (0.4-4.8)	1.3 (0.0-2.1)	1.4 (0.0-1.7)
Green	2.3 (0.6-3.5)	1.9 (0.0-3.3)	1.8 (0.0-2.0)
Ambient rain	0.9 (0.0-2.4)		

Table 3-11. Nitrate (mg/L NO_3^--N) in harvested rainwater from full-scale Kynar®-coated Galvalume® roof. The range of values for 2 rain events collected in 2010 is shown.

Roof type	First-flush	Tank 1	Tank 2 [a]
Kynar®-coated Galvalume®	0.0-1.2	0.0-1.2	0.0
Ambient rain	0.0-1.2		

[a] Only the September 7, 2010 event had enough rainfall to reach Tank 2. Thus, only one data point is shown for Tank 2.

Nitrite is an inorganic ion that can cause blue baby syndrome in infants. Table 3-12 summarizes the median, minimum, and maximum nitrite concentrations for the pilot-scale roofs. Table 3-13 summarizes the range of nitrite concentrations for the full-scale Kynar®-coated Galvalume® roof. Similar to nitrate, the nitrite concentrations decreased from the first-flush to the subsequent tank(s), with final values of nitrite that were similar to that of ambient rain. Nitrite concentrations in rainwater harvested after the first-flush for the pilot-scale roofs ranged from 0.00 to 0.04 mg/L NO_2^--N in 2009, comparable to the range of 0.00 to 0.06 mg/L NO_2^--N observed in 2010; nitrite concentrations in the rainwater harvested after the first-flush for the full-scale Kynar®-coated Galvalume® roof ranged from 0.00 to 0.01 mg/L NO_2^--N. According to the 2010 data, the harvested rainwater after the first-flush from the Galvalume® roof showed nitrite concentrations that were comparable to those from the Kynar®-coated-Galvalume® roof. All post-first-flush nitrite concentrations are well below the USEPA drinking water MCL for nitrite (1 mg/L NO_2^--N). In the April 18, 2009 rain event, only the first-flush of the Galvalume® roof yielded a nitrite concentration higher than the drinking water regulation; this was not reproduced in subsequent rain events of 2009 and 2010, which showed 0.00 to 0.09 mg/L NO_2^--N in the first-flush from the Galvalume® roof. However, nitrite concentrations in harvested rainwater can vary based on geographical location, and we recommend that those employing harvested rainwater for potable use should occasionally check the nitrite concentration in their harvested rainwater.

Table 3-12. Nitrite (mg/L NO_2^--N) in harvested rainwater from pilot-scale roofs. Median (minimum-maximum) values for 6 rain events collected in 2009 (Mendez et al., 2010) and 2010 (current study) are shown.

Roof type	First-flush	Tank 1	Tank 2
Shingle	0.05 (0.01-0.21)	0.02 (0.00-0.05)	0.02 (0.01-0.03)
Galvalume®	0.03 (0.00-1.13)	0.02 (0.00-0.03)	0.02 (0.00-0.02)
Tile	0.04 (0.01-0.24)	0.02 (0.01-0.04)	0.02 (0.00-0.03)
Cool	0.03 (0.00-0.34)	0.02 (0.00-0.04)	0.01 (0.01-0.02)
Green	0.04 (0.02-0.05)	0.02 (0.01-0.06)	0.02 (0.01-0.04)
Ambient rain	0.01 (0.00-0.03)		

Table 3-13. Nitrite (mg/L NO_2^--N) in harvested rainwater from full-scale Kynar®-coated Galvalume® roof. The range of values for 2 rain events collected in 2010 is shown.

Roof type	First-flush	Tank 1	Tank 2 [a]
Kynar®-coated Galvalume®	0.00-0.02	0.00-0.01	0.00
Ambient rain	0.00-0.01		

[a] Only the September 7, 2010 event had enough rainfall to reach Tank 2. Thus, only one data point is shown for Tank 2.

DOC is a general measure of the organic material dissolved in a water. While some DOC compounds are directly harmful to human health (e.g., certain pesticides), other DOC compounds can react with chlorine during disinfection to produce DBPs that are harmful to human health. USEPA regulations of DBPs in drinking water have focused on trihalomethanes (THMs) and haloacetic acids (HAAs). Humans can be exposed to these DBPs through ingestion of and showering, bathing, and swimming in water treated with chlorine (Nieuwenhuijsen et al., 2009). Strong epidemiological evidence exists for a relationship between certain DBPs and bladder cancer, and more limited evidence exists for a relationship between certain DBPs and other cancers, such as colorectal, liver, kidney, brain, lung, and breast cancers (Nieuwenhuijsen et al., 2009). Some evidence exists for a relationship between certain DBPs and small for gestational age/intrauterine growth retardation and preterm delivery (Nieuwenhuijsen et al., 2009). Thus, the chlorination of harvested rainwater containing DOC could produce DBPs that are harmful to human health.

Table 3-14 summarizes the median, minimum, and maximum DOC concentrations for the pilot-scale roofs. Table 3-15 summarizes the range of DOC concentrations for the full-scale Kynar®-coated Galvalume® roof. DOC concentrations in the rainwater harvested after the first-flush for the pilot-scale roofs ranged from 2.3 mg/L to 37.3 mg/L in 2009, comparable to the range of 0.8 to 34.8 mg/L observed in 2010; DOC concentrations in the rainwater harvested after the first-flush for the full-scale Kynar®-coated Galvalume® roof ranged from 1.2 to 1.4 mg/L. According to the 2010 data, the harvested rainwater after the first-flush from the Galvalume® roof showed DOC concentrations that were comparable to those from the Kynar®-coated Galvalume® roof. Most of the data showed that DOC concentrations decreased from the first-flush through the first and second tanks. The shingle roof, however, showed an increasing trend in DOC concentration from the first-flush to the first tank for all rain events in 2009; for the three rain events, average DOC concentrations were 0.5 mg/L in the first-flush and 12 mg/L in the first tank. This was not consistent in 2010, which showed that in all rain events, DOC concentrations in the harvested rainwater from the shingle roof had a significant decrease from the first-flush to the first and second tanks; for the three rain events, average DOC concentrations were 22 mg/L in the first-flush and 4 mg/L in the first tank. It is possible that the capacity for and kinetics of DOC leaching from a shingle roof change as the roof ages. Berdahl et al. (2008) discuss the changes in roofing materials due to photodegradation via UV light from the sun, elevated temperature, moisture, and microbial growth; thus, since roofing materials change with age, it is possible that the amount and rate of DOC leaching from a shingle roof also change with age.

Most of the data from 2009 and 2010 showed that the green roof yielded the highest DOC concentration in the second tank, while the Galvalume® and cool roofs yielded the lowest DOC concentration in the second tank. If the post-first-flush water were disinfected by chlorination prior to potable use, higher DOC concentrations (i.e., from the green roof) would be likely to produce higher concentrations of DBPs. While the shingle roof yielded relatively high DOC concentrations post-first-flush in 2009 (when the roof was brand new), the post-first-flush DOC concentrations in 2010 were much more reasonable; thus, the concentrations of DBPs formed in rainwater harvested from a shingle roof also might decrease as the roof ages. Additional data are needed to verify the trend of decreasing DOC concentrations from a shingle roof over time.

Table 3-14. DOC (mg/L) in harvested rainwater from pilot-scale roofs. Median (minimum-maximum) values for 6 rain events collected in 2009 (Mendez et al., 2010) and 2010 (current study) are shown.

Roof type	First-flush	Tank 1	Tank 2
Shingle	8.9 (0.1-27.5)	7.5 (2.7-15.4)	10.2 (2.8-13.4)
Galvalume®	4.2 (1.1-30.0)	2.7 (0.8-11.4)	2.5 (2.3-7.4)
Tile	5.3 (0.4-16.7)	3.9 (2.1-11.6)	6.2 (3.2-10.1)
Cool	9.0 (5.6-17.3)	5.6 (2.0-14.0)	4.9 (2.3-5.8)
Green	25.3 (12.5-36.4)	22.4 (2.6-37.3)	25.5 (7.8-35.1)
Ambient rain	3.3 (3.0-4.7)		

Table 3-15. DOC (mg/L) in harvested rainwater from full-scale Kynar®-coated Galvalume® roof. The range of values for 2 rain events collected in 2010 is shown.

Roof type	First-flush	Tank 1	Tank 2 [a]
Kynar®-coated Galvalume®	1.3-3.1	1.2-1.4	1.3
Ambient rain	0.0-1.4		

[a] Only the September 7, 2010 event had enough rainfall to reach Tank 2. Thus, only one data point is shown for Tank 2.

TC and FC are groups of indicator microorganisms whose presence has traditionally been used to signal the possible presence of pathogenic (disease-causing) microorganisms in water. Tables 3-16 and 3-18 summarize the median, minimum, and maximum TC and FC concentrations, respectively, for the pilot-scale roofs. Tables 3-17 and 3-19 summarize the range of TC and FC concentrations, respectively, for the full-scale Kynar®-coated Galvalume® roof. TC and FC concentrations generally decreased from the first-flush to the first and second tanks. The rainwater harvested after the first-flush often had detectable TC and FC, indicating the potential presence of pathogenic microorganisms. Thus, we recommend disinfection prior to potable use.

The green roof showed low coliform concentrations in the harvested rainwater after the first-flush for the April 18, 2009 and June 11, 2009 rain events, with TC concentrations from 7 to 12 colony forming units per one-hundred milliliters (CFU/100mL) and FC concentrations of <1 CFU/100mL. This was not true of the July 23, 2009 rain event, which showed much higher coliform concentrations in the harvested rainwater after the first-flush from the green roof; TC concentrations from 833 to 1300 CFU/100mL and FC concentrations from 270 to 390 CFU/100mL were observed. The inter-event variability in FC and TC concentrations in the harvested rainwater after the first-flush from the green roof also occurred in 2010. For the July 8, 2010 rain event, the rainwater harvested after the first-flush from the green roof showed non-detectable TC and FC concentrations; for the September 22, 2010 event, the rainwater harvested after the first-flush from the green roof also had non-detectable FC concentrations but showed a high TC concentration of 667 CFU/100mL. Fire-ant colonizations of the green roofs have been observed from time to time, which might be linked to increases in coliform concentrations; in future samplings, the timing of fire-ant colonizations will be noted.

Ambient rainwater from the pilot-scale study contained TC concentrations from 547 to 648 CFU/100mL and FC concentrations from 3 to 33 CFU/100mL in 2009, comparable to the range of TC from 340 to 620 CFU/100mL and FC from 43 to 200 CFU/100mL observed in 2010; the ambient rainwater from the full-scale study had a range of TC from 268 to 45500 [2] CFU/100mL and FC from <1 to 73 CFU/100mL. Another study (Yaziz et al., 1989) found no TC or FC in ambient rain collected from one meter above the ground. Our ambient sample also was collected approximately one meter above the ground, but the sampler was left open overnight to collect early morning rain events. The higher TC and FC concentrations in our ambient samples might be due to overnight contamination, including airborne deposition or presence of wildlife.

The TC and FC concentrations from the Galvalume® and Kynar®-coated Galvalume® roofs were similar (after leaving out the anomalous September 24, 2010 data for the coated roof). The rainwater harvested from the Galvalume® roof often showed lower FC concentrations as compared to the other pilot-scale roofs (except the green roof). Since metals tend to have higher surface temperatures in sunlight as compared to higher emissivity materials (Bretz et al., 1998), these higher temperatures might have inactivated some of the FC on the Galvalume® roof.

Table 3-16. TC (CFU/100mL) in harvested rainwater from pilot-scale roofs. Median (minimum-maximum) values for 5 [a] rain events collected in 2009 (Mendez et al., 2010) and 2010 (current study) are shown.

Roof type	First-flush	Tank 1	Tank 2
Shingle	2470 (1500-8100)	800 (203-6933)	256 (177-733)
Galvalume®	767 (300-1267)	167 (<1-770)	416 (117-500)
Tile	1680 (1017-5617)	832 (225-983)	567 (293-783)
Cool	1882 (1683-5450)	917 (130-3750)	226 (150-867)
Green	333 (13-1233)	12 (<1-1300)	8 (7-833)
Ambient rain	550 (340-648)		

[a] TC concentrations from June 30, 2010 were not measured.

[2] The high TC and FC concentrations in the harvested rainwater from the full-scale Kynar®-coated Galvalume® roof are from the September 24, 2010 rain event. Rain was expected during the weekend so the tanks were left at the site for three days before collection. As a result, there likely was microbial growth in the tanks during that period.

Table 3-17. TC (CFU/100mL) in harvested rainwater from full-scale Kynar®-coated Galvalume® roof. The range of values for 2 rain events collected in 2010 is shown.

Roof type	First-flush	Tank 1	Tank 2 [a]
Kynar®-coated Galvalume®	3367-47000	317-20500	277
Ambient rain	268-45500		

[a] Only the September 7, 2010 event had enough rainfall to reach Tank 2. Thus, only one data point is shown for Tank 2.

Table 3-18. FC (CFU/100mL) in harvested rainwater from pilot-scale roofs. Median (minimum-maximum) values for 5 [a] rain events collected in 2009 (Mendez et al., 2010) and 2010 (current study) are shown.

Roof type	First-flush	Tank 1	Tank 2
Shingle	167 (32-400)	50 (<1-87)	25 (9-32)
Galvalume®	13 (2-17)	3 (<1-10)	1 (<1-6)
Tile	30 (10-93)	9 (5-73)	1 (1-8)
Cool	35 (25-317)	16 (<1-22)	7 (6-8)
Green	<1 (<1-550)	<1 (<1-390)	<1 (<1-270)
Ambient rain	33 (3-200)		

[a] FC concentrations from June 30, 2010 were not measured.

Table 3-19. FC (CFU/100mL) in harvested rainwater from full-scale Kynar®-coated Galvalume® roof. The range of values for 2 rain events collected in 2010 is shown.

Roof type	First-flush	Tank 1	Tank 2 [a]
Kynar®-coated Galvalume®	<1-267	<1-37	33
Ambient rain	<1-73		

[a] Only the September 7, 2010 event had enough rainfall to reach Tank 2. Thus, only one data point is shown for Tank 2.

The presence of certain metals in drinking water is a nuisance (e.g., staining of fixtures), while the presence of other metals in drinking water poses a direct threat to human health. A total of 9 metals were analyzed for the harvested rainwater, including aluminum (Al), arsenic (As), cadmium (Cd), chromium (Cr), copper (Cu), iron (Fe), lead (Pb), selenium (Se), and zinc (Zn). Al, Fe, and Zn are nuisance metals, while the ingestion of As, Cd, Cr, Pb, and Se can be harmful to human health; Cu is both a nuisance metal and problematic from a health standpoint. Tables 3-20 to 3-37 summarize the median, minimum, and maximum metal concentrations for the pilot-scale roofs and the range of metal concentrations for the full-scale Kynar®-coated Galvalume® roof. For 2009 and 2010, most of the data from the pilot- and full-scale studies showed that metal concentrations decreased from the first-flush through the first and second tanks, with final metal concentrations that were close to those of ambient rain. Below, the notable exceptions to this are discussed. As, Cd, and Se were often undetectable.

The rainwater harvested after the first-flush from the pilot-scale green roof had the highest As and Pb concentrations (Tables 3-22 and 3-32, respectively) as compared to the other roofing materials. The rainwater harvested after the first-flush from the Galvalume® and green roofs had the highest Zn concentrations as compared to the other roofing materials (Table 3-36). The elevated As, Pb, and Zn from the green roofs suggest that the composition of the growing media must be carefully evaluated if the harvested rainwater is intended for potable use. The rainwater harvested after the first-flush from the shingle roof had the highest Cu concentrations (Table 3-28). Asphalt fiberglass shingles can be a source of copper in harvested rainwater because copper is often added to them (including the GAF-Elk shingles used in this study) to prevent the growth of algae and moss.

The metal concentrations for all roofs in the rainwater harvested after the first-flush are compared to USEPA MCLs or action levels in Tables 3-38 and 3-39. With the exception of Al and Fe, the 2009 and 2010 data show that all metal concentrations in the rainwater harvested after the first-flush for the pilot-scale roofs meet USEPA standards for drinking water (Table 3-38). For the rainwater harvested after the first-flush, all of the pilot-scale roofs violate the Al drinking water standard, and only the green pilot-scale roof does not violate the Fe drinking water standard. All metal concentrations (including Al and Fe) in the rainwater harvested after the first-flush for the full-scale Kynar®-coated Galvalume® roof meet USEPA standards for drinking water (Table 3-39).

While the rainwater harvested after the first-flush from the Galvalume® roof showed higher metal concentrations (Al, Cr, Cu, Fe, Pb, and Zn) than did the Kynar®-coated-Galvalume® roof, this often coincided with higher metal concentrations in the ambient rain at the pilot-scale Galvalume® roof site. All metals, except Cd and Cr, showed a decrease in concentration in harvested rainwater at the pilot-scale site from 2009 to 2010, which is consistent with a decrease in metal concentrations in ambient rain from 2009 to 2010.

Table 3-20. Al (µg/L) in harvested rainwater from pilot-scale roofs. Median (minimum-maximum) values for 6 rain events collected in 2009 (Mendez et al., 2010) and 2010 (current study) are shown.

Roof type	First-flush	Tank 1	Tank 2
Shingle	343.66 (42.36-3349.00)	176.63 (69.15-374.87)	270.33 (17.63-717.80)
Galvalume®	612.23 (20.42-2049.67)	92.91 (14.27-472.87)	205.82 (23.75-554.87)
Tile	650.36 (196.89-1780.00)	232.14 (54.66-939.50)	228.90 (31.27-532.13)
Cool	636.14 (121.99-3756.00)	514.14 (210.49-847.33)	151.32 (77.59-513.17)
Green	134.16 (49.96-282.13)	127.78 (63.03-182.07)	141.02 (94.59-181.87)
Ambient rain	170.74 (4.15-558.83)		

Table 3-21. Al (µg/L) in harvested rainwater from full-scale Kynar®-coated Galvalume® roof. The range of values for 2 rain events collected in 2010 is shown.

Roof type	First-flush	Tank 1	Tank 2 [a]
Kynar®-coated Galvalume®	0.06-12.21	5.31-6.68	0.06
Ambient rain	11.54-55.38		

[a] Only the September 7, 2010 event had enough rainfall to reach Tank 2. Thus, only one data point is shown for Tank 2.

Table 3-22. As (μg/L) in harvested rainwater from pilot-scale roofs. Median (minimum-maximum) values for 6 rain events collected in 2009 (Mendez et al., 2010) and 2010 (current study) are shown.

Roof type	First-flush	Tank 1	Tank 2
Shingle	0.60 (<0.29 -4.20)	<0.29 (<0.29-0.67)	<0.29 (<0.29-0.65)
Galvalume®	0.39 (<0.29 -0.97)	<0.29 (<0.29-0.34)	<0.29 (<0.29-0.30)
Tile	0.61 (<0.29 -2.69)	<0.29 (<0.29-1.33)	<0.29 (<0.29-0.50)
Cool	0.37 (<0.29 -1.06)	<0.29 (<0.29-0.46)	<0.29 (<0.29-<0.29)
Green	4.49 (2.98-8.45)	6.16 (2.24-7.92)	6.05 (3.48-8.38)
Ambient rain	<0.29 (<0.29 -<0.29)		

Table 3-23. As (μg/L) in harvested rainwater from full-scale Kynar®-coated Galvalume® roof. The range of values for 2 rain events collected in 2010 is shown.

Roof type	First-flush	Tank 1	Tank 2 [a]
Kynar®-coated Galvalume®	<0.01-<0.01	<0.01-<0.01	<0.01
Ambient rain	<0.01-<0.01		

[a] Only the September 7, 2010 event had enough rainfall to reach Tank 2. Thus, only one data point is shown for Tank 2.

Table 3-24. Cd (μg/L) in harvested rainwater from pilot-scale roofs. Median (minimum-maximum) values for 6 rain events collected in 2009 (Mendez et al., 2010) and 2010 (current study) are shown.

Roof type	First-flush	Tank 1	Tank 2
Shingle	<0.10 (<0.10-0.14)	<0.10 (<0.10-<0.10)	<0.10 (<0.10-<0.10)
Galvalume®	<0.10 (<0.10-0.34)	<0.10 (<0.10-<0.10)	<0.10 (<0.10-<0.10)
Tile	<0.10 (<0.10-2.91)	<0.10 (<0.10-<0.10)	<0.10 (<0.10-<0.10)
Cool	<0.10 (<0.10-0.44)	<0.10 (<0.10-<0.10)	<0.10 (<0.10-<0.10)
Green	<0.10 (<0.10-4.46)	<0.10 (<0.10-<0.10)	<0.10 (<0.10-<0.10)
Ambient rain	<0.10 (<0.10-0.15)		

Table 3-25. Cd (μg/L) in harvested rainwater from full-scale Kynar®-coated Galvalume® roof. The range of values for 2 rain events collected in 2010 is shown.

Roof type	First-flush	Tank 1	Tank 2 [a]
Kynar®-coated Galvalume®	<0.07-<0.07	<0.07-<0.07	<0.07
Ambient rain	<0.07-<0.07		

[a] Only the September 7, 2010 event had enough rainfall to reach Tank 2. Thus, only one data point is shown for Tank 2.

Table 3-26. Cr (µg/L) in harvested rainwater from pilot-scale roofs. Median (minimum-maximum) values for 6 rain events collected in 2009 (Mendez et al., 2010) and 2010 (current study) are shown.

Roof type	First-flush	Tank 1	Tank 2
Shingle	1.02 (<0.12-5.00)	0.18 (<0.12-1.70)	0.35 (<0.12-0.66)
Galvalume®	3.69 (1.03-12.52)	0.78 (0.29-5.33)	0.75 (0.16-3.29)
Tile	1.73 (0.61-6.59)	0.49 (0.15-2.93)	0.58 (0.21-0.89)
Cool	0.98 (0.23-3.15)	0.40 (0.20-0.57)	<0.12 (<0.12-0.44)
Green	0.61 (0.22-1.61)	0.34 (0.19-1.94)	0.72 (0.20-1.71)
Ambient rain	0.26 (<0.12-3.02)		

Table 3-27. Cr (µg/L) in harvested rainwater from full-scale Kynar®-coated Galvalume® roof. The range of values for 2 rain events collected in 2010 is shown.

Roof type	First-flush	Tank 1	Tank 2 [a]
Kynar®-coated Galvalume®	0.17-0.27	0.11-0.14	0.08
Ambient rain	0.06-0.29		

[a] Only the September 7, 2010 event had enough rainfall to reach Tank 2. Thus, only one data point is shown for Tank 2.

Table 3-28. Cu (µg/L) in harvested rainwater from pilot-scale roofs. Median (minimum-maximum) values for 6 rain events collected in 2009 (Mendez et al., 2010) and 2010 (current study) are shown.

Roof type	First-flush	Tank 1	Tank 2
Shingle	184.71 (38.71-600.30)	18.43 (5.64-45.75)	21.09 (1.67-72.16)
Galvalume®	2.57 (<0.63-9.88)	1.76 (<0.63-4.84)	2.36 (1.10-4.65)
Tile	5.38 (<0.63-36.85)	1.91 (<0.63-19.05)	3.89 (<0.63-14.35)
Cool	3.44 (<0.63-12.80)	1.87 (<0.63-5.16)	0.80 (<0.63-2.11)
Green	3.64 (<0.63-9.01)	5.22 (<0.63-6.98)	5.83 (<0.63-12.39)
Ambient rain	<0.63 (<0.63-11.70)		

Table 3-29. Cu (µg/L) in harvested rainwater from full-scale Kynar®-coated Galvalume® roof. The range of values for 2 rain events collected in 2010 is shown.

Roof type	First-flush	Tank 1	Tank 2 [a]
Kynar®-coated Galvalume®	<0.02-<0.02	<0.02-<0.02	<0.02
Ambient rain	<0.02-<0.02		

[a] Only the September 7, 2010 event had enough rainfall to reach Tank 2. Thus, only one data point is shown for Tank 2.

Table 3-30. Fe (µg/L) in harvested rainwater from pilot-scale roofs. Median (minimum-maximum) values for 6 rain events collected in 2009 (Mendez et al., 2010) and 2010 (current study) are shown.

Roof type	First-flush	Tank 1	Tank 2
Shingle	299.34 (10.26-2105.00)	120.09 (41.97-342.47)	236.87 (10.62-480.93)
Galvalume®	611.36 (17.77-1687.67)	65.54 (8.94-323.93)	148.31 (40.94-563.00)
Tile	627.64 (136.75-1488.33)	186.04 (14.54-761.57)	153.00 (20.16-364.47)
Cool	335.31 (43.67-3535.00)	428.84 (106.65-721.43)	116.55 (59.87-341.80)
Green	74.82 (27.85-222.30)	51.34 (20.33-78.61)	55.58 (32.69-71.65)
Ambient rain	232.25 (12.40-1056.00)		

Table 3-31. Fe (µg/L) in harvested rainwater from full-scale Kynar®-coated Galvalume® roof. The range of values for 2 rain events collected in 2010 is shown.

Roof type	First-flush	Tank 1	Tank 2 [a]
Kynar®-coated Galvalume®	6.23-23.83	4.93-7.88	4.10
Ambient rain	12.12-42.00		

[a] Only the September 7, 2010 event had enough rainfall to reach Tank 2. Thus, only one data point is shown for Tank 2.

Table 3-32. Pb (µg/L) in harvested rainwater from pilot-scale roofs. Median (minimum-maximum) values for 6 rain events collected in 2009 (Mendez et al., 2010) and 2010 (current study) are shown.

Roof type	First-flush	Tank 1	Tank 2
Shingle	0.91 (<0.12-5.19)	0.35 (<0.12-0.87)	0.54 (<0.12-1.19)
Galvalume®	2.56 (<0.12-6.40)	0.30 (<0.12-1.08)	1.48 (0.27-5.65)
Tile	2.68 (0.81-13.62)	0.85 (<0.12-8.72)	1.57 (0.49-2.89)
Cool	3.56 (0.26-11.51)	1.33 (0.72-2.49)	0.58 (0.50-1.28)
Green	8.38 (5.71-39.69)	3.09 (1.36-5.39)	2.62 (1.04-4.22)
Ambient rain	0.67 (<0.12-0.94)		

Table 3-33. Pb (µg/L) in harvested rainwater from full-scale Kynar®-coated Galvalume® roof. The range of values for 2 rain events collected in 2010 is shown.

Roof type	First-flush	Tank 1	Tank 2 [a]
Kynar®-coated Galvalume®	<0.01-0.21	<0.01-<0.01	<0.01
Ambient rain	<0.01-1.54		

[a] Only the September 7, 2010 event had enough rainfall to reach Tank 2. Thus, only one data point is shown for Tank 2.

Table 3-34. Se (µg/L) in harvested rainwater from pilot-scale roofs. Median (minimum-maximum) values for 6 rain events collected in 2009 (Mendez et al., 2010) and 2010 (current study) are shown.

Roof type	First-flush	Tank 1	Tank 2
Shingle	0.26 (0.20-1.33)	<0.14 (<0.14-0.21)	<0.14 (<0.14-0.21)
Galvalume®	0.21 (<0.14-0.91)	<0.14 (<0.14-0.24)	<0.14 (<0.14-0.19)
Tile	0.28 (0.15-1.16)	<0.14 (<0.14-0.37)	<0.14 (<0.14-0.27)
Cool	0.31 (0.19-0.90)	<0.14 (<0.14-0.31)	<0.14 (<0.14-0.22)
Green	0.36 (<0.14-0.39)	0.35 (<0.14-0.50)	0.31 (0.28-0.50)
Ambient rain	<0.14 (<0.14-0.16)		

Table 3-35. Se (µg/L) in harvested rainwater from full-scale Kynar®-coated Galvalume® roof. The range of values for 2 rain events collected in 2010 is shown.

Roof type	First-flush	Tank 1	Tank 2 [a]
Kynar®-coated Galvalume®	0.15-0.21	0.10-0.18	0.05
Ambient rain	0.07-0.14		

[a] Only the September 7, 2010 event had enough rainfall to reach Tank 2. Thus, only one data point is shown for Tank 2.

Table 3-36. Zn (µg/L) in harvested rainwater from pilot-scale roofs. Median (minimum-maximum) values for 6 rain events collected in 2009 (Mendez et al., 2010) and 2010 (current study) are shown.

Roof type	First-flush	Tank 1	Tank 2
Shingle	58.92 (17.15-160.57)	12.02 (7.96-81.95)	24.56 (6.29-84.77)
Galvalume®	709.53 (208.29-852.13)	184.99 (128.77-297.79)	178.38 (77.46-362.13)
Tile	164.83 (44.81-542.47)	69.02 (2.42-313.67)	73.44 (19.70-118.17)
Cool	203.56 (88.63-483.33)	50.02 (37.93-121.97)	43.47 (15.81-98.70)
Green	399.35 (152.29-786.37)	314.93 (76.28-525.17)	278.48 (89.34-353.27)
Ambient rain	14.74 (0.86-108.97)		

Table 3-37. Zn (µg/L) in harvested rainwater from full-scale Kynar®-coated Galvalume® roof. The range of values for 2 rain events collected in 2010 is shown.

Roof type	First-flush	Tank 1	Tank 2 [a]
Kynar®-coated Galvalume®	97.99-178.79	57.38-176.99	75.37
Ambient rain	1.36-69.61		

[a] Only the September 7, 2010 event had enough rainfall to reach Tank 2. Thus, only one data point is shown for Tank 2.

Table 3-38. Comparison of metal concentrations (µg/L) in harvested rainwater from 2009 (Mendez et al., 2010) and 2010 (current study) of the pilot-scale roofs with USEPA drinking water standards.

Metal	Primary USEPA MCL (µg/L)	2009 metal concentrations in first and second tanks from all pilot-scale roofs (µg/L)	2010 metal concentrations in first and second tanks from all pilot-scale roofs (µg/L)
Arsenic	10	<0.29 to 8.38	0.03 to 6.73
Cadmium	5	<0.10	<0.01 to 0.04
Chromium	100	<0.12 to 2.93	0.09 to 5.33
Selenium	50	<0.14 to 0.50	0.02 to 0.37
	USEPA Action Level (µg/L)		
Copper	1300	<0.63 to 72.16	<0.02 to 12.43
Lead	15	<0.12 to 8.72	<0.01to 5.65
	Secondary USEPA MCL(µg/L)		
Aluminum	50-200	73.97 to 939.50	14.27 to 580.59
Iron	300	40.94 to 761.57	8.94 to 429.65
Zinc	5000	8.25 to 525.17	2.42 to 397.19

Table 3-39. Comparison of metal concentrations (µg/L) in harvested rainwater from 2010 of the full-scale Kynar®-coated Galvalume® roof with USEPA drinking water standards.

Metal	Primary USEPA MCL (µg/L)	2010 metal concentrations in first and second tanks from full-scale Kynar®-coated Galvalume® roof (µg/L)
Arsenic	10	0.02 to 0.05
Cadmium	5	<0.01 to 0.06
Chromium	100	0.08 to 0.14
Selenium	50	0.05 to 0.18
	USEPA Action Level (µg/L)	
Copper	1300	<0.02
Lead	15	<0.01
	Secondary USEPA MCL(µg/L)	
Aluminum	50-200	<0.1 to 6.68
Iron	300	4.10to 7.88
Zinc	5000	57.38 to 176.99

4 Task 6. Microbial diversity of harvested rainwater

The overall diversity of the microorganisms in the harvested rainwater was assessed by two molecular techniques: T-RFLP and sequencing of the 16S rRNA gene. T-RFLP was used to determine if there was a major change in the overall diversity (i.e., number of different microorganisms and relative abundance of each microorganism) of the microbial community between roofing materials. Sequencing was used to identify microorganisms present in the water at the genus level. This information was used to examine the harvested rainwater for the presence of gram-positive versus gram-negative organisms since gram-positive bacteria are often more difficult to disinfect; the communities also were examined for organisms that might be human pathogens (i.e., cause disease in humans).

In the Appendix, the detailed methods and raw electropherograms are shown for T-RFLP (Figures 9-1 to 9-6). Table 4-1 summarizes the Shannon-Weaver index (SWI) calculated from the T-RFLP data. The SWI is a diversity index that includes both the richness (i.e., number of different microorganisms present) and evenness (i.e., relative abundance of different organisms in the community) of the community. Table 4-2 summarizes the values of the Sørenson index (SI), which describes the similarity between the microbial communities of the different roofing materials. An SI of 0 indicates that two samples had no microorganisms in common, and an SI of 1 indicates that two samples contained all of the same microorganisms.

Table 4-1. SWI values from T-RFLP for the harvested rainwater after the first-flush from all pilot-scale roofs[a] and ambient rain[b].

Green	2.8
Galvalume®	2.3
Shingle	1.8
Tile	1.8
Cool	2.4
Ambient rain	1.5

[a] Harvested rainwater after the first-flush from April 18, 2009.
[b] Ambient rain from July 23, 2009.

Table 4-2. SI values from T-RFLP for the harvested rainwater after the first-flush from all pilot-scale roofs [a] and ambient rain [b].

	Cool	Tile	Shingle	Galvalume®	Green
Ambient rain	0.38	0.43	0.29	0.35	0.41
Green	0.61	0.34	0.34	0.65	1.00
Galvalume®	0.63	0.41	0.41	1.00	
Shingle	0.57	0.71	1.00		
Tile	0.57	1.00			
Cool	1.00				

[a] Harvested rainwater after the first-flush from April 18, 2009.
[b] Ambient rain from July 23, 2009.

As shown in Table 4-1, the ambient rain had the lowest SWI value of 1.5 as compared to the harvested rainwater from all the roofs, indicating that the ambient rain had lower microbial diversity than did the harvested rainwater. This was expected because microorganisms may attach and accumulate on roof surfaces, which might increase the microbial diversity in the harvested rainwater. The highest SWI value of 2.8 suggests that the harvested rainwater from the green roof had a more diverse microbial community as compared to the other roofing materials, and this value is consistent with higher SWI values from soils (Dunbar et al., 2000).

As shown in Table 4-2, the low SI values of 0.29 to 0.43 between ambient rain and harvested rainwater indicate differences between the microbial communities. This was expected because deposition of microorganisms and microbial growth on the roof surfaces could significantly alter the microbial community present on the roofs. The highest SI value of 0.71 was seen between the shingle and tile roofs suggesting similar communities between the two roofing materials.

A library of sequence information was assembled for rainwater harvested from each pilot-scale roof, which allowed us to examine what types of bacteria are present in rainwater harvested from different roofing materials. The discussion of each library focuses on those populations that represent more than 5% of the library. The 16S rRNA gene clone library for the harvested rainwater after the first-flush of the green roof (Table 4-3) showed dominant organisms (greater than 5% of the clone library) to be *Rubrobacter* (8.0%), *Brevibacterium* (9.3%), *Leifsonia* (5.3%), *Staphylococcus* (6.7%), *Bacillus* (10.7%), *Rhizobium* (9.3%), and *Acidovorax* (10.7%). The 16S rRNA gene clone library for the harvested rainwater after the first-flush of the Galvalume® metal roof (Table 4-4) showed dominant organisms to be cyanobacteria (20.0%), *Staphylococcus* (21.4%), *Acidovorax* (12.9%), and *Massilia* (10.0%). The 16S rRNA gene clone library for the harvested rainwater after the first-flush of the asphalt fiberglass shingle roof (Table 4-5) showed dominant organisms to be cyanobacteria (21.3%), *Staphylococcus* (16.0%), *Bacillus* (10.7%), and *Massilia* (16.0%). The 16S rRNA gene clone library for the harvested rainwater after the first-flush of the concrete tile roof (Table 4-6) showed dominant organisms to be *Brevibacterium* (16.0%), cyanobacteria (14.8%), *Staphylococcus* (11.1%), *Bacillus* (7.4%), *Sphingomonas* (6.2%), *Janthinobacterium* (6.2%), and *Massilia* (12.3%). The 16S rRNA gene clone library for the harvested rainwater after the first-flush of the cool roof (Table 4-7) showed dominant organisms to be *Brevibacterium* (20.7%), actinobacteria (5.4%), *Staphylococcus* (23.9%), and *Bacillus* (5.4%).

Staphylococcus was identified in the harvested rainwater after the first-flush from all roofs. *Staphylococcus* is a group of gram-positive bacteria that has been identified in humans and animals; some species are antibiotic-resistant and pathogenic to humans (Safdar and Maki, 2002). *Bacillus* was identified in the harvested rainwater after the first-flush from all roofs. *Bacillus* is a group of gram-positive, spore-forming bacteria that is commonly found in soil; some *Bacillus* species are pathogenic to humans.

Cyanobacteria were identified in the harvested rainwater after the first-flush from all roofs, with the green roof having the lowest percentage of these microorganisms; cyanobacteria are phototrophic bacteria that evolve oxygen.

Brevibacterium was identified in the harvested rainwater after the first-flush from all roofs. *Brevibacterium* is a group of gram-positive bacteria that has been found in dairy products, human skin, and blood cultures (Funke and Carlotti, 1994). *Rubrobacter* was identified in the harvested rainwater after the first-flush from the green roof. *Rubrobacter* is a group of gram-positive bacteria (Schabereiter-Gurtner et al., 2001) that has been identified in soils (Saul et al., 2005), and some species are resistant to gamma radiation (Ferreira et al., 1999). *Leifsonia* was identified in the harvested rainwater after the first-flush from the green, Galvalume® metal, and concrete tile roofs. *Leifsonia* is a group of gram-positive bacteria that has been found in soils (Suzuki et al., 1999).

Acidovorax was identified in the harvested rainwater after the first-flush from the green and Galvalume® metal roofs. *Acidovorax* is a group of gram-negative bacteria that is commonly found in soil. *Rhizobium* was identified in the harvested rainwater after the first-flush from the green, Galvalume® metal, and cool roofs. *Rhizobium* is a group of gram-negative bacteria that is common in soils. *Janthinobacterium* was identified in the harvested rainwater after the first-flush from the Galvalume® metal, concrete tile, and cool roofs. *Janthinobacterium* is a group of gram-negative bacteria that is found in soil and water. *Massilia* was identified in the harvested rainwater after the first-flush from all roofs except the green roof. *Massilia* is a group of gram-negative bacteria that has been found in air samples (Weon et al., 2008).

The 16S rRNA gene clone libraries allow us to draw several broad conclusions. First, as expected, soil bacteria are an important component of the microbial communities of harvested rainwater. Second, genera containing human pathogens are present in harvested rainwater. Third, the presence of gram-positive bacteria in the harvested rainwater will have implications for disinfection practices, since these microorganisms are more difficult to inactivate. Based on these results, we recommend that harvested rainwater be disinfected prior to potable use.

Table 4-3. 16S rRNA gene clone library for the harvested rainwater after the first-flush from the green roof.

Shaded populations represent more than 5.0% of the clone library.

Phylum	Class	Order	Family	Genus	Number of clones	Percent of clone library
Actinobacteria	Actinobacteridae	Rubrobacterales	Rubrobacterineae	*Rubrobacter* sp.[a]	6	8.0
		Actinomycetales	Brevibacteriaceae	*Brevibacterium*	7	9.3
			Intrasporangiaceae	*Phycicoccus*	2	2.7
			Micrococcaceae	*Leifsonia* sp.	4	5.3
				Mycobacterium sp.	3	4.0
			Micrococcineae	*Tetrasphaera japonica*	2	2.7
Bacteroidetes	Flavobacteria	Flavobacteriales	Flavobacteriaceae	Flexibacteraceae sp.	1	1.3
	Bacteroidetes bacterium				1	1.3
	Sphingobacteria	Sphingobacteriales	Sphingobacteriaceae	*Sphingobacterium* sp.	3	4.0
Cyanobacteria	Unclassified cyanobacterium				1	1.3
Firmicutes	Bacilli	Bacillales	Staphylococcaceae	*Staphylococcus*	5	6.7
			Bacillaceae	*Bacillus*	8	10.7
			Paenibacillaceae	*Paenibacillus* sp.	3	4.0
			Thermoactinomyces	Thermoactinomyces sp.	1	1.3
			Thermoactinomycetaceae	*Laceyella*	1	1.3
Proteobacteria	Alphaproteobacteria	Rhodospirillales	Acetobacteraceae	*Roseomonas*	2	2.7
			Rhodobacteraceae	*Rhodobacter* sp.	1	1.3
		Sphingomonadales	Sphingomonadaceae	*Sphingomonas*	1	1.3
		Rhizobiales	Rhizobiaceae	*Rhizobium* sp.	7	9.3
			Beijerinckiaceae	*Chelatococcus*	3	4.0
			Hyphomicrobiaceae	Hyphomicrobiaceae bacterium	1	1.3
	Betaproteobacteria	Burkholderiales	Comamonadaceae	*Acidovorax* sp.	8	10.7
	Gammaproteobacteria	Enterobacteriales	Enterobacteriaceae	*Enterobacter*	1	1.3
		Chromatiales	Chromatiaceae	*Rheinheimera* sp.	1	1.3
		Legionellales	Legionellaceae	*Legionella*	2	2.7

[a] sp. stands for species.

Table 4-4. 16S rRNA gene clone library for the harvested rainwater after the first-flush from the Galvalume® metal roof.

Shaded populations represent more than 5.0% of the clone library.

Phylum	Class	Order	Family	Genus	Number of clones	Percent of clone library
Actinobacteria	Actinobacteridae	Actinomycetales	Brevibacteriaceae	*Brevibacterium*	3	4.3
			Microbacteriaceae	*Leifsonia* sp.[a]	1	1.4
Bacteroidetes	Cytophagia	Cytophagales	Cytophagaceae	Flexibacteraceae bacterium	1	1.4
				Cytophagaceae bacterium	3	4.3
				Hymenobacter sp.	1	1.4
Cyanobacteria	Unclassified cyanobacterium				14	20.0
Deinococcus-thermus	Deinococci	Deinococcales	Deinococcaceae	*Deinococcus* sp.	2	2.9
Firmicutes	Bacilli	Bacillales	Staphylococcaceae	*Staphylococcus*	15	21.4
			Bacillaceae	*Bacillus*	2	2.9
Proteobacteria	Alphaproteobacteria	Rhizobiales	Rhizobiaceae	*Rhizobium* sp.	2	2.9
	Betaproteobacteria	Burkholderiales	Comamonadaceae	*Acidovorax* sp.	9	12.9
			Burkholderiaceae	Burkholderiaceae bacterium	1	1.4
			Oxalobacteraceae	*Janthinobacterium* sp.	1	1.4
				Herbaspirillum sp.	2	2.9
				Massilia sp.	7	10.0
				Duganella	2	2.9
				Oxalobacteraceae bacterium	1	1.4
	Gammaproteobacteria	Enterobacteriales	Enterobacteriaceae	*Shigella*	1	1.4
		Pseudomonadales	Pseudomonadaceae	*Pseudomonas*	2	2.9

[a] sp. stands for species.

Table 4-5. 16S rRNA gene clone library for the harvested rainwater after the first-flush from the asphalt fiberglass shingle roof.

Shaded populations represent more than 5.0% of the clone library.

Phylum	Class	Order	Family	Genus	Number of clones	Percent of clone library
Actinobacteria	Actinobacteridae	Solirubrobacterales	Patulibacteraceae	*Patulibacter*	1	1.3
		Actinomycetales	Brevibacteriaceae	*Brevibacterium*	3	4.0
			Frankineae	*Blastococcus*	1	1.3
			Micrococcaceae	*Actinoplanes*	1	1.3
			Acidimicrobineae	*Iamia*	1	1.3
				Acidimicrobineae bacterium	1	1.3
Bacteroidetes	Flavobacteria	Bacteroidales	Porphyromonadaceae	*Tannerella*	2	2.7
Cyanobacteria	Unclassified cyanobacterium				16	21.3
Firmicutes	Bacilli	Bacillales	Staphylococcaceae	*Staphylococcus*	12	16.0
			Bacillaceae	*Bacillus*	8	10.7
			Paenibacillaceae	*Paenibacillus* sp.	1	1.3
	Alphaproteobacteria	Rhizobiales	Aurantimonadaceae	*Aurantimonas*	2	2.7
			Rhizobiaceae	*Rhizobium* sp.	1	1.3
			Methylobacteriaceae	*Methylobacterium*	2	2.7
		Sphingomonadales	Sphingomonadaceae	*Sphingomonas* sp.	3	4.0
			Chitinophagaceae	*Flavisolibacter*	1	1.3
	Betaproteobacteria	Burkholderiales	Comamonadaceae	*Diaphorobacter*	2	2.7
			Burkholderiaceae	*Ralstonia* sp.	2	2.7
			Oxalobacteraceae	*Massilia*	12	16.0
	Gammaproteobacteria	Enterobacteriales	Enterobacteriaceae	*Enterobacter*	1	1.3
		Pseudomonadales	Pseudomonadaceae	*Pseudomonas*	1	1.3
		Xanthomonadales	Xanthomonadaceae	*Xanthomonas*	1	1.3

[a] sp. stands for species.

Table 4-6. 16S rRNA gene clone library for the harvested rainwater after the first-flush from the concrete tile roof.

Shaded populations represent more than 5.0% of the clone library.

Phylum	Class	Order	Family	Genus	Number clones	Percent of clone library
Actinobacteria	Actinobacteridae	Actinomycetales	Brevibacteriaceae	*Brevibacterium*	13	16.0
Actinobacteria	Actinobacteridae	Actinomycetales	Geodermatophilaceae	*Blastococcus* sp. [a]	1	1.2
Actinobacteria	Actinobacteridae	Actinomycetales	Microbacteriaceae	*Leifsonia* sp.	2	2.5
Actinobacteria	Actinobacteridae	Uncultured actinobacterium			1	1.2
Bacteroidetes	Cytophagia	Cytophagales	Cytophagaceae	Flexibacteraceae bacterium	1	1.2
Bacteroidetes	Cytophagia	Cytophagales	Cytophagaceae	*Hymenobacter* sp.	2	2.5
Bacteroidetes	Bacteroidia	Bacteroidales	Porphyromonadaceae	Porphyromonadaceae bacterium	1	1.2
Cyanobacteria	Unclassified cyanobacterium				12	14.8
Deinococcus-thermus	Deinococci	Deinococcales	Trueperaceae	*Stenotrophomonas*	1	1.2
Firmicutes	Bacilli	Bacillales	Staphylococcaceae	*Staphylococcus*	9	11.1
Firmicutes	Bacilli	Bacillales	Bacillaceae	*Bacillus*	6	7.4
Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae	*Sphingomonas*	5	6.2
Proteobacteria	Alphaproteobacteria	Rhizobiales	Bradyrhizobiaceae	Bradyrhizobiaceae bacterium	1	1.2
Proteobacteria	Alphaproteobacteria	Rhizobiales	Methylocystaceae	*Albibacter*	1	1.2
Proteobacteria	Alphaproteobacteria	Rhodobacterales	Rhodobacteraceae	*Rubellimicrobium*	2	2.5
Proteobacteria	Betaproteobacteria	Burkholderiales	Oxalobacteraceae	*Janthinobacterium* sp.	5	6.2
Proteobacteria	Betaproteobacteria	Burkholderiales	Oxalobacteraceae	*Massilia* sp.	10	12.3
Proteobacteria	Betaproteobacteria	Burkholderiales	Comamonadaceae	*Diaphorobacter*	1	1.2
Proteobacteria	Betaproteobacteria	Burkholderiales	Burkholderiaceae	*Leptothrix* sp.	1	1.2
Proteobacteria	Betaproteobacteria	Burkholderiales	Burkholderiaceae	*Ralstonia*	1	1.2
Proteobacteria	Gammaproteobacteria	Enterobacteriales	Enterobacteriaceae	*Citrobacter*	1	1.2
Proteobacteria	Gammaproteobacteria	Pseudomonadales	Pseudomonadaceae	*Pseudomonas*	2	2.5
Proteobacteria	Gammaproteobacteria	Xanthomonadales	Xanthomonadaceae	*Xanthomonas*	1	1.2
Proteobacteria	Gammaproteobacteria	Xanthomonadales	Xanthomonadaceae	*Stenotrophomonas*	1	1.2

[a] sp. stands for species.

Table 4-7. 16S rRNA gene clone library for the harvested rainwater after the first-flush from the cool roof.

Shaded populations represent more than 5.0% of the clone library.

Phylum	Class	Order	Family	Genus	Number of clones	Percent of clone library
Actinobacteria	Actinobacteridae	Actinomycetales	Brevibacteriaceae	*Brevibacterium*	19	20.7
			Propionibacteriaceae	*Propionibacterium*	2	2.2
			Intrasporangiaceae	*Phycicoccus*	2	2.2
			Micrococcaceae	*Micrococcus* sp.[a]	2	2.2
		Uncultured actinobacterium			5	5.4
Bacteroidetes	Cytophagia	Cytophagales	Cytophagaceae	Flexibacteraceae bacterium	1	1.1
Cyanobacteria		Unclassified cyanobacterium			4	4.3
		Nostocales	Microchaetaceae	*Coleodesmium*	3	3.3
Firmicutes	Bacilli	Bacillales	Staphylococcaceae	*Staphylococcus*	22	23.9
			Bacillaceae	*Bacillus*	5	5.4
Proteobacteria	Alphaproteobacteria	Sphingomonadales	Chitinophagaceae	*Flavisolibacter*	2	2.2
			Sphingomonadaceae	*Sphingomonas*	2	2.2
		Rhodospirillales	Acetobacteraceae	*Roseomonas*	2	2.2
		Rhizobiales	Aurantimonadaceae	*Fulvimarina* sp.	1	1.1
			Methylocystaceae	*Methylopila* sp.	1	1.1
			Beijerinckiaceae	*Chelatococcus*	1	1.1
	Betaproteobacteria	Burkholderiales	Oxalobacteraceae	Uncultured Oxalobacteraceae	1	1.1
				Janthinobacterium sp.	2	2.2
				Massilia sp.	2	2.2
				Herbaspirillum sp.	3	3.3
				Duganella	2	2.2
			Burkholderiaceae	*Burkholderia* sp.	1	1.1
				Ralstonia	1	1.1
	Gammaproteobacteria	Enterobacteriales	Enterobacteriaceae	*Erwinia* sp.	1	1.1
		Oceanospirillales	Halomonadaceae	*Halomonas* sp.	2	2.2
		Xanthomonadales	Xanthomonadaceae	*Lysobacter* sp.	2	2.2
				Stenotrophomonas	1	1.1

[a] sp. stands for species.

5 Task 7. Lab-scale studies of roofing materials

Roofing materials are subject to changes over time due to photodegradation via UV light from the sun, elevated temperature, moisture, and microbial growth (Berdahl et al., 2008). Thus, since roofing materials change with age, it also is possible that the release of certain elements and compounds from a roofing material changes as the roof ages.

These lab-scale studies examined the contaminants released into harvested rainwater from coupons of new and artificially aged roofing materials. Three materials were tested: asphalt fiberglass shingle, Galvalume® metal, and concrete tile (same manufacturers as those in the pilot-scale study). Six coupons (4 in by 4 in) of each material were prepared. Three coupons of each material were artificially aged in a pressure aging vessel (Gilson Company Inc., Lewis Center, Ohio) at 2.1 megapascal (MPa) and 100°C for 20 hours. This apparatus was designed to simulate 5-10 years of aging for asphalt samples, and the standard practice for accelerated aging of asphalt was followed (American Association of State Highway and Transportation Officials [AASHTO], 2009). Then, the samples were exposed to UV light from a 160-watt panel for 24 hours (UV Panel HP, American DJ, Los Angeles, California).

Eighteen coupon experiments were performed (3 coupons for each new material and 3 coupons for each artificially aged material) with water from a residential rainwater harvesting system that treats with filtration (25-µm and 5-µm filters) and UV disinfection (Novatek 3G #10 Blue MB UV bulb, Pentair Inc., Minneapolis, Minnesota; TrojanUVMax C, Danaher Corporation, Washington, DC). We designed an artificial rainfall apparatus to mimic local rainfall rates for two-year intensities, with a flow of 13 milliliters per minute (mL/min) over each roofing coupon (Figure 5-1A). Twenty-three macro intravenous drip sets were connected in parallel (Figure 5-1B) and suspended in a grid above the roofing coupon. The water dripped onto the coupon and was recirculated over the coupon for 24 hours to determine which contaminants would be released from the roofing materials. Two separate rainfall units were powered by a multi-channel peristaltic pump (Cole Parmer, L/S variable speed digital drive with 2-channel pump head) so that two experiments could be run simultaneously. After recirculation, pH, conductivity, turbidity, TSS, DOC, nitrate, nitrite, and metals were measured as described section 3 of this report. The average and standard deviation for each water quality parameter were calculated using data from the triplicate coupons (new or artificially aged coupons) for each roofing material. For instance, the three new metal roofing coupons yielded the following pH values: 7.1, 6.4, and 6.6. The average ($\frac{7.1+6.4+6.6}{3} = 6.7$) and standard deviation of the triplicate coupons $\sqrt{\frac{(7.1-6.7)^2+(6.4-6.7)^2+(6.6-6.7)^2}{2}}$ are summarized in Figure 5-2.

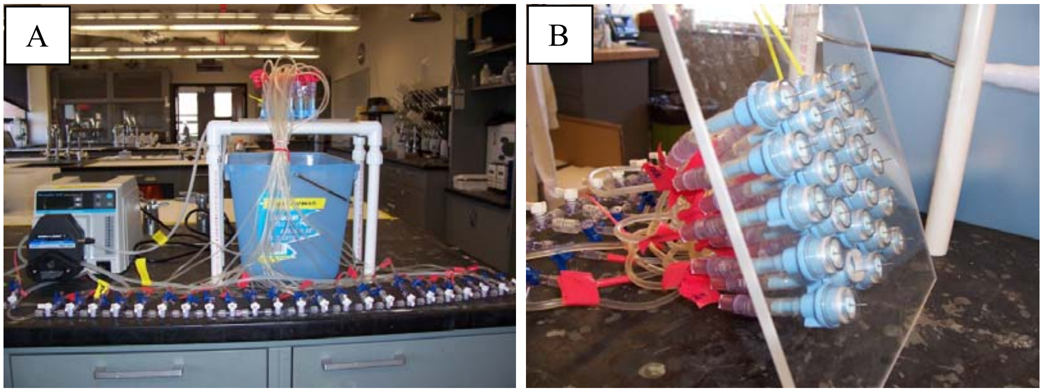

Figure 5-1. Lab-scale testing apparatus. A. Overall system with peristaltic pump and 23 tubes to simulate rain onto a roofing coupon. B. Grid of macro intravenous drip sets.

The aging process did not appreciably affect pH (Figure 5-2). The new and artificially aged coupons yielded near-neutral pH values, and all roofing materials resulted in a pH increase over that of the starting rainwater (before it was exposed to the roofing coupons).

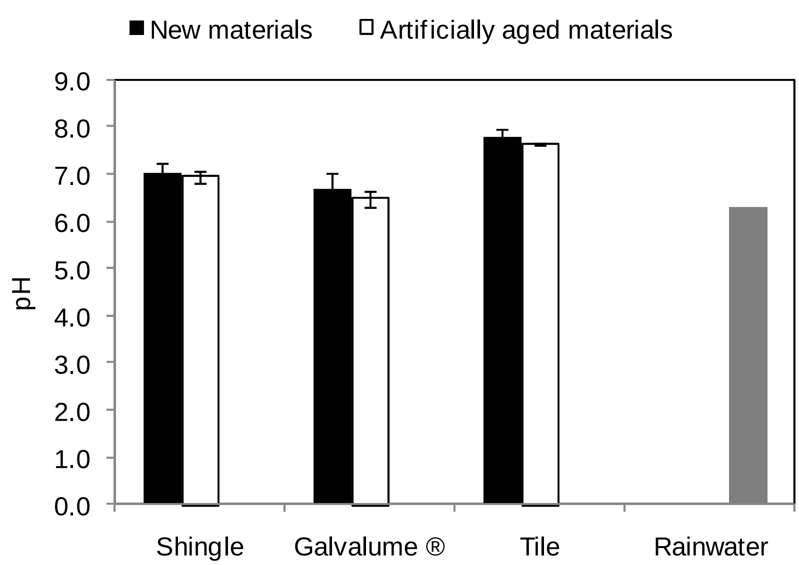

Figure 5-2. pH in the coupon study, including the starting rainwater and the new and artificially aged roofing coupons. Error bars represent one standard deviation from triplicate coupon experiments.

The aging process affected conductivity from the asphalt fiberglass shingle roof coupons but did not appreciably affect conductivity from the Galvalume® metal and concrete tile roofing coupons (Figure 5-3). The aged shingle and new and aged concrete tile coupons yielded increased conductivity values as compared to that of the starting rainwater.

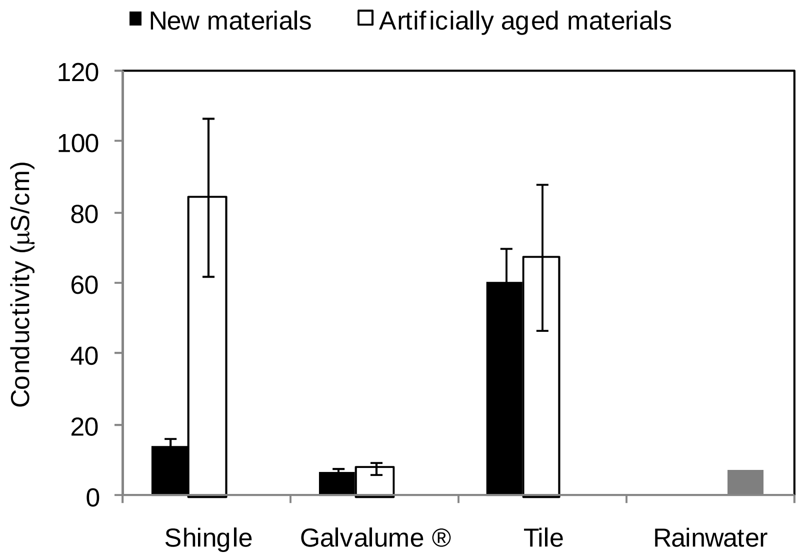

Figure 5-3. **Conductivity in the coupon study, including the starting rainwater and the new and artificially aged roofing coupons. Error bars represent one standard deviation from triplicate coupon experiments.**

The aging process slightly increased the average release of particulate matter from the asphalt fiberglass shingle, Galvalume® metal, and concrete tile roofing coupons (Figures 5-4 and 5-5). All roofing materials yielded an increase in particulate matter concentrations as compared to that of the starting rainwater.

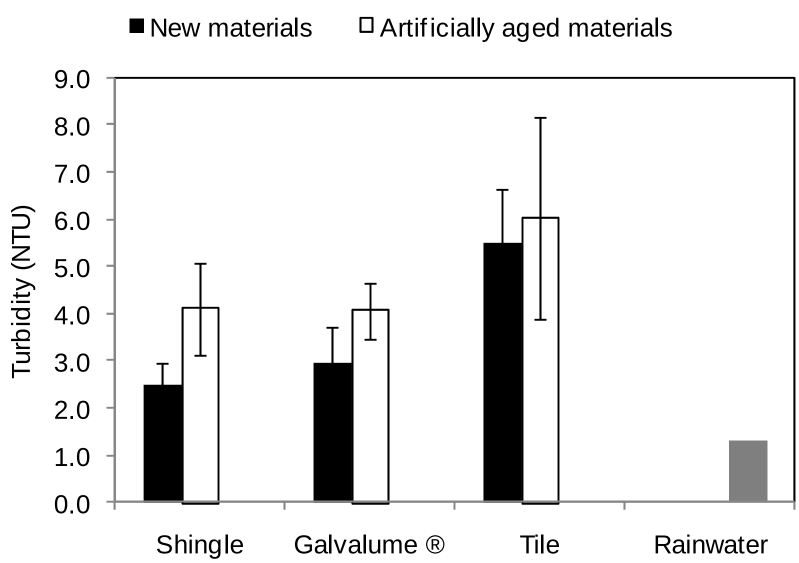

Figure 5-4. **Turbidity in the coupon study, including the starting rainwater and the new and artificially aged roofing coupons. Error bars represent one standard deviation from triplicate coupon experiments.**

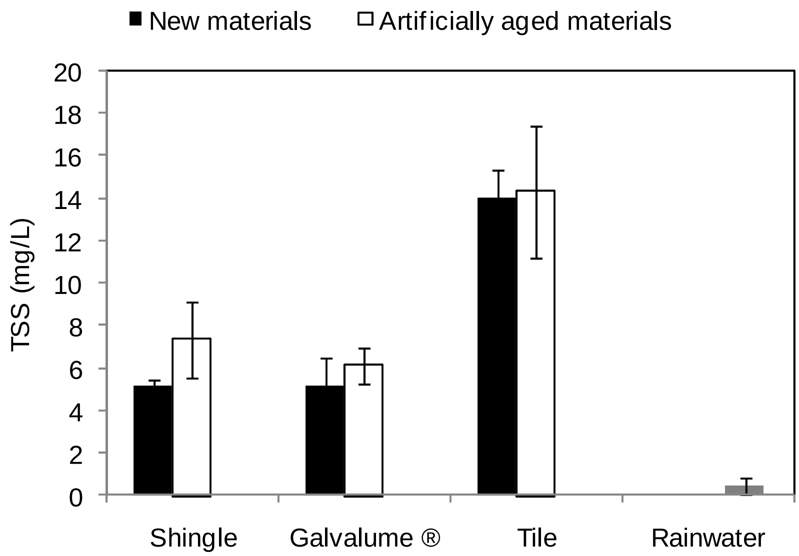

Figure 5-5. TSS in the coupon study, including the starting rainwater and the new and artificially aged roofing coupons. Error bars represent one standard deviation from triplicate coupon experiments.

The release of organic matter from the asphalt fiberglass shingle, Galvalume® metal, and concrete tile roofing coupons increased after the aging process (data not shown), but we believe that this is an artifact of using the pressure aging vessel. This vessel is routinely used to age asphalt samples and could have contaminated the roofing coupons with additional organic matter during the aging process.

The aging process slightly increased the average release of nitrite from the asphalt fiberglass shingle and Galvalume® metal roofing coupons (Figure 5-6). The error bars, particularly for the shingle roofing coupons, indicate variability in nitrite release from the triplicate coupons.

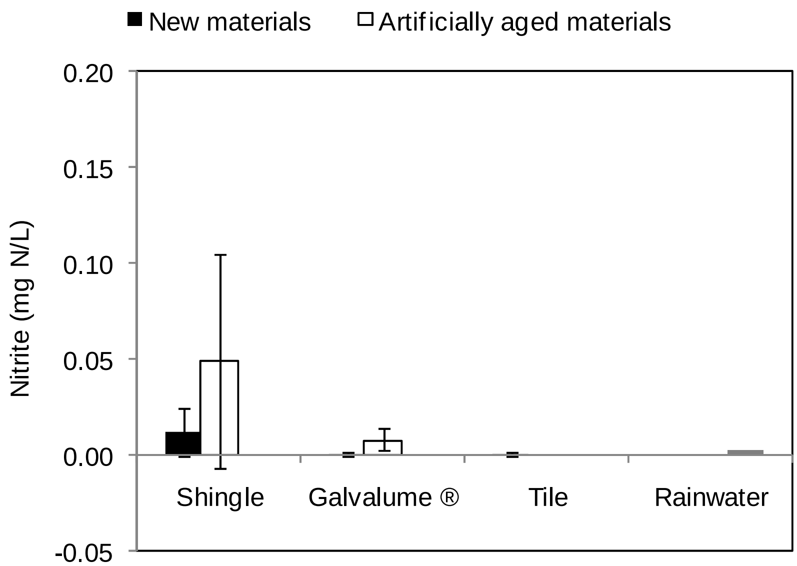

Figure 5-6. Nitrite in the coupon study, including the starting rainwater and the new and artificially aged roofing coupons. Error bars represent one standard deviation from triplicate coupon experiments.

The aging process decreased the average nitrate released from the asphalt fiberglass shingle, Galvalume® metal, and concrete tile roofing coupons, although the error bars indicate considerable variation among the triplicate coupons (Figure 5-7). The nitrate concentrations from the new and artificially aged roofing coupons were generally lower than that of the starting rainwater (Figure 5-7), suggesting that these roofing materials act as a sink for nitrate. The bulk of nitrate in harvested rainwater likely comes from atmospheric deposition, as previous work has shown that nitrate concentrations in harvested rainwater generally increase with the length of the antecedent dry period (Mendez et al., 2011).

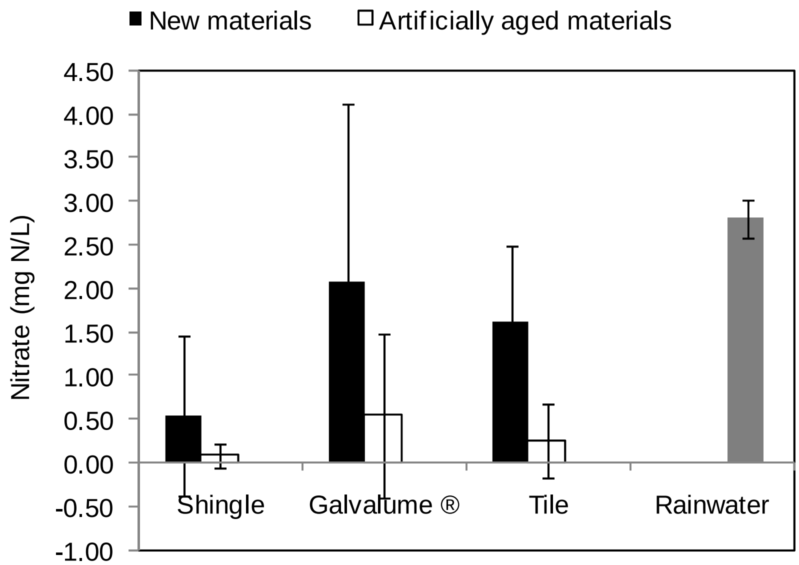

Figure 5-7. Nitrate in the coupon study, including the starting rainwater and the new and artificially aged roofing coupons. Error bars represent one standard deviation from triplicate coupon experiments.

The aging process did not appreciably affect As, Cd, Cr, Cu, Pb, and Se from the asphalt fiberglass shingle, Galvalume® metal, and concrete tile roofing coupons (Table 5-1). Most of the concentrations of these same metals were not appreciably different from those in the starting rainwater (shaded in Table 5-1). However, the concrete tile coupons yielded slightly higher concentrations of As, Cd, Cr, and Se than those in the starting rainwater (Table 5-1).

The aging process did affect Al, Fe, and Zn from the roofing coupon materials (Table 5-1). In general, the aging process caused the roofing coupons to decrease the release of Al, Fe, and Zn, except that the release of Fe and Zn increased from the artificially aged asphalt fiberglass shingle roofing coupons as compared to the new asphalt fiberglass shingle roofing coupons. Consistent with the pilot-scale observations, the Galvalume® metal roofing coupons showed the highest Zn concentrations and the concrete tile roofing coupons showed the highest Al concentrations.

Overall, the artificial aging process that we employed appeared to have the most effect on the asphalt fiberglass shingles. The artificial aging process increased the conductivity and the concentration of particulate matter from the asphalt fiberglass shingle roofing coupons to a greater extent than it did for the Galvalume® metal and concrete tile roofing coupons; it also caused an increase in the release of Fe and Zn from the asphalt fiberglass shingle roofing coupons. These short-term tests suggest that harvested rainwater quality will change as an asphalt fiberglass shingle roof ages, and the pilot-scale roofs should continue to be monitored to verify this trend.

Table 5-1. Metals in the coupon study, including the starting rainwater and the new and artificially aged roofing coupons. Values were calculated from triplicate coupon experiments. Average concentration (standard deviation) shown in μg/L. Shaded portions indicate metal concentrations that were not appreciably higher than those in the starting rainwater.

	Shingle		Galvalume®		Tile	
Metal	**New**	**Artificially aged**	**New**	**Artificially aged**	**New**	**Artificially aged**
Arsenic	0.94 (0.15)	0.79 (0.08)	0.54 (0.08)	0.57 (0.02)	1.69 (0.27)	1.30 (0.13)
Cadmium	0.06 (0.01)	0.05 (0.02)	0.07 (0.05)	0.04 (0.02)	0.02 (0.01)	0.02 (0.01)
Chromium	0.63 (0.15)	0.58 (0.10)	0.66 (0.08)	0.55 (0.04)	4.00 (0.75)	2.51 (0.14)
Copper	0.01 (0)	0.01 (0)	0.01 (0)	0.01 (0)	0.01 (0)	0.01 (0)
Lead	0.01 (0)	0.01 (0)	0.01 (0)	0.01 (0)	0.01 (0)	0.01 (0)
Selenium	0.03 (0)	0.03 (0)	0.04 (0.02)	0.03 (0)	0.08 (0.02)	0.11 (0.02)
Aluminum	2.94 (4.95)	0.08 (0)	7.64 (6.86)	0.08 (0)	287.95 (127.34)	180.45 (72.13)
Iron	4.82 (1.93)	10.17 (11.07)	5.59 (0.65)	3.97 (0.54)	151.29 (118.39)	71.48 (60.92)
Zinc	153.51 (10.08)	186.54 (16.20)	476.94 (37.21)	327.91 (27.96)	14.41 (15.80)	1.17 (1.61)

6 Conclusions and recommendations

This study complements our previous work (Mendez et al., 2010) through the collection of additional data regarding the impact of roofing material on the quality of harvested rainwater. Specifically we assessed harvested rainwater quality from five pilot-scale roofs (asphalt fiberglass shingle, Galvalume®, concrete tile, cool, and green) and one full-scale Kynar®-coated Galvalume® roof. We assessed microbial community diversity of the rainwater harvested from the pilot-scale roofs by T-RFLP and 16S rRNA gene sequencing. We performed lab-scale studies with new and artificially aged coupons of asphalt fiberglass shingle, Galvalume®, and concrete tile to examine how the release of contaminants might change as these roofing materials age. As discussed below, several important conclusions can be drawn from our work.

- As with our previous study (Mendez et al., 2010), our current study shows that harvested rainwater quality generally increases with roof flushing, indicating the importance of an effective first-flush diverter. However, the rainwater harvested after the first-flush from all of the pilot-scale roofs did contain some contaminants at levels above USEPA drinking water standards (i.e., turbidity, TC, FC, Fe[3], and Al); the harvested rainwater after the first-flush from the full-scale Kynar®-coated Galvalume® roof exceeded the turbidity, TC, and FC standards. The pH of the harvested rainwater after the first-flush generally complied with the range specified by the USEPA secondary standard, but some of the events from the Galvalume®, concrete tile, green, and Kynar®-coated Galvalume® roofs produced rainwater after the first-flush with pH values that were outside the range specified by the standard. The quality of rainwater harvested for potable use at a private residence is not regulated by the TCEQ, and, thus, the USEPA drinking water standards do not have to be met. However, to best protect the public health, we recommend the use of a first-flush diverter and additional treatment prior to potable use of harvested rainwater.

[3] Only the green pilot-scale roof did not violate the Fe standard.

- Although metal roofs are commonly recommended for rainwater harvesting applications, our data show that concrete tile and cool roofs also are good candidate roofing materials for rainwater harvesting applications. While the Kynar®-coated Galvalume® roof often showed lower concentrations of individual contaminants as compared to the Galvalume® roof, this was generally attributable to lower contaminant concentrations in the ambient rain at the site of the Kynar®-coated Galvalume® roof. Thus, we expect Galvalume® and Kynar®-coated Galvalume® roofs in good condition to yield similar harvested rainwater qualities for similar ambient rain compositions, but additional data are needed to demonstrate this.

- DOC concentrations must be considered when choosing a roofing material and disinfection strategy for a rainwater harvesting application. As with our previous study (Mendez et al., 2010), our current study shows that green roofs yield the highest DOC concentrations in harvested rainwater as compared to asphalt fiberglass shingle, Galvalume®, Kynar®-coated Galvalume®, concrete tile, and cool, which could lead to high concentrations of DBPs after chlorination. While our previous study (Mendez et al., 2010) showed that the new pilot-scale asphalt fiberglass shingle roof produced the second highest concentrations of DOC in harvested rainwater, our current study showed that the 1-year-old pilot-scale asphalt fiberglass shingle roof produced DOC concentrations more in line with those from the Galvalume®, Kynar®-coated Galvalume®, concrete tile, and cool roofs. This trend of decreasing DOC concentrations from aging shingle roofs must be verified, as it has significant implications for DBP concentrations. Thus, we recommend that rainwater harvested from a green roof and probably an asphalt fiberglass shingle roof not be disinfected with chlorine.

- Our previous study (Mendez et al., 2010) and our current study both show that the rainwater harvested from the Galvalume® roof had lower concentrations of fecal indicator bacteria as compared to the other roofing materials. This suggests that the Galvalume® roof might have an advantage over other roofing materials in terms of producing rainwater with lower concentrations of human pathogens (i.e., microorganisms that cause disease in humans). This needs to be studied in detail by monitoring the survival of multiple pathogens on the various roofing materials.

- The quality of commercial growing media must be carefully examined if green roofs were to be used in potable rainwater harvesting applications. Our previous study (Mendez et al., 2010) and our current study both show measurable concentrations of arsenic and lead in the rainwater harvested from the green roof. In particular, the highest arsenic concentration in the rainwater harvested after the first-flush from the green roof was 8 μg/L, which is close to the 10 μg/L USEPA drinking water standard.

- Rainwater harvested from all of the pilot-scale roofing materials showed diverse microbial communities. The community in the rainwater harvested from the green roof showed the highest microbial diversity, and the communities in the rainwater harvested from each of the pilot-scale roofs were more similar to each other than they were to that of ambient rain.

- According to the 16S rRNA gene clone libraries, the rainwater harvested from each pilot-scale roof contained genera associated with human pathogens (e.g., *Staphylococcus* and *Bacillus*) and genera associated with soil (e.g., *Acidovorax* and *Rhizobium*). Many of the genera found in the harvested rainwater were gram-positive bacteria, indicating that sufficient disinfection practices must be in place to inactivate these generally more recalcitrant microorganisms.

- We employed an artificial aging process to rapidly examine the effect of aging on contaminant release from three common roofing materials (asphalt fiberglass shingle, Galvalume®, and concrete tile). The artificial aging process appeared to have the most effect on the asphalt fiberglass shingles, causing a greater increase in conductivity and the concentration of particulate matter released from the asphalt fiberglass shingles as compared to Galvalume® and concrete tile; it also caused an increase in the release of iron and zinc from the asphalt fiberglass shingles. Thus, the potential exists for changes in harvested rainwater quality as the roofing material ages, particularly for asphalt fiberglass shingles.

7 Acknowledgements

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8 References

American Association of State Highway and Transportation Officials (AASHTO), 2009, Standard practice for accelerated aging of asphalt binder using a pressurized aging vessel (PAV), Washington D.C.

Berdahl, P., Akbari, H., Levinson, R., and Miller, W.A., 2008, Weathering of roofing materials – an overview, Report LBNL-59724, Lawrence Berkeley National Laboratory, Berkeley, CA.

Bretz, S., Akbari, H., and Rosenfeld, A., 1998, "Practical issues for using solar-reflective materials to mitigate urban heat islands," *Atmospheric Environment* 32(1): 95-101.

Dunbar, L., Ticknor, L.O., and Kuske, C.R., 2000, "Assessment of microbial diversity in four southwestern United States soils by 16S rRNA gene terminal restriction fragment analysis," *Applied and Environmental Microbiology* 66(7): 2943-2950.

Ferreira, A.C., Nobre, M.F., Moore, E., Rainey, F.A., Battista, J.R., and daCosta, M.S., 1999, "Characterization and radiation resistance of new isolates of *Rubrobacter radiotolerans* and *Rubrobacter xylanophilus*," *Extremophiles* 3(4): 235–238.

Funke, G., and Carlotti, A., 1994, "Differentiation of *Brevibacterium* spp. encountered in clinical specimens," *Journal of Clinical Microbiology* 32(7): 1729-1732.

Mendez, C.B., Afshar, B.R., Kinney, K., Barrett, M.E., and Kirisits, M.J., 2010, Effect of roof material on water quality for rainwater harvesting systems, Texas Water Development Board, Austin, TX.

Mendez, C.B., Klenzendorf, J.B., Afshar, B.R., Simmons, M.T., Barrett, M.E., Kinney, K.A., and Kirisits, M.J., 2011, "The effect of roofing material on the quality of harvested rainwater," *Water Research*, in press.

Nieuwenhuijsen, M.J., Smith, R., Golfinopoulos, S., Best, N., Bennett, J., Aggazzotti, G., Righi, E., Fantuzzi, G., Bucchini, L., Cordier, S., Villanueva, C.M., Moreno, V., La Vecchia, C., Bosetti, C., Vartiainen, T., Rautiu, R., Toledano, M., Iszatt, N., Grazuleviciene R., and Kogevinas, M., 2009, "Health impacts of long-term exposure to disinfection by-products in drinking water in Europe: HIWATE," *Journal of Water and Health* 7(2): 185-207.

Reiter, B., Pfeifer, U., Schwab, H., and Sessitsch, A., 2002, "Response of endophytic bacterial communities in potato plants to infection with *Erwinia carotovora* subsp. *Atroseptica,"Applied and Environmental Microbiology* 68(5): 2261-2268.

Safdar, N., and Maki, D.G., 2002, "The commonality of risk factors for nosocomial colonization and infection with antimicrobial-resistant *Staphylococcus aureus*, enterococcus, gram-negative bacilli, *Clostridium difficile*, and *Candida,*" *Annals of Internal Medicine* 136(11): 834-844.

Saul, D.J., Aislabie, J., Brown, C.E., Harris, L., and Foght, J.M., 2005, "Hydrocarbon contamination changes the bacterial diversity of soil from around Scott Base, Antarctica," *FEMS Microbiology Ecology* 53(1): 141–155.

Schabereiter-Gurtner, C., Piñar, G., Vybiral, D., Lubitz, W., and Rölleke, S., 2001, *"Rubrobacter*-related bacteria associated with rosy discolouration of masonry and lime wall paintings*," Archives of Microbiology* 176(5): 347–354.

Simmons, G., Hope, V., Lewis, G., Whitmore, J., and Gao, W., 2001, "Contamination of potable roof-collected rainwater in Auckland, New Zealand," *Water Research* 35(6): 1518-1524.

Singh, T., and Kalra, Y.P., 1975, "Specific conductance method for in situ estimation of total dissolved solids," *Journal of the American Water Works Association* 67(2): 99-100.

Suzuki, K., Suzuki, M., Sasaki, J., Park, Y.H., and Komagata, K., 1999, "*Leifsonia* gen. nov., a genus for 2,4-diaminobutyric acid-containing actinomycetes to accommodate "*Corynebacterium aquaticum*" Leifson 1962 and *Clavibacter xyli* subsp. *cynodontis* Davis *et al*. 1984," *Journal of General Applied Microbiology* 45(5): 253–262.

TWDB, 2005, The Texas manual on rainwater harvesting. Austin, TX.

TWDB, 2006, Rainwater harvesting potential and guidelines for Texas. Austin, TX.

Turpeinen R., Kairesalo, T., and Haggblom, M.M., 2004, "Microbial community structure and activity in arsenic-, chromium- and copper-contaminated soils," *Federation of European Microbiological Societies Microbiology Ecology* 47(1): 39-50.

USEPA, 2009. "Drinking water contaminants," Accessed 10 September 2009. http://www.epa.gov/safewater/contaminants/index.html

Weon, H.Y., Kim, B.Y., Son, J.A., Jang, H.B., Hong, S.K., Go, S.J., and Kwon, S.W., 2008, "*Massilia aerilata* sp. nov., isolated from an air sample," *Journal of Systematic and Evolutionary Microbiology* 58: 1422–1425.

Yaziz, M., Gunting, H., Sapari, N., and Ghazali, A., 1989, "Variations in rainwater quality from roof catchments," *Water Research* 23(6): 761-765.

9 Appendix

9.1 Description of molecular methods

For the 2009 rain events in Mendez et al. (2010), deoxyribonucleic acid (DNA) was extracted from sediments in the first-flush, first tank, and second tank. Sediments were allowed to settle overnight in the tanks and pipetted into microcentrifuge tubes. DNA was isolated from each sediment sample using two kits: UltraClean Microbial DNA Isolation and UltraClean Soil DNA (MoBio, Carlsbad, California). For the 2010 rain events of the current study, rainwater samples were filtered using 0.45-micrometer (µm) filter membranes, and DNA was extracted with the Ultraclean Water Isolation kit (MoBio, Carlsbad, California).

For T-RFLP, DNA was amplified by the polymerase chain reaction (PCR) using primers 8F (FAM-labeled) and 926R to target the 16S rRNA gene. Each 50-microliter (µL) PCR mixture contained 1.25 unit (U) Taq polymerase, 5 µL of 10× PCR buffer, 0.05 millimolar (mM) concentrations of each deoxynucleoside triphosphate (dNTP), 0.5 micromolar (µM) concentrations of each primer, 1 µL of DNA, and 38.5 µL of water. Each reaction was placed in a PTC-200 Peltier Thermal Cycler (MJ Research, Watertown, Massachusetts) and incubated under the following conditions: 94 degrees Celsius (°C) for 3 minutes, 32 cycles at 94°C for 30 seconds, 52°C for 30 seconds, 72°C for 1 minute, followed by a final extension at 72°C for 7 minutes. The resulting PCR amplicon was run on a 1 percent agarose gel at 70 milliamp (mA) for 45 minutes, stained with SYBR® Gold for 20 minutes, and imaged with a Kodak 1D Image Analyzer (Eastman Kodak Company, Rochester, New York). Amplification of the appropriately sized product (approximately 920 base pairs [bp]) was verified by comparison to a DNA ladder.

Only the April 18, 2009 rain event yielded successful amplification of the 16S rRNA gene from the rainwater harvested after the first-flush for all roofs using the UltraClean Soil DNA kit. In addition, only the July 23, 2009 rain event yielded successful amplification of the 16S rRNA gene from ambient rain. PCR might have been inhibited by the presence of humic substances and metals in the harvested rainwater, which are common inhibitors in environmental samples. Since successful amplification of DNA is key to proceeding with T-RFLP and sequencing, DNA from the harvested rainwater after the first-flush from April 18, 2009 and the ambient rain from July 23, 2009 were used to describe the microbial diversity in the pilot-scale samples. Rather than spending additional time to optimize PCR to get additional T-RFLP data, we chose to focus our efforts on the 16S rRNA gene clone libraries, which provide more detailed information about the microbial communities.

The PCR amplicon was treated with the Klenow enzyme. Each 50-µL PCR amplicon was incubated with 6.25 µL of 10× PCR buffer, 0.063 mM of each dNTP, and 5 U Klenow enzyme. The tubes were put in the thermal cycler at 20°C for 1 hour. The amplicon was cleaned using a

MoBio Ultraclean® PCR Clean-up kit (Carlsbad, California). The DNA concentration of the PCR product was measured spectrophotometrically at 260 nanometer (nm) (NanoDrop ND-1000 spectrophotometer, NanoDrop Technologies, Wilmington, Delaware). After DNA quantification, 100 nanograms (ng) of amplicon were digested with 40 U HhaI in a 20-µL reaction at 37°C for 3 hours. At the end of the digestion, HhaI was inactivated by exposing the reactions to 65°C for 20 minutes. The digested amplicon was cleaned with Microcon YM-30 filters (Millipore, Bedford, Massachusetts), resuspended in tris(hydroxymethyl)aminomethane-ethylenediaminetetraacetic acid (TE) buffer at pH 8. Samples were submitted to the Institute for Cellular and Molecular Biology (ICMB) Core Research Facility at the University of Texas at Austin for fragment size analysis with a 3739 DNA analyzer (Applied Biosystems, Foster City, California). DNA fragments were analyzed with the GeneMarker 1.70 software (SoftGenetics, State College, Pennsylvania). Fragments with lengths between 60 and 600 bp and heights of greater than 40 relative fluorescence units were included in the fragment analysis.

The microbial diversity and similarity of the samples was assessed by analyzing the DNA fragment patterns with the SWI and SI. The microbial diversity of each sample was determined by the SWI, and the similarity between samples was determined by the SI using ±0.5bp comparisons.

The SWI (Reiter et al., 2002) can be calculated as follows:

$$SWI = -\sum P_i ln P_i$$

$$P_i = \frac{n_i}{N}$$

n_i = height of a peak
N = sum of all peak heights in a T-RFLP profile

SI (Turpeinen et al., 2004) can be calculated as follows:

$$SI = \frac{2c}{a + b}$$

a = number of fragments in the first sample
b = number of fragments in the second sample
c = number of fragments shared between the samples

For cloning and sequencing, DNA was amplified by PCR with unlabeled primers 8F and 926R to target the 16S rRNA gene. Each 50-µl PCR mixture contained 1.25 U Taq polymerase, 5 µL of 10× PCR buffer, 0.05 mM concentrations of each dNTP, 0.5 µM concentrations of each primer, 1 µL of DNA, and 38.5 µL of water. Each reaction was placed in a PTC-200 Peltier Thermal Cycler (MJ Research, Watertown, Massachusetts) and incubated under the following conditions: 94°C for 3 minutes, 32 cycles at 94°C for 30 seconds, 52°C for 30 seconds, 72°C for 1 minute, followed by a final extension at 72°C for 7 minutes. The resulting PCR amplicon was run on a 1 percent agarose gel at 70 mA for 45 minutes, stained with SYBR® Gold for 20 minutes, and imaged with a Kodak 1D Image Analyzer (Eastman Kodak Company, Rochester, New York). Amplification of the appropriately sized product (approximately 920 bp) was verified by comparison to a DNA ladder. The target amplicon was gel-purified with a MinElute Gel

Extraction Kit (QIAGEN, Valencia, California). A TOPO TA cloning kit (Invitrogen, Carlsbad, California) was used according to the manufacturer's instructions. For each pilot-scale roofing material, 96 clones were picked individually using sterile toothpicks and transferred from Lysogeny Broth (LB) agar plates to the wells of a 96-well round bottom plate (Costar, Cambridge, Massachusetts) containing 200-µL of medium consisting of LB broth and 10% glycerol. The 96-well plates were incubated at 37°C for 12 hours, sealed with aluminum, and shipped overnight on dry ice to Beckman Coulter Genomics (Danvers, Massachusetts) for insert sequencing in one direction. The sequences were submitted to BLAST (blastn and megablast queries at http://blast.ncbi.nlm.nih.gov/Blast.cgi) to identify library sequences most closely matching the query sequence, and the phylogenetic information for each clone was retrieved (phylum, class, order, family, and genus).

9.2 T-RFLP electropherograms

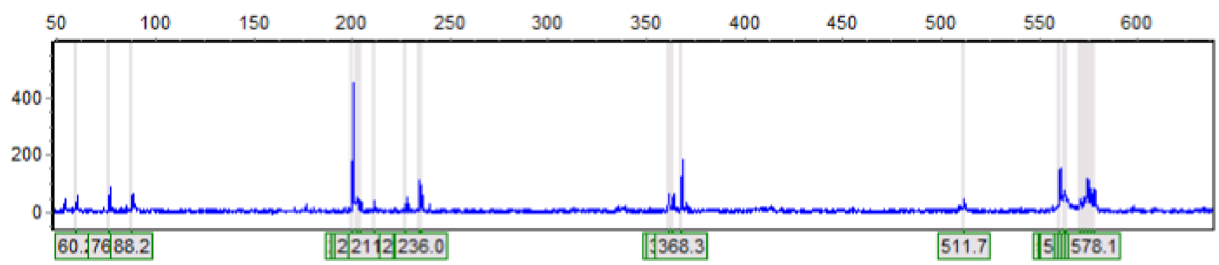

Figure 9-1. DNA fragments from T-RFLP for green roof (tank 1). Note: x-axis shows base pairs and y-axis shows peak heights (relative fluorescence units).

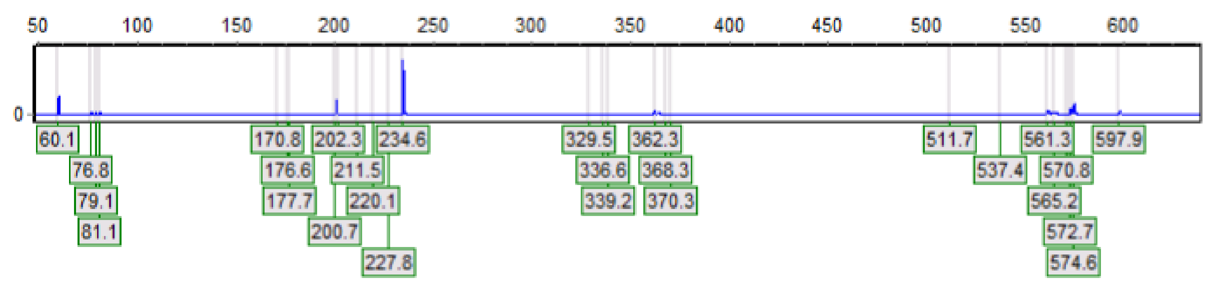

Figure 9-2. DNA fragments from T-RFLP for Galvalume® metal roof (tank 1). Note: x-axis shows base pairs and y-axis shows peak heights (relative fluorescence units).

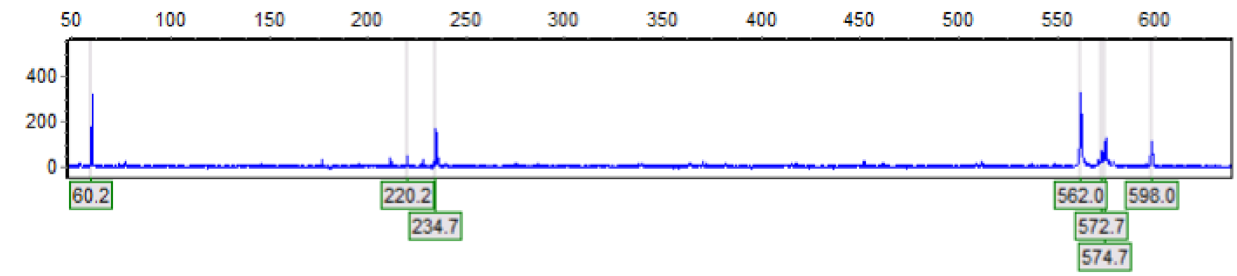

Figure 9-3. DNA fragments from T-RFLP for shingle roof (tank 2). Note: x-axis shows base pairs and y-axis shows peak heights (relative fluorescence units).

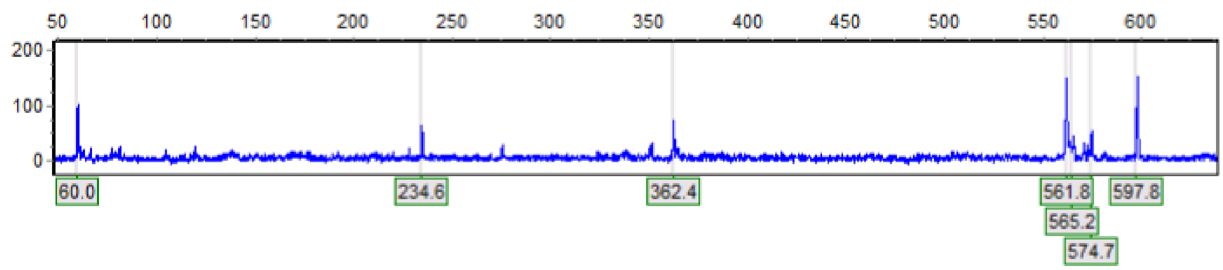

Figure 9-4. DNA fragments from T-RFLP for tile roof (tank 2). Note: x-axis shows base pairs and y-axis shows peak heights (relative fluorescence units).

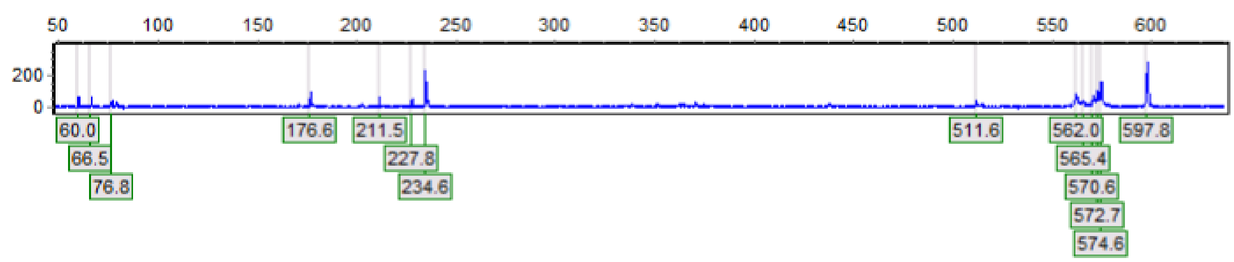

Figure 9-5. DNA fragments from T-RFLP for cool roof (tank 2). Note: x-axis shows base pairs and y-axis shows peak heights (relative fluorescence units).

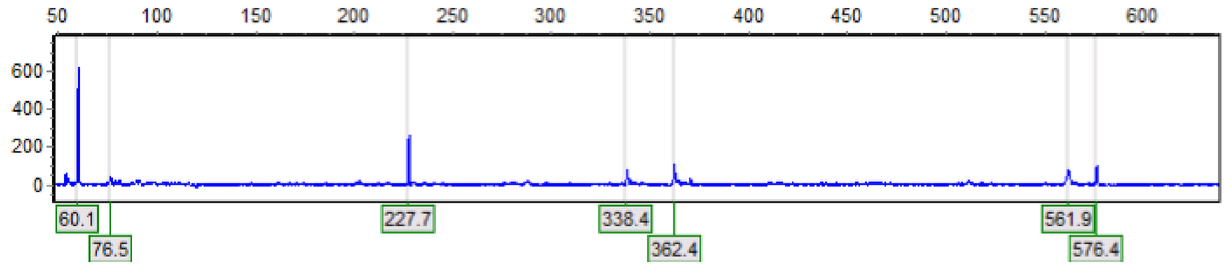

Figure 9-6. DNA fragments from T-RFLP for ambient rain. Note: x-axis shows base pairs and y-axis shows peak heights (relative fluorescence units).

9.3 Review comments and responses

Table 9-1. Responses to review comments.

- Overall, the report has done a good job of presenting the research study in a clear, concise, and factual manner. It is also remarkably free of typographical and other errors. However, the tone of the report is very scientific and may need to be softened somewhat to make it more suitable for TWDB's intended audience: the general public. Accordingly, consideration should be given to explaining, in general terms, some of the more sophisticated concepts and techniques used in the study and the implications of the results for users interested in harvesting rainwater. For example, a simple description of T-RFLP and sequencing of the 16S rRNA gene and its implication for or importance in rainwater harvesting would be most useful to members of the public (we would guess, many) who do not have the necessary scientific background and may not be familiar with these profiling techniques.
  - We have addressed this comment throughout the text. We have added simple explanations for each parameter measured, and we have more simply described the implications of the results to the user.

- Because the report contains many abbreviations and acronyms that are frequently used throughout the report, please consider adding a list of abbreviations at the beginning of the report for the convenience of the reader.
  - A list of abbreviations has been added to the front matter.

- Please consider including a map showing the locations of the Kynar®, pilot, and green roofs used in the study. It would be useful to the reader.
  - A map showing the locations of the roofs has been added (Figure 3-2).

- Please consider describing in more detail the methodology used to derive the error bars shown in Figures 5-2 through 5-7.
  - A few sentences describing how the average and standard deviation were calculated were added to page 29. ("The average and standard deviation for each water quality parameter were calculated using data from the triplicate coupons

(new coupons or artificially aged coupons) for each roofing material. For instance, the three new metal roofing coupons yielded the following pH values: 7.1, 6.4, and 6.6. The average ($\frac{7.1+6.4+6.6}{3} = 6.7$) and standard deviation of the triplicate coupons $\sqrt{\frac{(7.1-6.7)^2+(6.4-6.7)^2+(6.6-6.7)^2}{2}}$ are summarized in Figure 5-2.")

- Because Texas does not currently regulate water quality of harvested rainwater for potable use, please add a statement - somewhere prominently – that use of USEPA Maximum Contaminant Levels in the report is made for reference purposes only and is not meant to imply that the standards have to be met or are regulated by the Texas Commission on Environmental Quality.
    - This is an excellent point that we have added to the report in the Executive Summary, Introduction, Results (Task 5. Additional Sampling of Pilot- and Full-scale Roofs), and Conclusions and Recommendations sections. (For example, "The quality of harvested rainwater is not regulated by the Texas Commission on Environmental Quality, and, thus, the USEPA drinking water standards do not have to be met by rainwater harvested for potable use. However, to best protect the public health, we recommend the use of a first-flush diverter and additional treatment prior to potable use of harvested rainwater.")

- Page 2, section 2, second paragraph, line 4. Please describe Kynar® and how it is different from or similar to Galvalume®.
    - This has been added to Section 3. "Kynar® is a polyvinylidene fluoride (PVDF) resin-based coating that is often applied to Galvalume® or to galvanized steel; it is used as a roof coating for a variety of reasons including its resistance to corrosion, impact, abrasion, UV light, and particle accumulation."

- Page 5, first paragraph, line 10. Please add a period after "ambient rain".
    - This has been done.

- Page 5, first paragraph. As is done in the last paragraph on page 7 relating to the increase in TSS, please consider including possible explanations for the increases in pH. If this is an important issue, please discuss in the "Conclusions and Recommendations" section of the report.
    - Many other reports in the literature have shown an increase in pH after rainwater contacts a roofing surface. We have added information on this to the text (p. 5) and also mentioned it in the Conclusions and Recommendations section. "Chemical reactions between the rainwater and roofing material components (e.g., limestone in the asphalt fiberglass shingles or concrete) can lead to an increase in harvested rainwater pH, allowing the rainwater harvested after the first-flush to meet the USEPA (2009) secondary drinking water standard for pH (6.5-8.5). However, some roofing materials (e.g., Galvalume®, concrete tile, green, and Kynar®-coated Galvalume®) produced rainwater after the first-flush with pH values that were outside of the range specified by the standard. In those cases, the user might consider treatment for pH (e.g., using a concrete-lined storage tank,

adding limestone to the storage tank, adjusting the pH by chemical addition)."

- Page 5, second paragraph. Please consider adding a discussion on the importance and implication of high conductivity in harvested rainwater.
    - Additional information has been added (p. 6). "Conductivity is a measure of the ability of a solution to conduct electricity; a solution with a higher concentration of charged constituents (e.g., Na^+, Cl^-) will have a higher conductivity. A conductivity standard is not specified by the USEPA for drinking water, but conductivity is correlated to total dissolved solids (TDS), which is regulated by a USEPA secondary standard (500 mg/L). Waters with high concentrations of TDS can have a disagreeable taste or color… Using a correlation from the literature (Singh and Kalra, 1975), the estimated TDS of rainwater harvested after the first-flush for all of the roofs in this study met the secondary standard for TDS."

- Page 6, last paragraph. Please define "nephelometric turbidity units".
    - Nephelometric turbidity units are the standard unit of measure for turbidity, as listed on page 7.

- Page 8, last paragraph, line 11. Please change "maximum contaminant limit" to "maximum contaminant level".
    - This has been changed (p. 9).

- Page 8, last paragraph. Please consider explaining the possible sources of nitrates detected in the water samples.
    - The nitrate concentrations following the first-flush were typically similar to those present in ambient rain. The investigation of the sources of nitrate in ambient rain is outside the scope of the current report.

- Page 10, end of first paragraph. Please consider including a short explanation or possible reasons for the observation that "the capacity for and kinetics of DOC leaching from a shingle roof change as the roof ages."
    - Possible reasons have been added to the text (p. 12). "Berdahl et al. (2008) discuss the changes in roofing materials due to photodegradation via UV light from the sun, elevated temperature, moisture, and microbial growth; thus, since roofing materials change with age, it is possible that the amount and rate of DOC leaching from a shingle roof also change with age."

- Page 10, last paragraph. Please consider explaining how chlorination produces disinfection by-products, the nature of these by-products, and how they can be detrimental to human health.
    - A paragraph on these topics has been added (p.11)

- Page 13, second full paragraph, second line. Please consider rewriting the sentence to read "…As and Pb concentrations (Tables 3-22 and 3-32, respectively) as compared to other roofing materials."
    - This has been changed as suggested (p. 14).

- Page 13, paragraph 2. Is there any chemical data available from the manufacturer of the composition shingle roof that might explain the presence of the elevated levels of copper detected in the harvested rainwater samples?
  - "Asphalt fiberglass shingles can be a source of copper in harvested rainwater because copper is often added to them (including the GAF-Elk shingles used in this study) to prevent the growth of algae and moss." This information has been added (p. 15).

- Pages 19 through 22. Please consider simplifying the methodology description to better suit the average reader of this report. The detailed methodology can be placed in an appendix.
  - As suggested, the detailed methodology for T-RFLP and 16S rRNA gene sequencing has been moved to the appendix.

- Page 23, first full paragraph, second line. Please consider explaining or describing gram-positive bacteria.
  - The following was added (p. 20) to describe why the presence of gram-positive versus gram-negative organisms is important. "The communities were examined for presence of gram-positive versus gram-negative organisms since gram-positive bacteria are often more difficult to disinfect."

- Page 29, second paragraph. If water was recirculated through the lab-scale system, would it not have caused an increase in the concentration of the contaminants that were analyzed? Please clarify.
  - Yes, our goal was to see if any of the contaminants of interest would be released from the roofing material to the water. We recirculated for 24 hours so that it would be easier to measure an increase in the contaminant concentration in the water phase. The following information was added (p. 29) to explain this. "The water dripped onto the coupon and was recirculated over the coupon for 24 hours to determine which contaminants would be released from the roofing materials."

- Page 32, last paragraph, line 5. Please consider adding an explanation on why the roofing material acts as a sink for nitrate.
  - We did not investigate the mechanism as part of this study, and it would be pure speculation to discuss the mechanism of nitrate uptake.

- Page 33, paragraph 2. Please consider adding an explanation on why the aging process causes a decrease in the release of certain elements. Is this just a matter of decreasing supply or could it be something else?
  - Possible reasons have been added to the text (p. 29). "Roofing materials are subject to changes over time due to photodegradation via UV light from the sun, elevated temperature, moisture, and microbial growth (Berdahl et al., 2008). Thus, since roofing materials change with age, it also is possible that the release of certain elements and compounds from the roofing material changes as the roof ages."

- Page 34, Conclusions and Recommendations, first bullet item, line 7. Because Texas does not currently regulate water quality of harvested rainwater, please replace the sentence "This indicates that harvested rainwater must be treated…" with a sentence that recommends the user to consider treating the rainwater before using it for potable purposes.
  - We have addressed this as suggested (p. 35).

- Page 35, paragraph 2. Please explain why Galvalume® roofs are observed to have lower bacteria values than the other roofs that were tested.
  - We added a potential reason for this to the Results section (p. 13). "The rainwater harvested from the Galvalume® roof often showed lower FC concentrations as compared to the other pilot-scale roofing materials (except the green roof). Since metals tend to have higher surface temperatures in sunlight as compared to higher emissivity materials (Bretz et al., 1998), these higher temperatures might have inactivated some of the FC on the Galvalume® roof."

- Page 36, Acknowledgements. Please acknowledge the U.S. Army Corps of Engineers for the funding ($50,000) that they provided for the study.
  - This has been added (p. 37).

- Page 37. Please remove the extra space between the references, Weon and others and Yaziz and others.
  - The extra spaces have been removed.