

Supporting Information for:
**Improved contaminant removal in vegetated stormwater biofilters amended
with biochar**

Bridget A. Ulrich,^a Megan Loehnert,^a and Christopher P. Higgins^{a,†}

^a Department of Civil and Environmental Engineering, Colorado School of Mines
1500 Illinois St, Golden, CO 80401

† To whom correspondence should be addressed.

E-mail: chiggins@mines.edu, Phone: (720)-984-2116, Fax: (303)-273-3413

This document consists of 12 pages, 1 method, 5 tables, and 3 figures.

Method S1. Additional details for analysis of TOrCs by LC-MS/MS.....	S2
Table S1. Abbreviations and standard sources for TOrCs and isotope surrogates.....	S3
Table S2. Electrospray ionization parameters for LC-MS/MS analysis TOrCs and isotope surrogates.....	S4
Figure S1. Representative result from 24 hour water retention experiments.....	S5
Figure S2. Removal of additional TOrCs throughout the dosing experiments.....	S6
Table S3. P values for two-tailed student t tests on column influent and effluent TOrC concentrations.....	S7
Figure S3. Total organic carbon (TOC) levels throughout the dosing experiment.....	S9
Table S4. Additional influent and effluent concentrations from contaminant removal survey tests.....	S10
Table S5. P values for the two-tailed student t test for the final contaminant removal survey test.....	S11

Method S1. Trace organic contaminants (TOrcs) were analyzed by liquid chromatography tandem mass spectrometry (LC-MS/MS). TOrcs were separated on a 50 x 3.0 mm Luna C18 column with a 3 μ m particle size (Phenomenex), using a gradient elution method with an overall flow rate of 0.4 mL/min and methanol as the organic solvent (adapted from a previously described method).¹ TOrcs were analyzed by electrospray ionization (ESI) in positive or negative mode using a 3200 QTrap MS/MS (AB Sciex). The ESI positive eluent contained 2 mM ammonium formate and 0.1 vol% formic acid, and the ESI negative eluent contained 4 mM ammonium acetate. Isotope dilution was carried out to account for matrix effects and losses during sample preparation. Immediately prior to LC-MS/MS analysis, samples were diluted with isotope surrogate in 2 mL microcentrifuge tubes (1800 μ L sample diluted with 200 μ L of μ g/L isotope surrogates in methanol) and centrifuged at 13,000 RPM for 10 min before being transferred to autosampler vials. A subset of un-centrifuged samples was also analyzed during each run, confirming that particle-associated TOrcs were not being removed by centrifugation. Standards for native compounds and isotope surrogates are listed in Table S1, and electrospray ionization parameters are listed in Table S2.

Table S1. Abbreviations and standard sources for TOrCs and their isotope surrogates.

Compound	Abbreviation	Native standard source, purity	Surrogate, purity	Surrogate source
Atrazine	ATZ	Fluka, Pestanal®	Atrazine-d ₅ , 99% D	CDN Isotopes
Desethylatrazine	DEA	Fluka, Pestanal®		
Deisopropylatrazine	DIA	Fluka, Pestanal®		
Simazine	SIM			
5-Methyl-benzotriazole	Me-BTA	Sigma-Aldrich, analytical standard	Benzotriazole-d ₄ , 99% D	CDN Isotopes
Benzotriazole	BTA	Alfa Aesar, 99%		
Diuron	-	Fluka, analytical standard	Diuron-d ₆ , 99% D	CDN Isotopes
1-(3,4-dichlorophenyl)-3-methylurea	DCPMU	Fluka, analytical standard		
Fipronil	-	Fluka, Pestanal®	Fipronil des F3, >99%	EQ Laboratories, Inc.
Fipronil sulfone	FSO	Fluka, Pestanal®		
Fipronil sulfide	FSI	Fluka, Pestanal®		
Fipronil desulfinyl	-	Fluka, Pestanal®		
Oryzalin	-	Chem Service, 99%		
2,4-Dichlorophenoxyacetic acid	2,4-D	Fluka, Pestanal®	2,4-D-d ₃ , 98% D	CDN Isotopes
Prometon	-	Fluka, Pestanal®	Prometon-d ₃ , 99% D	CDN Isotopes
tris(3-chloro-2-propyl)phosphate	TCP	Fluka, analytical standard	TCEP-d ₁₂ , 97% D Sigma Aldrich	
tris(3-chloro-ethyl)phosphate	TCEP	Fluka, analytical standard		

Table S2. Electrospray ionization parameters for all analytes. For each parent ion (Q1), the transition for first product ion (Q3) listed was used for quantitation, and the transition for the second product ion was used for confirmation when possible.

Compound	Ionization mode	Q1 [m/z]	Q3 [m/z]	DP [V]	EP [V]	CEP [V]	CE [V]	CXP [V]
Atrazine	+	216.089	174.2, 103.9	41	3.5	12	23	4
Atrazine-d ₇	+	221.107	179.2	41	5.5	12	29	4
Desethylatrazine	+	188.125	146.2, 104.1	36	4	10	23	4
Deisopropylatrazine	+	174.109	146.2, 132.3	41	4.5	12	27	4
5-Methyl-benzotriazole	+	134.097	77.1, 79.1	36	9.5	12	33	4
Benzotriazole	+	120.08	65.1	46	11.5	12	29	4
Benzotriazole-d ₄	+	124.098	69.1	46	12	10	31	4
Diuron	+	233.081	72.0, 74.2	41	6.5	14	35	4
Diuron-d ₆	+	239.09	78.0	36	4.5	14	37	4
DCPMU	+	221.023	164.2, 127.0	41	3.5	12	19	4
Prometon	+	226.167	142.2, 184.3	51	3	12	33	4
Prometon-d ₃	+	229.279	145.1	46	3	18	31	4
TCCP	+	329.036	99.1	31	7	12	35	4
TCEP	+	284.966	223.1	36	5.5	18	17	4
TCEP-d ₁₂	+	297.03	232.2	36	8	16	19	4
Simazine	+	202.54	132.0, 124.0	36	5	12	25	4
2,4-D	-	218.927	160.6, 124.9	-15	-4.5	-16	-18	0
2,4-D-d ₃	-	223.938	166	-20	-4.5	-12	-20	0
Fipronil	-	434.98	330, 249.9	-40	-5	-18	-22	-4
Fipronil-desF3	-	380.922	330	-20	-5	-18	-20	-4
Fipronil sulfone	-	450.941	415, 282	-30	-4.5	-18	-20	-4
Fipronil sulfide	-	418.896	261.8, 382.9	-25	-4.5	-14	-44	-4
Oryzalin	-	345.087	281, 77.9	-50	-4.5	-20	-26	-4

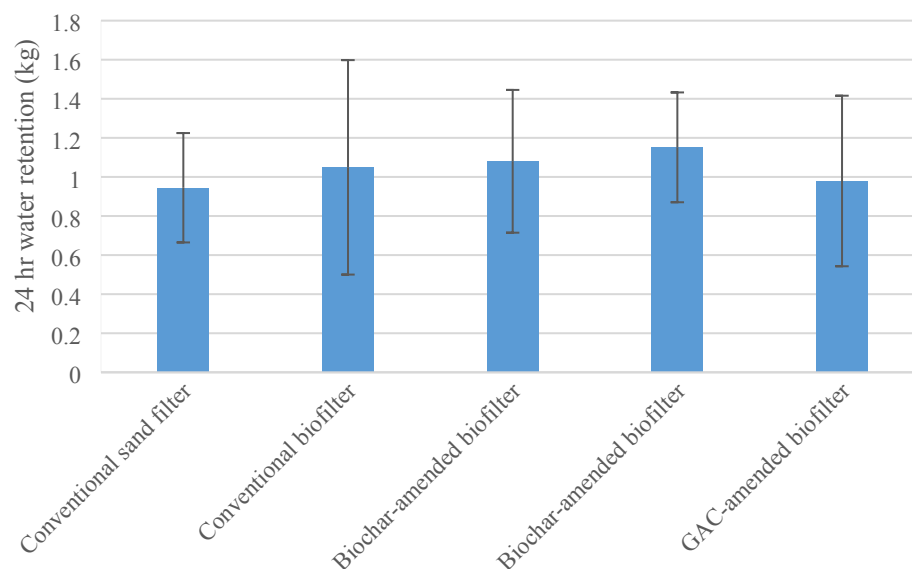


Figure S1. Representative result from 24 hour water retention experiments (water retention determined gravimetrically as the difference between the initial column dry weight and weight following 24 hours of draining from saturation). Error bars represent 95% confidence intervals from a two-tailed student t test. Though some variation was observed among conditions, differences did not appear to be statistically significant.

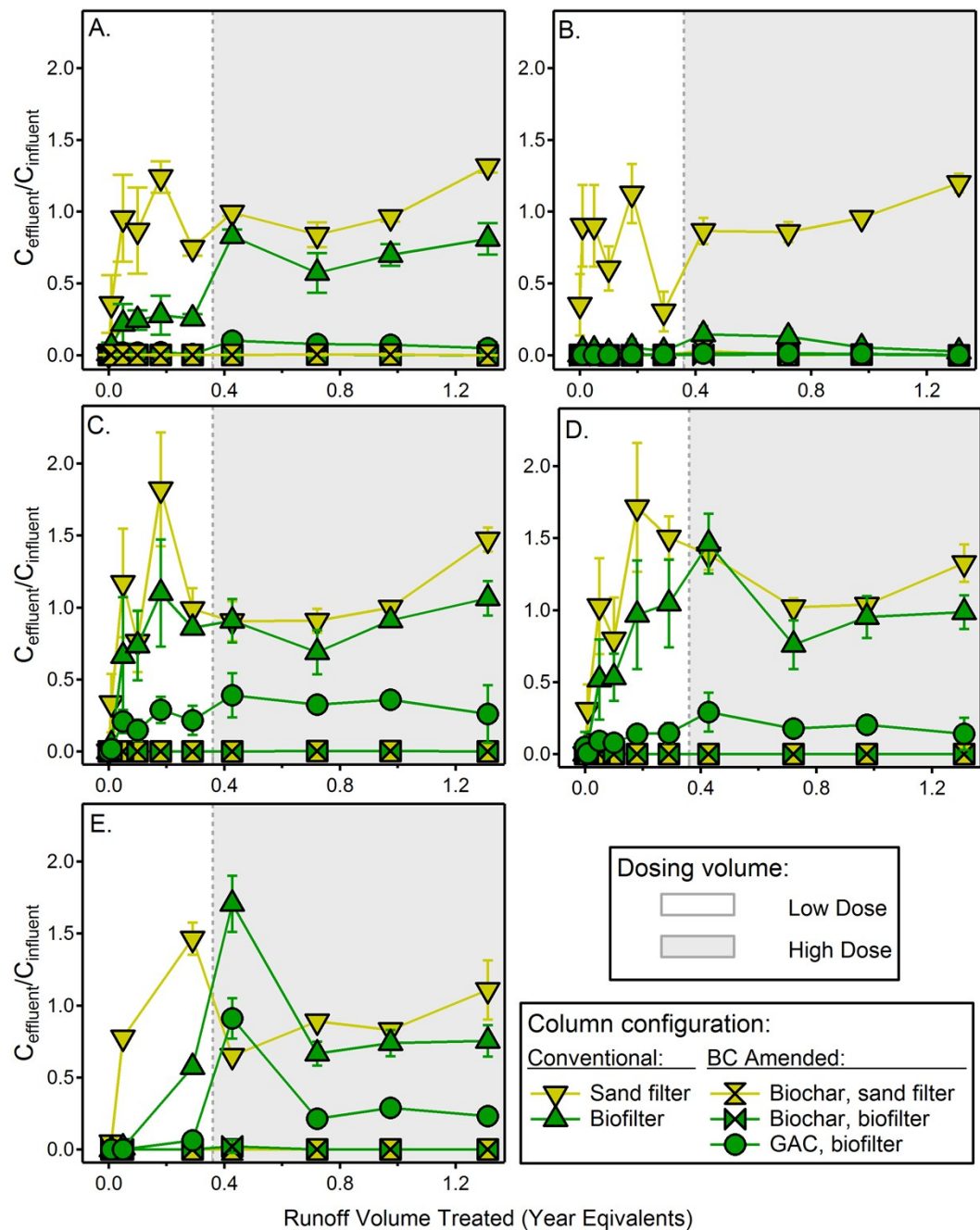


Figure S2. Removal (influent-normalized effluent concentration) of (A) benzotriazole, (B) diuron, (C) prometon, (D) simazine, and (E) fipronil throughout column dosing experiments. Error bars represent 95% confidence intervals from a two-tailed student t test.

Table S3. P values for two-tailed student t tests on column influent and effluent TOxC concentrations during the final high volume dose (A = unamended sand filters, B = unamended biofilters, C = biochar-amended sand filters, D = biochar-amended biofilters, E = GAC-amended biofilters). Cases where differences were statistically significant ($p < 0.05$) are indicated in bold (NA indicates cases where concentrations were below the 0.01 $\mu\text{g/L}$ detection limit).

Methyl-Benzotriazole		Influent	A	B	C	D	E
Influent		-	6.40E-03	3.66E-03	1.41E-04	1.52E-04	1.49E-04
A		6.40E-03	-	5.82E-04	5.53E-05	5.37E-05	6.17E-05
B		3.66E-03	5.82E-04	-	8.33E-04	8.25E-04	9.63E-04
C		1.41E-04	5.53E-05	8.33E-04	-	6.45E-01	1.15E-03
D		1.52E-04	5.37E-05	8.25E-04	6.45E-01	-	9.99E-03
E		1.49E-04	6.17E-05	9.63E-04	1.15E-03	9.99E-03	-

Atrazine		Influent	A	B	C	D	E
Influent		-	2.21E-02	9.26E-01	NA	NA	1.27E-03
A		2.21E-02	-	6.32E-02	NA	NA	2.31E-04
B		9.26E-01	6.32E-02	-	NA	NA	1.74E-03
C		NA	NA	NA	-	NA	NA
D		NA	NA	NA	NA	-	NA
E		1.27E-03	2.31E-04	1.74E-03	NA	NA	-

Benzotriazole		Influent	A	B	C	D	E
Influent		-	5.35E-03	6.68E-02	NA	NA	3.23E-05
A		5.35E-03	-	1.30E-03	NA	NA	2.04E-05
B		6.68E-02	1.30E-03	-	NA	NA	5.23E-04
C		NA	NA	NA	-	NA	NA
D		NA	NA	NA	NA	-	NA
E		3.23E-05	2.04E-05	5.23E-04	NA	NA	-

Diuron		Influent	A	B	C	D	E
Influent		-	6.77E-03	1.51E-05	NA	NA	8.57E-06
A		6.77E-03	-	2.70E-05	NA	NA	4.66E-05
B		1.51E-05	2.70E-05	-	NA	NA	6.47E-02
C		NA	NA	NA	-	NA	NA
D		NA	NA	NA	NA	-	NA
E		8.57E-06	4.66E-05	6.47E-02	NA	NA	-

Prometon		Influent	A	B	C	D	E
Influent		-	8.26E-04	3.92E-01	1.29E-06	1.29E-06	1.34E-03
A		8.26E-04	-	2.10E-02	5.16E-05	5.17E-05	1.05E-03
B		3.92E-01	2.10E-02	-	3.94E-04	3.91E-04	6.28E-05
C		1.29E-06	5.16E-05	3.94E-04	-	1.40E-01	6.27E-03
D		1.29E-06	5.17E-05	3.91E-04	1.40E-01	-	6.23E-03
E		1.34E-03	1.05E-03	6.28E-05	6.27E-03	6.23E-03	-

Table S3 Continued.

Simazine	Influent	A	B	C	D	E
Influent	-	7.03E-02	9.07E-01	1.10E-03	1.09E-03	2.90E-03
A	7.03E-02	-	4.39E-02	2.53E-04	2.54E-04	7.69E-04
B	9.07E-01	4.39E-02	-	4.52E-04	4.46E-04	1.30E-04
C	1.10E-03	2.53E-04	4.52E-04	-	7.84E-01	8.85E-03
D	1.09E-03	2.54E-04	4.46E-04	7.84E-01	-	8.60E-03
E	2.90E-03	7.69E-04	1.30E-04	8.85E-03	8.60E-03	-

TCEP	Influent	A	B	C	D	E
Influent	-	8.57E-02	6.07E-01	1.55E-03	1.51E-03	5.46E-03
A	8.57E-02	-	2.12E-01	1.06E-03	1.05E-03	3.07E-03
B	6.07E-01	2.12E-01	-	2.09E-03	2.14E-03	1.60E-03
C	1.55E-03	1.06E-03	2.09E-03	-	4.47E-01	1.03E-02
D	1.51E-03	1.05E-03	2.14E-03	4.47E-01	-	1.10E-02
E	5.46E-03	3.07E-03	1.60E-03	1.03E-02	1.10E-02	-

TCPP	Influent	A	B	C	D	E
Influent	-	3.68E-01	1.22E-02	5.99E-04	5.95E-04	1.07E-03
A	3.68E-01	-	3.30E-03	9.32E-04	9.16E-04	1.99E-03
B	1.22E-02	3.30E-03	-	1.86E-02	1.90E-02	5.58E-02
C	5.99E-04	9.32E-04	1.86E-02	-	5.70E-01	1.03E-02
D	5.95E-04	9.16E-04	1.90E-02	5.70E-01	-	1.12E-02
E	1.07E-03	1.99E-03	5.58E-02	1.03E-02	1.12E-02	-

2,4-D	Influent	A	B	C	D	E
Influent	-	3.07E-01	6.63E-02	4.10E-04	4.18E-04	2.10E-03
A	3.07E-01	-	5.95E-02	2.80E-05	2.72E-05	5.86E-05
B	6.63E-02	5.95E-02	-	4.80E-04	4.70E-04	8.74E-03
C	4.10E-04	2.80E-05	4.80E-04	-	3.91E-01	1.45E-03
D	4.18E-04	2.72E-05	4.70E-04	3.91E-01	-	1.45E-03
E	2.10E-03	5.86E-05	8.74E-03	1.45E-03	1.45E-03	-

Fipronil	Influent	A	B	C	D	E
Influent	-	3.28E-01	1.02E-02	4.72E-06	4.76E-06	1.16E-04
A	3.28E-01	-	4.80E-02	1.69E-03	1.70E-03	5.45E-03
B	1.02E-02	4.80E-02	-	4.53E-04	4.52E-04	2.34E-03
C	4.72E-06	1.69E-03	4.53E-04	-	2.91E-01	2.42E-03
D	4.76E-06	1.70E-03	4.52E-04	2.91E-01	-	2.35E-03
E	1.16E-04	5.45E-03	2.34E-03	2.42E-03	2.35E-03	-

Table S3 Continued.

Oryzalin Influent	Influent	A	B	C	D	E
	-	1.74E-01	4.60E-04	1.01E-07	1.01E-07	3.78E-05
A	1.74E-01	-	5.73E-03	3.02E-03	3.02E-03	5.00E-03
B	4.60E-04	5.73E-03	-	1.25E-03	1.25E-03	4.04E-03
C	1.01E-07	3.02E-03	1.25E-03	-	3.86E-01	8.81E-03
D	1.01E-07	3.02E-03	1.25E-03	3.86E-01	-	8.75E-03
E	3.78E-05	5.00E-03	4.04E-03	8.81E-03	8.75E-03	-

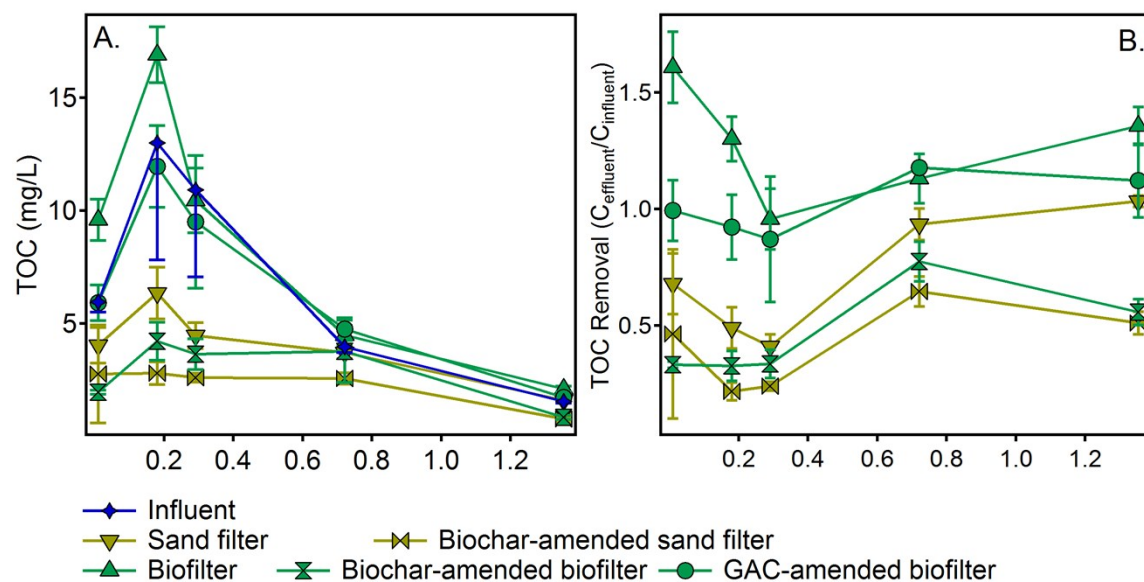


Figure S3. (A) Total organic carbon (TOC) influent and effluent concentrations, and (B) influent-normalized effluent compositions for columns throughout the dosing experiment.

Table S4. Concentrations of total organic carbon (TOC), total nitrogen (TN), *E. Coli*, and total coliforms (TC) during the final low volume dosing test and the final high volume dosing test (nitrate and metals were only analyzed during the final high volume dose). Confidence intervals (95%) from a two-tailed student t test are shown in parentheses.

Contaminant	Test 1 (Final low volume dose)				Test 2 (Final high volume dose)			
	TOC (mg/L)	TN (mg/L)	<i>E. Coli</i> (MPN/ 100 mL)	TC (MPN/ 100 mL)	TOC (mg/L)	TN (mg/L)	<i>E. Coli</i> (MPN/ 100 mL)	TC (MPN/ 100 mL)
Influent	13.13 (1.31)	1.43 (0.44)	4.5 (2.6)	84.4 (40.1)	1.55 (0.10)	1.50 (0.11)	14.0 (3.5)	516.2 (256.7)
Conventional sand filter effluent	4.46 (0.83)	0.22 (0.07)	2.3 (1.7)	48.3 (7.3)	1.60 (0.06)	1.55 (0.06)	6.0 (2.9)	188.0 (25.9)
Conventional biofilter effluent	9.75 (1.99)	0.36 (0.09)	0.6 (0.3)	24.4 (8.7)	2.10 (0.17)	2.07 (0.18)	3.2 (0.7)	112.7 (80.0)
Biochar-amended sand filter effluent	2.86 (0.28)	0.21 (0.04)	0.8 (0.9)	28.7 (7.3)	0.79 (0.10)	0.70 (0.11)	4.9 (2.5)	128.7 (43.6)
Biochar-amended biofilter effluent	3.14 (0.93)	> 0.01 -	0.9 (1.2)	32.8 (13.5)	0.86 (0.12)	0.78 (0.13)	1.9 (1.1)	49.1 (24.7)
GAC-amended biofilter effluent	8.68 (4.09)	0.21 (0.75)	0.4 (0.6)	26.4 (15.0)	1.74 (0.34)	1.69 (0.35)	2.0 (2.5)	70.7 (4.4)

Table S5. Probability values for the two-tailed student t test for concentrations of conventional contaminants in the column influent and effluents during the final high volume dosing experiment (A = unamended sand filters, B = unamended biofilters, C = biochar-amended sand filters, D = biochar-amended biofilters, E = GAC-amended biofilters). “NA” indicates cases where probability values are not available because concentrations were below the detection limit (indicated in Table 2). Cases where differences were statistically significant ($p < 0.05$) are indicated in bold.

TOC		Influent	A	B	C	D	E
Influent		-	1.65E-01	3.53E-03	1.50E-04	6.10E-05	2.06E-01
A		1.65E-01	-	4.80E-03	5.32E-04	5.84E-04	3.22E-01
B		3.53E-03	4.80E-03	-	3.44E-04	3.98E-04	1.12E-02
C		1.50E-04	5.32E-04	3.44E-04	-	3.91E-02	5.27E-03
D		6.10E-05	5.84E-04	3.98E-04	3.91E-02	-	5.61E-03
E		2.06E-01	3.22E-01	1.12E-02	5.27E-03	5.61E-03	-

TN		Influent	A	B	C	D	E
Influent		-	1.63E-01	3.54E-03	1.50E-04	6.05E-05	2.05E-01
A		1.63E-01	-	4.76E-03	5.27E-04	5.76E-04	3.20E-01
B		3.54E-03	4.76E-03	-	3.44E-04	3.98E-04	1.12E-02
C		1.50E-04	5.27E-04	3.44E-04	-	3.90E-02	5.26E-03
D		6.05E-05	5.76E-04	3.98E-04	3.90E-02	-	5.60E-03
E		2.05E-01	3.20E-01	1.12E-02	5.26E-03	5.60E-03	-

Nitrate		Influent	A	B	C	D	E
Influent		-	4.33E-01	7.73E-01	1.98E-01	NA	6.96E-02
A		4.33E-01	-	3.47E-01	3.19E-02	NA	9.37E-03
B		7.73E-01	3.47E-01	-	8.76E-02	NA	1.92E-02
C		1.98E-01	3.19E-02	8.76E-02	-	NA	8.85E-03
D		NA	NA	NA	NA	-	NA
E		6.96E-02	9.37E-03	1.92E-02	8.85E-03	NA	-

TDP		Influent	A	B	C	D	E
Influent		-	NA	1.17E-02	NA	4.26E-01	4.67E-03
A		NA	-	NA	NA	NA	NA
B		1.17E-02	NA	-	NA	7.05E-03	8.63E-01
C		NA	NA	NA	-	NA	NA
D		4.26E-01	NA	7.05E-03	NA	-	1.65E-02
E		4.67E-03	NA	8.63E-01	NA	1.65E-02	-

Cu		Influent	A	B	C	D	E
Influent		-	1.03E-03	3.68E-03	NA	NA	1.20E-03
A		1.03E-03	-	5.71E-02	NA	NA	1.78E-01
B		3.68E-03	5.67E-02	-	NA	NA	3.93E-01
C		NA	NA	NA	-	NA	NA
D		NA	NA	NA	NA	-	NA

E	1.20E-03	1.78E-01	3.93E-01	NA	NA	-
---	-----------------	----------	----------	----	----	---

Table S5 continued.

Zn	Influent	A	B	C	D	E
Influent	-	6.43E-05	7.88E-05	4.77E-05	2.22E-04	2.47E-05
A	6.43E-05	-	1.11E-02	1.04E-01	1.84E-02	1.38E-02
B	7.88E-05	1.11E-02	-	4.78E-02	4.51E-01	1.76E-01
C	4.77E-05	1.04E-01	4.78E-02	-	1.08E-01	1.46E-02
D	2.22E-04	1.84E-02	4.51E-01	1.08E-01	-	6.63E-01
E	2.47E-05	1.38E-02	1.76E-01	1.46E-02	6.63E-01	-

E. Coli	Influent	A	B	C	D	E
Influent	-	2.47E-02	2.70E-03	3.24E-03	2.64E-03	6.01E-04
A	2.47E-02	-	1.05E-01	5.52E-01	9.76E-03	1.16E-01
B	2.70E-03	1.05E-01	-	1.85E-01	9.91E-02	2.82E-01
C	3.24E-03	5.52E-01	1.85E-01	-	5.02E-02	9.54E-03
D	2.64E-03	9.76E-03	9.91E-02	5.02E-02	-	9.18E-01
E	6.01E-04	1.16E-01	2.82E-01	9.54E-03	9.18E-01	-

TC	Influent	A	B	C	D	E
Influent	-	2.02E-02	1.58E-02	1.04E-02	7.33E-03	9.16E-03
A	2.02E-02	-	4.47E-02	3.33E-02	2.50E-06	9.52E-04
B	1.58E-02	4.47E-02	-	7.03E-01	6.91E-02	2.43E-01
C	1.04E-02	3.33E-02	7.03E-01	-	1.49E-02	3.00E-02
D	7.33E-03	2.50E-06	6.91E-02	1.49E-02	-	8.02E-02
E	9.16E-03	9.52E-04	2.43E-01	3.00E-02	8.02E-02	-

References:

- (1) Ulrich, B. A.; Im, E. A.; Werner, D.; Higgins, C. P. Biochar and activated carbon for enhanced trace organic contaminant retention in stormwater infiltration systems. *Environ. Sci. Technol.* **2015**, *49*, 6222–6230.