

Supplementary Information

Development of a Sensitive Direct Injection LC-MS/MS Method for the Detection of Glyphosate and Aminomethylphosphonic Acid (AMPA) in Hard Waters

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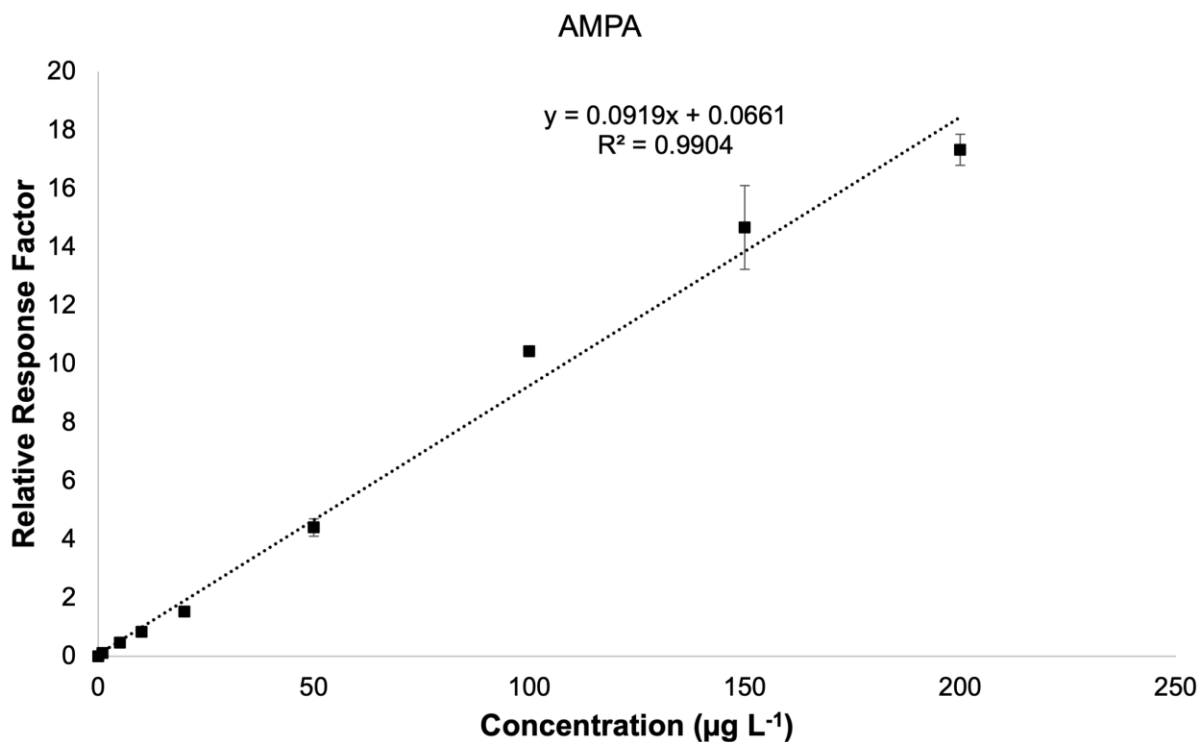
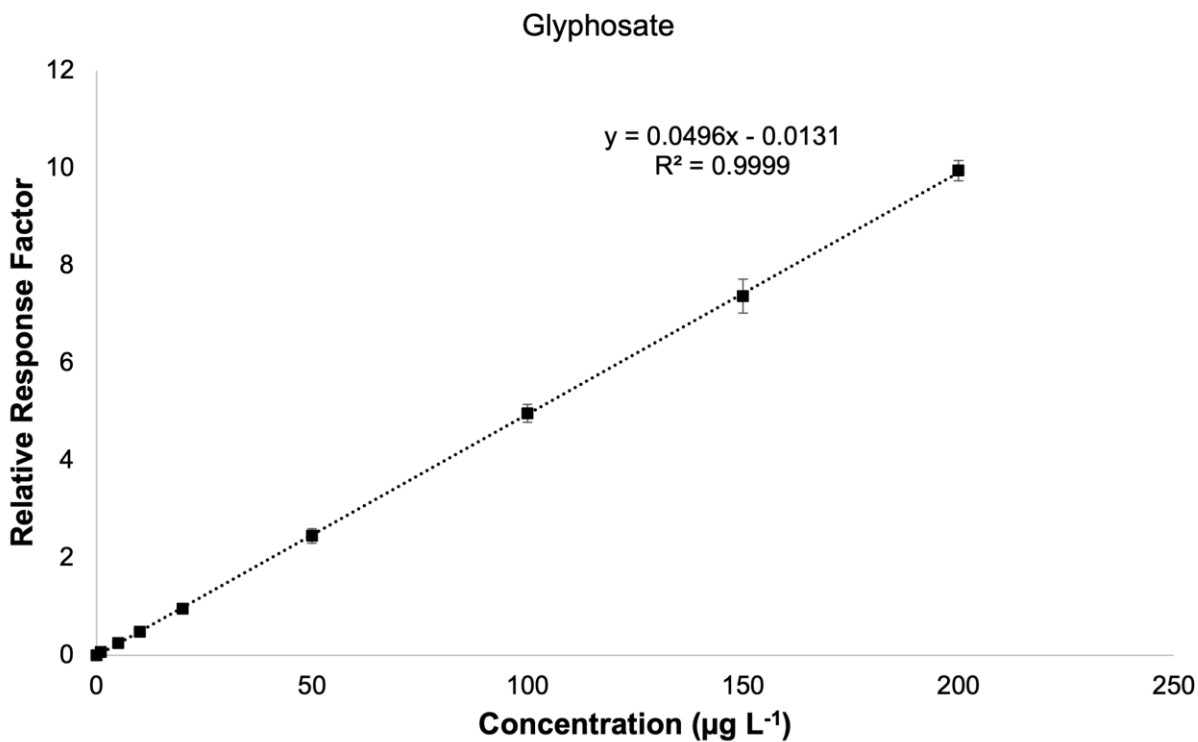


Fig. S1 Calibration curves generated in triplicate for both glyphosate (top) and AMPA (bottom) plotted relative response factor versus analyte concentration ($\mu\text{g L}^{-1}$). The R^2 values of 0.9999 for glyphosate and 0.9904 for AMPA demonstrate acceptable fit of data to a linear model, suggesting that the calibration responses are linear.

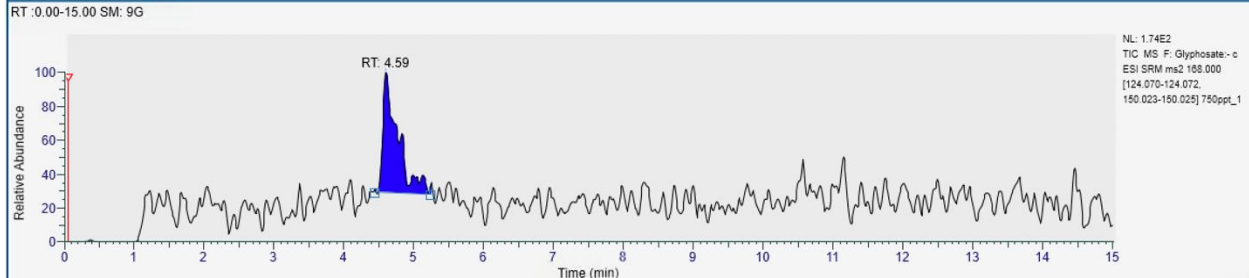
Table S1 Average predicted concentration and accuracy and precision for standards determined via a triplicate calibration curve analysis.

Glyphosate				AMPA			
Expected Conc. ($\mu\text{g L}^{-1}$)	Measured average Conc. (\pm SD) ($\mu\text{g L}^{-1}$)	Accuracy	RSD	Expected Conc. ($\mu\text{g L}^{-1}$)	Measured average Conc. (\pm SD) ($\mu\text{g L}^{-1}$)	Accuracy	RSD
0	N/F ^a	N/A ^a	N/A	0	N/F ^a	N/A ^a	20%
1	1.08 (\pm 0.09) ^b	108%	8%	1	1.1 (\pm 0.2)	119%	9%
5	5.0 (\pm 0.4)	100%	8%	5	5.1 (\pm 0.5)	102%	6%
10	9.6 (\pm 0.2)	96.2%	2%	10	9.1 (\pm 0.6)	91.2%	7%
20	19.1 (\pm 0.9)	95.7%	5%	20	17 (\pm 1)	82.8%	7%
50	50 (\pm 3)	99.0%	6%	50	48 (\pm 3)	94.9%	1%
100	101 (\pm 4)	101%	4%	100	112 (\pm 1)	112%	10%
150	149 (\pm 7)	100%	5%	150	160 (\pm 20)	105%	3%
200	202 (\pm 4)	101%	2%	200	186 (\pm 6)	93.2%	20%

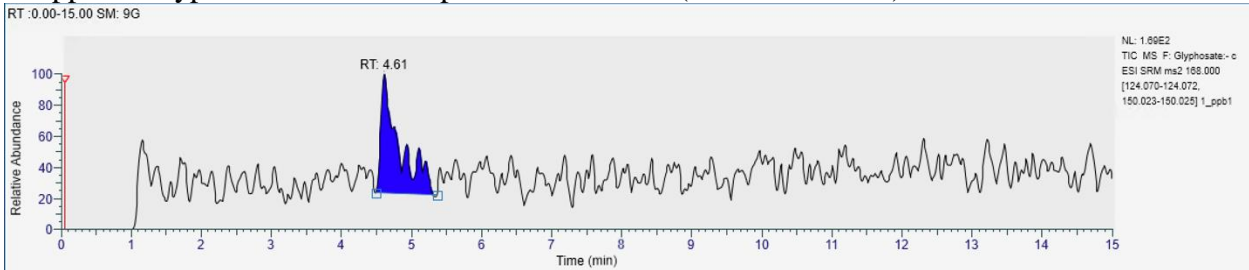
^aNo analyte peaks were observed at the 0 $\mu\text{g L}^{-1}$ standard for either analyte, which is represented by a N/F (for not found) and the accuracy is thus not applicable (N/A).

Fig. S2 Chromatograms of 0.75 ppb glyphosate, 1.0 ppb glyphosate, 1.0 ppb AMPA, and 2.5 ppb AMPA with respective areas under the curve and accuracy calculations.

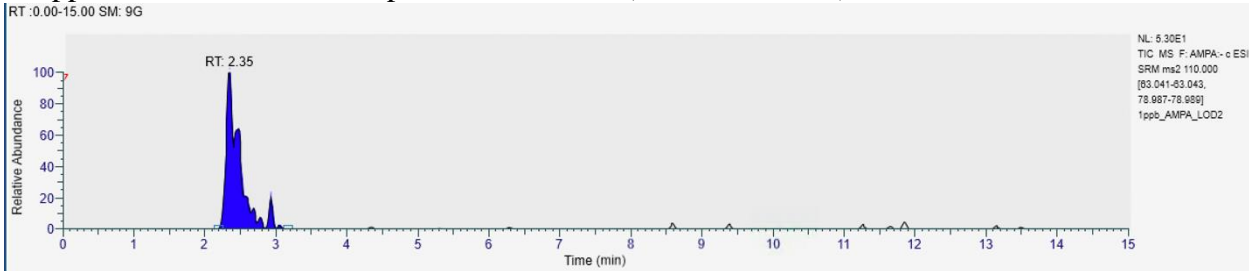
0.75 ppb of Glyphosate in EDTA Spiked Hard Water (Area = 1.795E3)



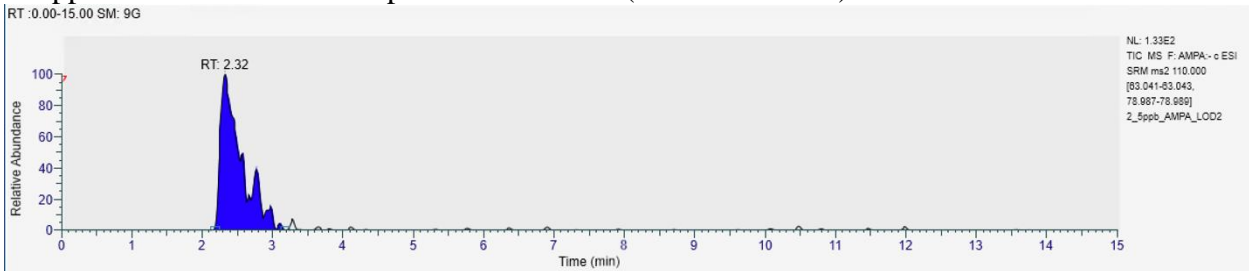
1.0 ppb of Glyphosate in EDTA Spiked Hard Water (Area = 2.309E3)



1.0 ppb of AMPA in EDTA Spiked Hard Water (Area = 8.888E2)



2.5 ppb of AMPA in EDTA Spiked Hard Water (Area = 2.754E3)



Glyphosate			AMPA		
Conc. ($\mu\text{g L}^{-1}$)	Average Conc. ($\mu\text{g L}^{-1}$)	Accuracy	Conc. ($\mu\text{g L}^{-1}$)	Average Conc. ($\mu\text{g L}^{-1}$)	Accuracy
0.75	0.71 (± 0.09)	94.9 ₈ %	1.0	1.12 (± 0.09)	111%
1.0	1.07 (± 0.06)	107%	2.5	2.51 (± 0.29)	100%

Table S2 Water Hardness of Haw River Water Samples.

Sample	Mg Hardness (mg L⁻¹ CaCO₃)	Ca Hardness (mg L⁻¹ CaCO₃)	Total Hardness (mg L⁻¹ CaCO₃)
EWWTTP A	7.40	10.7	18.1
EWWTTP B	6.90	9.20	16.1
SWWTTP	7.70	11.3	19.0
Saxapahaw	8.20	1.1.0	19.2
CCA	5.00	6.60	11.6
CCB	4.50	7.60	12.1
CCC	4.10	5.50	9.6
CCD	5.70	7.20	12.9
Confluence	5.90	9.80	15.7
Bynum	4.80	10.0	14.8
Haw 64	4.80	10.8	15.6
Jordan 64	4.00	8.30	12.3
JFP	4.80	8.00	12.8

*LOD of the hardness method is 0.05 mg/L of CaCO₃

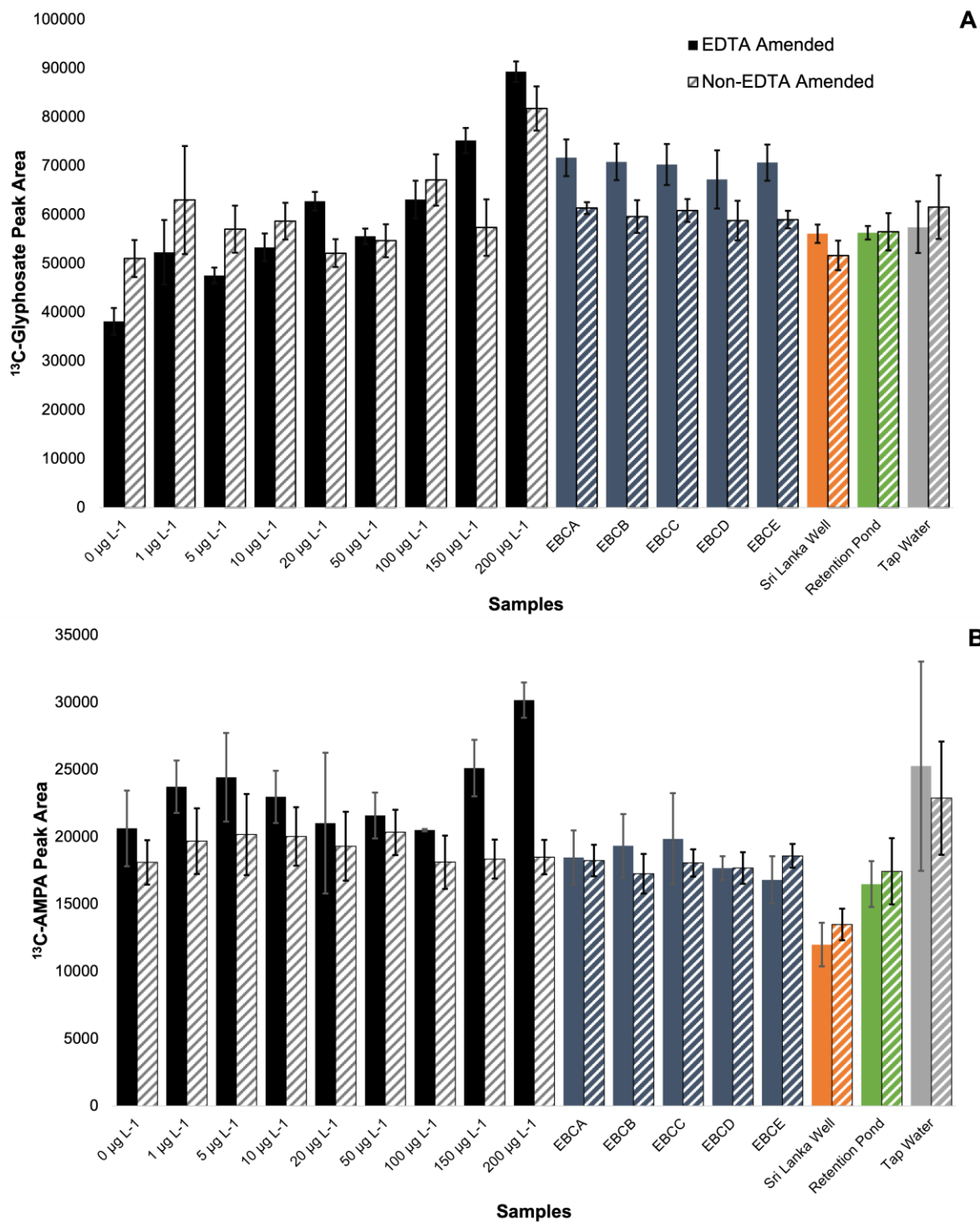


Fig. S3 Study of matrix effects on ISTD response of ¹³C-glyphosate (**A**) and ¹³C-AMPA (**B**) in calibration standards (black), Ellerbe Creek Samples (blues), a Sri Lankan Well Water Sample (orange), the Duke Retention Pond (green), and tap water (gray) with EDTA amended (solid color) and non-EDTA amended (cross-hatched). The responses of the glyphosate ISTD and AMPA ISTD were consistent across matrix types and standards, suggesting little matrix effects. The average response areas of both standards were frequently higher for EDTA spiked samples, than non-EDTA spiked samples, suggesting EDTA improved analytes response in samples. Additionally, isotope-dilution mass spectrometry was performed to account for any matrix effects which may have been encountered during analysis.

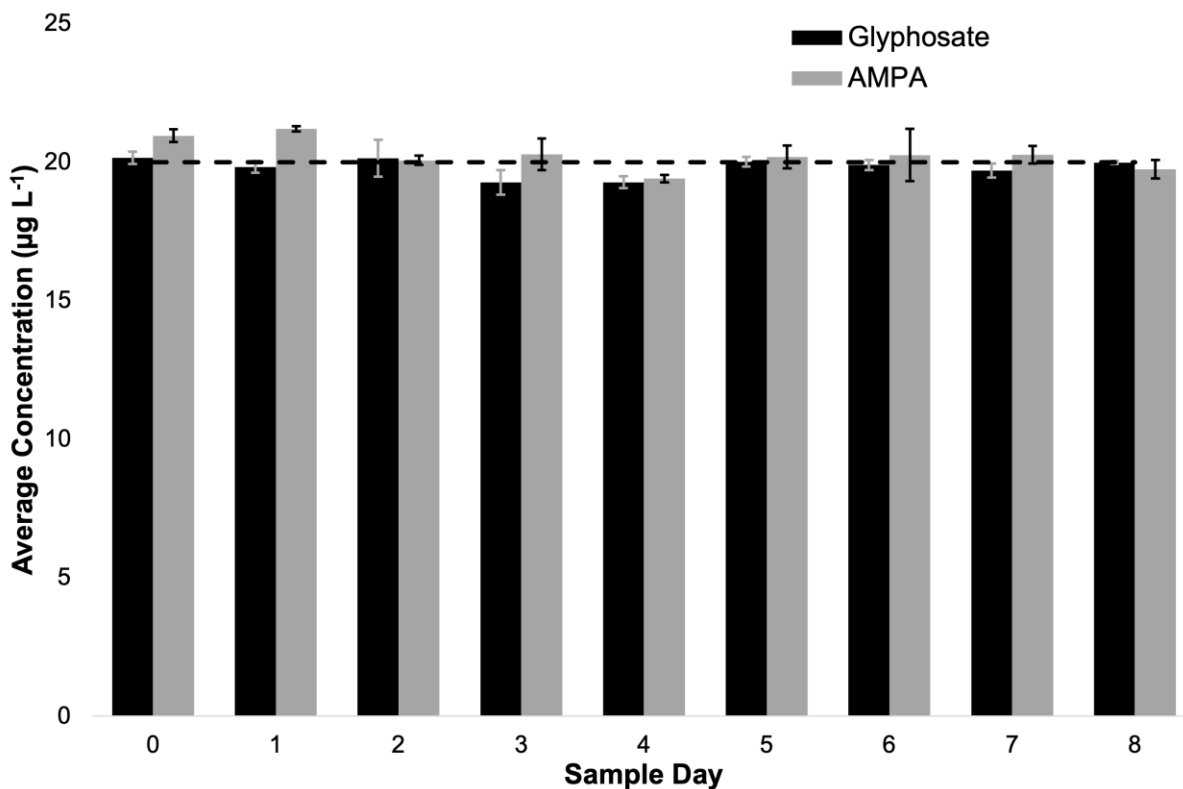


Fig. S4 Freeze-thaw stability tests analyzing the average concentration of glyphosate (black) and AMPA (gray) over multiple freeze/thaw events. The expected concentration was 20 µg L⁻¹ and is represented by the dashed lined, error bars represent standard deviation of 3 replicate measurements. The average concentration over 9 freeze/thaw cycles of both analytes was consistent, displaying no apparent decrease in measured concentration or impact of freezing and thawing on glyphosate and AMPA stability.

Table S3 Confidence Intervals for Haw River Glyphosate and AMPA Analyses

	Glyphosate			AMPA			
	Site	Avg. ($\mu\text{g L}^{-1}$)	Std Dev.	95% Confidence Interval ($\mu\text{g L}^{-1}$)	Avg. ($\mu\text{g L}^{-1}$)	Std Dev.	95% Confidence Interval ($\mu\text{g L}^{-1}$)
Non-EDTA Amended	Bynum	20.0	0.6	18.6 – 21.5	33.8	0.8	31.9 – 35.7
	CCA	19.3	0.2	18.8 – 19.7	29	2	23.6 – 35.1
	CCB	19	2	15.3 – 22.7	28	2	24.1 – 32.0
	CCC	21.8	0.8	19.8 – 23.9	33.8	0.4	32.7 – 34.8
	CCD	15	1	12.5 – 17.3	22	1	18.9 – 24.1
	Confluence	19.2	0.2	18.7 – 19.7	35	1	32.5 – 37.7
	EWWTTPA	18.7	0.4	17.6 – 19.7	15	2	10.8 – 18.8
	EWWTTPB	19.8	0.1	19.5 – 20.1	15.1	0.7	13.4 – 16.7
	Haw 64	19.6	0.3	18.8 – 20.4	32.4	0.5	31.1 – 33.6
	Jordan 64	19.0	0.1	18.7 – 19.4	32.6	0.4	31.7 – 33/5
	JFP	19.2	0.5	17.9 – 20.5	25	2	21.4 – 28.8
	Saxapahaw	19	1	15.6 – 22.5	32.2	0.4	31.1 – 33.3
SWWTP	19	1	16.4 – 22.1	36	1	32.6 – 38.7	
EDTA Amended	Glyphosate			AMPA			
	Site	Avg. ($\mu\text{g L}^{-1}$)	Std Dev.	95% Confidence Interval ($\mu\text{g L}^{-1}$)	Avg. ($\mu\text{g L}^{-1}$)	Std Dev.	95% Confidence Interval ($\mu\text{g L}^{-1}$)
	Bynum	21	1	17.9 – 23.6	20.3	0.9	18.0 – 22.5
	CCA	21	1	18.5 – 24.0	21.8	0.8	19.9 – 23.7
	CCB	22	1	19.7 – 24.9	21.7	0.3	21.0 – 22.4
	CCC	22	1	18.4 – 25.3	25	2	20.3 – 30.0
	CCD	22.5	0.6	21.1 – 23.9	26.1	0.7	24.3 – 28.0
	Confluence	22.4	0.4	21.3 – 23.5	24.3	0.4	23.3 – 25.3
	EWWTTPA	20.4	0.7	18.7 – 22.1	22	2	17.9 – 26.5
	EWWTTPB	20.3	0.8	18.3 – 22.3	20.4	1.0	17.9 – 23.0
	Haw 64	19.7	0.2	19.1 – 20.2	20.7	0.9	18.6 – 22.8
	Jordan 64	19.7	0.6	18.3 – 21.1	21	2	16.3 – 27.2
JFP	21.3	0.9	19.1 – 23.5	25	1	22.6 – 27.7	
Saxapahaw	20.4	0.7	18.5 – 22.2	22	1	19.2 – 24.4	
SWWTP	19.5	0.1	19.2 – 19.8	21.1	0.6	19.6 – 22.6	

Table S4 Water Hardness of Analyzed Water Samples.

Sample	Mg Hardness (mg L⁻¹ CaCO₃)	Ca Hardness (mg L⁻¹ CaCO₃)	Total Hardness (mg L⁻¹ CaCO₃)
Tap Water	2.62	<LOD	2.62
Retention Pond	9.00	34.00	43
Sri Lankan Well	124	39	163
Ellerbe Creek A	1.88	<LOD	1.88
Ellerbe Creek B	1.74	<LOD	1.74
Ellerbe Creek C	1.90	<LOD	1.90
Ellerbe Creek D	1.76	<LOD	1.76
Ellerbe Creek E	1.83	<LOD	1.83

*LOD of the hardness method is 0.05 mg/L of CaCO₃