Supporting Information

Exposure and Transport of Alkaloids and Phytoestrogens from Soybeans to Agricultural Soils and Streams in the Midwestern United States

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Supporting Information

Supporting Information (62 pages) includes; experimental methods, 12 tables and 20 figures.

The Contents

Tables

Figures

Experimental methods

S1. Chemicals and Reagents

Acetone (CAS: 67-64-1), ammonium formate (NH_4HCO_2) (CAS: 540-69-2), formic acid (FA) (CAS: 64-18-6), methanol (MeOH) (CAS: 67-56-1), analytical standard of coumestrol (CAS: 479-13-0), daidzein (CAS: 486-66-8), daidzin (CAS: 552-66-9), equol (CAS: 531-95-3), genistein (CAS: 446-72-0), genistin (CAS: 529-59-9), flazin (CAS: 100041-05-2), indole-3-acetic acid (CAS: 87-51-4), indole-3-carboxylic acid (CAS: 771-50-6), tyramine (CAS: 51-67-2), trigonelline (CAS: 535-83-1 (anhydrous)), metolachlor (CAS: 51218-45-2), atrazine (CAS: 1912-24-9), prochloraz (internal standard for surrogate recovery) (CAS: 67747-09-5, prochloraz-d4), and caffeine (CAS: 58-08-2), purchased from Sigma-Aldrich (Darmstadt, Germany). Senecionine (internal standard, for spiking experiments and method validation) (CAS: 130-01-8) purchased from Phytolab (Vestenbergsgreuth, Germany) and Sigma-Aldrich (Darmstadt, Germany). Oasis MCX and HLB 6 cc, 150 mg Sorbent, 30 µm particle size purchased from Waters (Milford, USA).

S2. Additional Sampling

To investigate the spatial resolution and effects of vegetation area on stream-water quality, a synoptic sampling was conducted during runoff conditions in upstream sampling points at three basins in Iowa (Clear Creek (n=5), West Branch Wapsinonoc Creek (n=2), and Old Mans Creek (n=5)) on December 29, 2019. For this synoptic effort, samples were collected at the U.S. Geological Survey (USGS) gaging station (most downstream sampling site) and two farther upstream sampling sites (upstream 1 and 2) for West Branch Wapsinonoc Creek, and five upstream sampling sites (upstream 1 - 5) for both Clear Creek and Old Mans Creek progressively upstream from the gaging station (Figure S2). All of the upstream sampling sites still have soybean production; however, the number soybean fields in the contributing drainage area and streams sizes decreased substantially. In addition, to determine potential temporal variations in stream chemistry during storm events, at least 5 samples were collected over a storm hydrograph at the three basins (Clear Creek (n=5), West Branch Wapsinonoc Creek (n=7), and Old Mans Creek (n=5)) in Iowa, during a storm event in November 2019; about 31 mm precipitation was recorded.[1] To better understand potential sources of phytotoxins to streams, two effluent samples were collected from a wastewater treatment plant

(WWTP) roughly 5.1 km upstream of the sampling site at the Muddy Creek reference basin (Figure S3). To demonstrate the potential of the side ditch to transport the phytotoxins and herbicides, four water samples were collected from a side ditch flowing through corn and soybean fields that discharged just above the sampling site at Old Mans Creek (Figure S4).

To determine and understand phytotoxin concentrations in soybean plant tissue and soil, samples of soybean plant and soils were collected from a soybean field adjacent to Clear Creek upstream from the Clear Creek gaging station (Figure S5). Plant tissue (n=8), surface soil (n=8), and near-surface (~0-10 cm) soil (n=8) samples were collected (in triplicate) from the same locations (approximately 60-100 m away from the field edge, Figure S1.E) on a monthly schedule. The whole plant samples, including roots were collected on October 18, 2019 (pre-harvest). However, after harvesting the soybean field, plant tissues residue remaining on the field were collected as a plant sample on November 19, 2019 (post-harvest 1); December 17, 2019 (post-harvest 2); and January 22, 2020 (post-harvest 3). Roots were included with plant tissue samples collected on October and November but were not able to be obtained in December and January due to frozen soil conditions.

S3. Sample Processing

S3.1 Stream water extraction

Stream water samples were processed using a previously described method.[2] In brief, water samples were filtered through a 0.7-µm nominal pore size glass-fiber filter (GF/F) [Whatman] to remove any suspended particles. A 1.0-L water sample was measured volumetrically. The pH of the water samples were adjusted to 3 using buffer solution of formic acid and ammonium formate solution for proper retention during solid phase extraction (SPE). Water samples prior to February 2020 were extracted using SPE cartridges of Oasis® MCX and HLB (6 cc, 500mg; Waters Corporation, Milford, Massachusetts) for alkaloids and phytoestrogen, respectively. Water samples (1 L) were extracted with MCX and HLB simultaneously, both cartridges connected in series, where MCX cartridge connected to the sample manifold and HLB cartridge connected to MCX cartridge using cartridge adapters. Samples were pumped at a flow rate of 10 mL/min. Samples were spiked with the surrogate compound prochloraz-d4 (from Sigma-Aldrich, Darmstadt, Germany) at a concentration of 100 ng/L. For alkaloid extraction, the Oasis® MCX cartridge was eluted with 5 mL of methanol and 10 mL of methanol–10% ammonia (3:1, v/v), respectively. For phytoestrogens, HLB cartridges were eluted with 5 mL of methanol and 10 mL of 50% methanol (1:1, v/v), respectively. The eluents of MCX and HLB cartridges were collected and combined in glass vials, then dried under nitrogen gas and stored in freezer at -20 °C prior to shipping. After February 2020, all water samples were extracted using only HLB cartridges, water samples were not loaded on two cartridges. Overall, the phytotoxins showed acceptable recovery (alkaloids: 96% using MCX cartridges and 90% using HLB cartridges; phytoestrogens 92% using HLB cartridges). The extracts were dried under a gentle stream of nitrogen in glass vials then shipped to the Department of Plant and Environmental Sciences, University of Copenhagen, Denmark, where extracts were reconstituted for analysis of the target compounds. Before analysis, the extracts were dissolved in 900 µL of methanol and spiked 100 µL with senecionine (100 ng/L) as an internal standard to account for potential matrix effects and instrument fluctuations.

S3.2 Plant and soil extraction

Plant tissues (2 out of 3 collected samples) were extracted using a previously described method. [3] Plant samples were freeze-dried overnight using a lyophilizer. Dry biomass content was determined. methanol (MeOH)/water solution (1.0 mL, 1:1 ratio), 100 µl of surrogate compound prochloraz-d4 (a concentration of 100 ng/L), and a single stainless steel homogenization bead (5 mm) were added to freeze-dried plant tissues in a microcentrifuge tube, and then placed on a mixer mill (Retsch) for 5 min at 30 Hz, sonicated 10 min, vortexed for 1 min, then centrifuged for 10 min at 10,000 rpm (4000 *g*). The supernatant was removed with a needle syringe and filtered through a 0.22-µm polytetrafluoroethylene (PTFE) filter (Fisher) into a glass vial. The extraction procedure was repeated sequentially two additional times by adding a volume of 1.0 mL of the MeOH:water solution for each subsequent extraction and repeating the extraction procedure (i.e., homogenization, sonication, vortex, centrifugation, and filtration). All three fractions were combined and filtered with a 0.22 µm PTFE filter (Fisher), and then passed through MCX SPE as described in section S3.1 (Stream water extraction). Compounds were eluted from the cartridges into glass vial and dried under nitrogen gas, and then stored at -20 °C prior to shipping. A spike-recovery test of the sequential extraction procedure yielded 98 ± 3% recovery of prochloraz-d4.

Soil samples (2 out of 3 collected samples) were extracted using a previously described method.[2] Soil samples (2.5 g fresh weight) were weighed into a 25 mL centrifuge tube, then spiked with 100 µL the surrogate compound prochloraz-d4 (at a concentration of 100 ng/L). MeOH (10 mL)was added to the tube, then the tube sonicated for 15 minutes and centrifuged for 10 min at 8000 rpm (2100 *g*). The supernatant was collected. This extraction was repeated. After that, 10 mL of MeOH:acetone (85: 15 v/v %) solvent was used for the third extraction. Finally, the three extracts were combined, centrifuged, and filtered with a 0.22 µm PTFE filter (Fisher), and then passed through MCX SPE as described in section S3.1 (Stream water extraction). Compounds were eluted from the cartridges into glass vial and dried under nitrogen gas, and then stored at -20 °C prior to shipping.

S4. Liquid Chromatography with Mass Spectrometry Analysis (LC-MS/MS)

The analysis was performed on a Waters ACQUITY Ultra Performance Liquid Chromatography (UPLC) I-Class System coupled with a Xevo TQD Triple Quadrupole Mass Spectrometer (MS/MS) (Milford, Massachusetts, USA). The separation was performed using a 50 mm × 2.1 mm I.D., 1.7 µm Waters Acquity UPLC HSS C18 Column at 35 °C. The mobile phase was comprosed of eluent A consisting of water and 0.1 % FA, and eluent B consisting of MeOH and 0.1 % FA. Gradient elution was used and programmed as follows: 90 % A from 0-4.0 min, 85 % A from 4.0 to 7.0 min, 80 % A from 7.0 to 8.0 min, 75 % A from 8.0 to 10.0 min, 50 % A from 10.0 to 15.0 min, 10 % A from 15.0 to 17.10 min, and 90 % A from 17.10 to 23.0 min. The flow rate was kept constant at 0.20 mL/min. The sample injection volume was 5 µL. MS/MS conditions were electrospray (ESI) ionization, positive mode, desolvation gas was set to 992 L/h at 497 °C, source temperature was set to 148 °C, and the cone gas flow was set to 20 L/h. The optimum capillary voltage was 3.5 kV. The cone voltage and collision energy were set at different values for each compound; the cone voltage ranged from 15-40 V and the collision energy ranged from 25-45 eV (Table S2). Data were collected in the multiple-reaction-monitoring (MRM) mode (Table S2); chromatograms of standards solution and samples of selected phytotoxins and herbicides are listed in Table S4. A 7-point surrogate-normalized external calibration curve was used to account for surrogate recovery and matrix effects during ionization. Curves were obtained by plotting measured analyte peak areas/internal standard peak area against corresponding analyte concentrations/internal standard concertation in the extracted matrix.

Linear regression was performed for each curve. Due to lack of analytical standards of ginsenine and soyalkaloid A for quantification indole-3-acetic acid and indole-3-carboxylic acid were used, respectively.

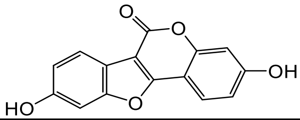
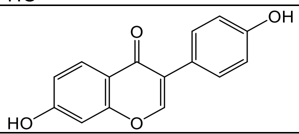
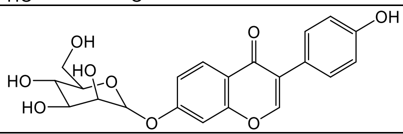
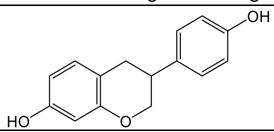
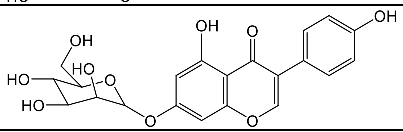
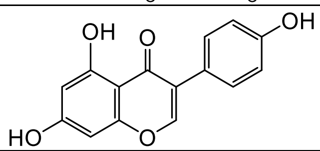
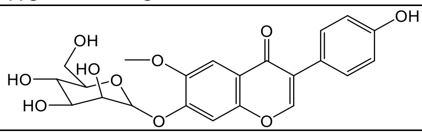
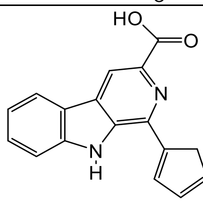
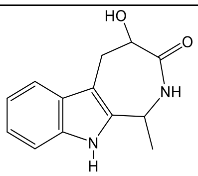
S5. Method Validation

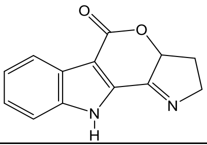
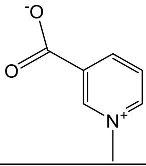
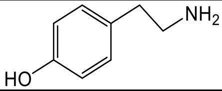
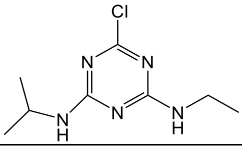
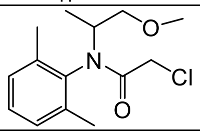
Field and laboratory blanks were used to determine recovery and matrix effects. Field blanks of plants (freeze-dried powder plant tissue of grass), soils (collected at the garden Water Plant of the University of Iowa) and water (using organic-free water (Thomas Scientific)) were collected; also laboratory water blanks consisted of deionized water. Alkaloids, phytoestrogens, and herbicides were not detected in any of the field blanks, or below the limits of detection (LOD). A new set of standard solutions was prepared to evaluate matrix effects, which were calculated from the ratio of the mean peak area of an analyte in post-extraction spiked samples to the mean peak area of the same analyte in standard solutions. Two concentration levels (25 and 100 µg/L) of phytotoxins were tested in triplicate. For recovery, freeze-dried powder of narrow-leaved or blue lupine (*Lupinus angustifolius* L.) plant, sandy soil from Vejle (N55°41'54.1, E9°25'34.8) – Denmark, and deionized water were used to evaluate the extraction efficiency. The recovery was calculated by comparing the response of post-spiked solution with a pre-spiked extract using equation 1; the non-spiked extract was regarded as background extract and subtracted in the calculations (Table S3). For this study, surrogate recovery of prochloraz-d4 ranged from 82 to 96%. Data were not corrected for recovery and matrix effects. For testing the recovery of alkaloids, phytoestrogens, herbicides and the surrogate compound prochloraz-d4 spiked for laboratory water samples (n=3), and then analyzed for assessing their initial recovery. Using HLB cartridges, the mean recovery rate of surrogates (prochloraz-d4) in the blank water, soil, and plant were 96 ±8% (n=3), 89 ±6% (n=3) and 94 ±8% (n=3) respectively. Using MCX cartridges, the mean recovery for alkaloids was 96% from 94% to 103% with a relative standard deviation (RSD) of 3%; however, using HLB cartridges their mean recovery was 90% ranging from 88 to 97% with an RSD of 4%. The mean recovery for phytoestrogens was 92% (ranging from 89 to 97%) with an RSD of 4%. The mean recovery for herbicides was 98% (ranging from 90 to 102%) with an RSD of 4%.

$$\text{Recovery (\%)} = \left(\frac{(\text{pre}-\text{spiked extract}) - (\text{non}-\text{spiked extract})}{(post-\text{spiked extract}) - (\text{non}-\text{spiked extract})}\right) * 100 \quad \text{Eq 1}$$

Tables:

Table S1. Physical-chemical properties of the investigated phytotoxins and herbicides.

Phytotoxins and herbicides	Molecular Structure [a]	Molecular Formula	Molecular Weight [g/mole]	Log K_{ow}	pK_a	Water solubility (g/L)
Coumestrol		$C_{15}H_{8}O_{5}$	268.2	1.57[b]	8.25	0.25[b]
Daidzein		$C_{15}H_{10}O_{4}$	254.2	2.55[b]	7.2 [d]	0.57[b]
Daidzin		$C_{21}H_{20}O_{9}$	416.4	0.54[b]	6.9	3.20[b]
Equol		$C_{15}H_{14}O_{3}$	242.3	3.67[b]	10 [d]	0.07[b]
Genistein		$C_{15}H_{10}O_{5}$	270.2	2.84[b]	6.51	0.23[b]
Genistin		$C_{21}H_{20}O_{10}$	432.4	0.83[b]	6.12	1.42[b]
Glycitin		$C_{22}H_{22}O_{10}$	446.4	0.10[b]	9.6	4.88[b]
Flazin		$C_{17}H_{12}N_{2}O_{4}$	308.3	2.2[b]	3.9	0.12[b]
Ginsenine		$C_{13}H_{13}O_{2}N_{2}$	229.1	-[g]	-	-

Soyalkaloid A		$C_{13}H_{11}O_2N_2$	227.1	-	-	-
Trigonelline		$C_7H_7NO_2$	137.1	-2.53[b]	2.1	900[b]
Tyramine		$C_8H_{11}NO$	137.2	0.86[b]	9.6	616[b]
Atrazine		$C_8H_{14}ClN_5$	215.7	2.61[c]	1.7 [f]	0.03[e, f]
Metolachlor		$C_{15}H_{22}ClNO_2$	283.8	3.13[c]	-	0.53[e, f]

a. Structures made in ChemDraw V.16.0.
b. calculation with EPI-WIN (version 4.11) ref. [4]
c. ref. [5]
d. ref. [6]
e. ref. [7]
f. ref. [8]
g. data not available

Table S2. Retention time (Rt), precursor ions, quantification, and qualification ions for MS/MS parameters of selected phytotoxins and herbicides (atrazine and metolachlor).

phytotoxins	Rt (min)	Precursor Ions (m/z)	Quantification ions			Qualification ions		
			MS/MS (m/z)	CV (V)	CE (eV)	MS/MS, m/z	CV (V)	CE (eV)
Coumestrol	10.86	269.05	213.05	30	35	197.0	30	45
Daidzein	8.84	255.06	199.04	40	45	137.0	30	55
Daidzin	8.56	417.12	255.07	15	25	223.0	25	35
Equol	9.97	243.10	123.04	25	35	119.0	35	45
Genistein	8.62	271.06	215.01	35	40	153.0	40	50
Genistin	9.36	431.09	271.04	20	25	215.0	30	35
Glycitin	9.20	447.13	382.95	30	35	322.0	30	45
Flazin	11.05	308.07	247.10	30	35	236.0	30	40
Ginsenine [a]	10.63	229.09	169.12	30	30	115.0	30	35
Soyalkaloid A [a, b]	7.89	227.08	219.00	30	30	203.0	30	35
Trigonelline	2.22	138.05	94.06	25	30	120.0	30	35
Tyramine	1.29	138.00	121.00	20	30	95.0	30	35
Atrazine	10.39	216.10	174.05	35	40	138.0	35	50
Metolachlor	11.22	284.14	252.11	25	35	176.0	30	40

The MS/MS parameters adapted from: a. ref [9] and b. ref [10, 11].

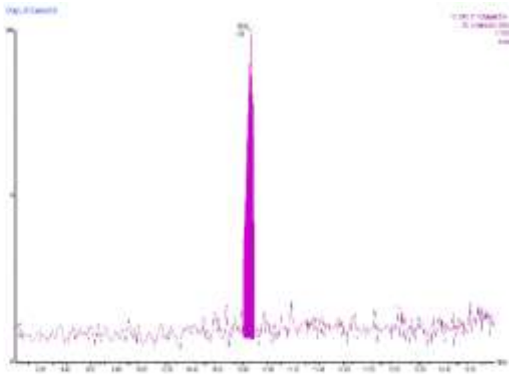
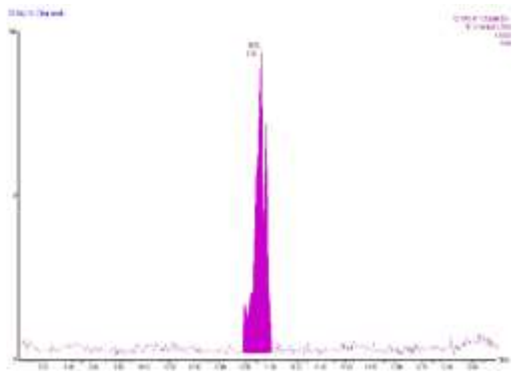
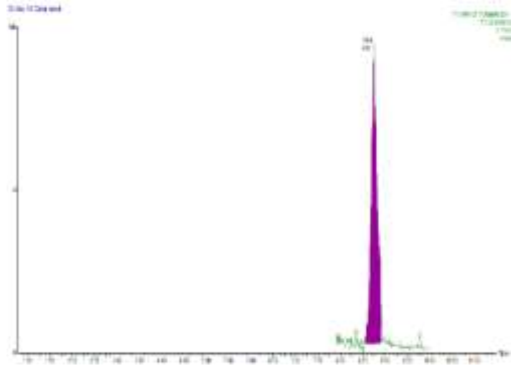
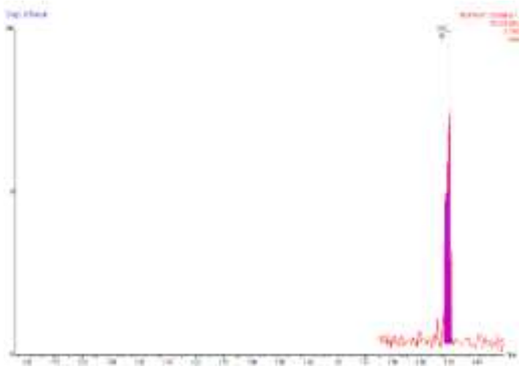
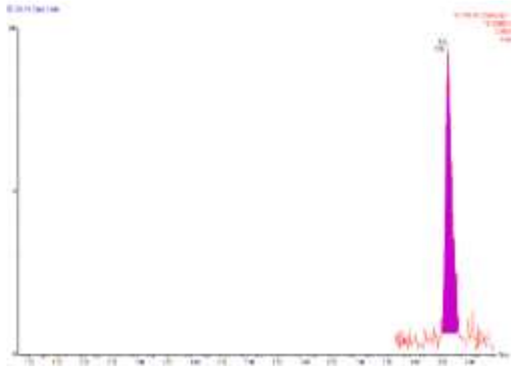
Table S3. Recovery, precision (RSDs) and matrix effects of the phytotoxins and herbicides (atrazine and metolachlor) in plant, soil and water.

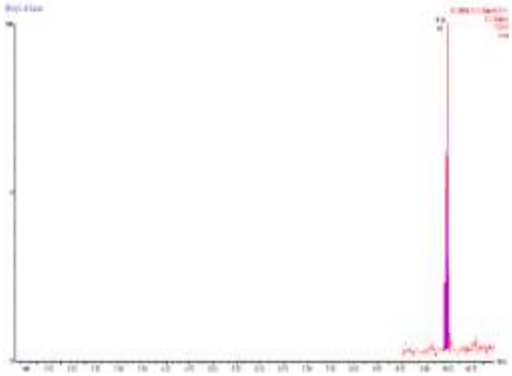
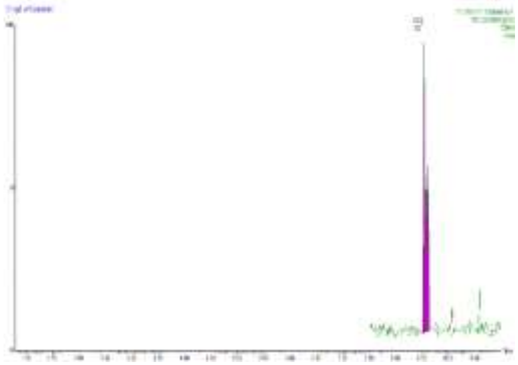
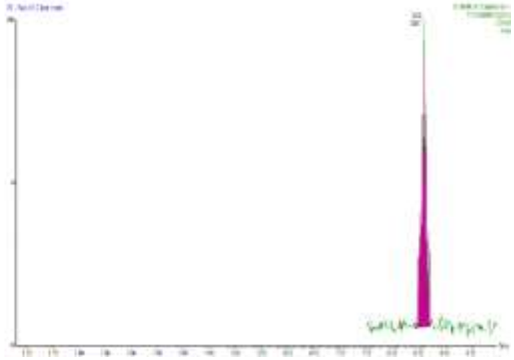
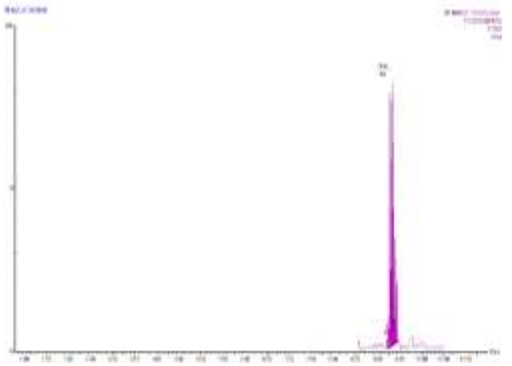
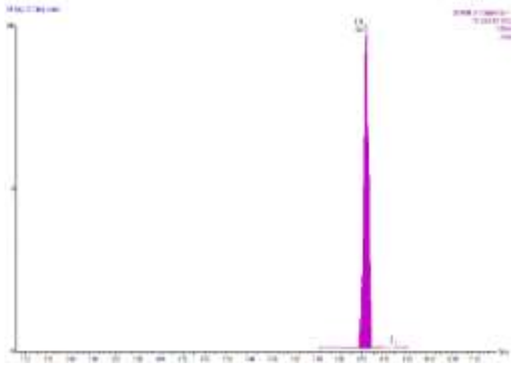
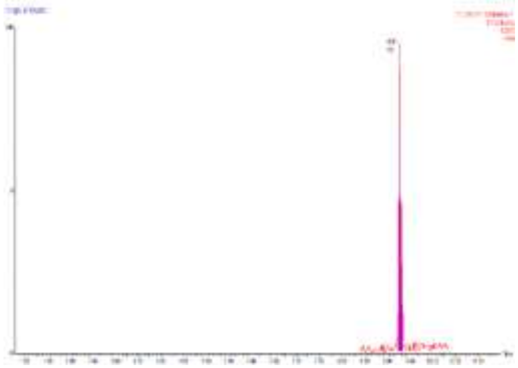
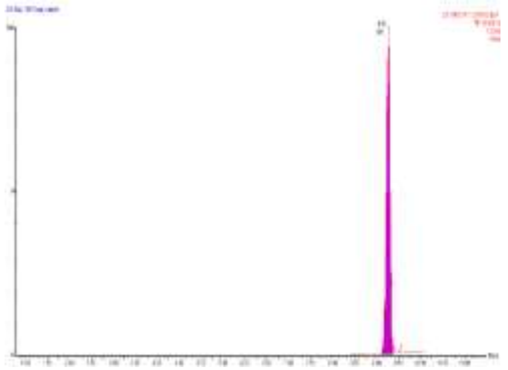
Phytotoxins	LOD			LOQ			Recovery% ±RSD			Matrix effect[a]		
	Plant [ng/g]	Soil [ng/kg]	Water [ng/L]	Plant [ng/kg]	Soil [ng/kg]	Water [ng/L]	Plant	Soil	Water	Plant	Soil	Water
Coumestrol	0.1	0.01	0.9	0.5	0.03	2.6	88±7	89±7	90±6	-10±8	10±7	8±7
Daidzein	1.0	0.06	1.0	4.5	0.1	3.3	80±8	87±8	88±9	-11±6	15±10	10±8
Daidzin	1.0	0.02	0.8	3.3	0.07	2.9	83±7	90±9	93±5	-9±7	9±5	10±8
Equol	2.0	0.01	0.8	6.7	0.04	2.6	76±7	83 ±10	72±7	-8±6	17±9	8±6
Genistein	0.2	0.02	0.8	2.1	0.05	3.1	70±9	84 ±10	70±7	-10±8	16±10	10±8
Genistin	1.0	0.08	0.6	4.1	0.12	2.7	87±8	84±90	93±8	-8±6	19±6	9±5
Glycitin	1.0	0.05	0.5	3.6	0.11	2.7	70±10	70±8	74±6	-13±10	11±8	9±7
Flazin	0.5	0.01	1.1	2.7	0.03	3.1	89±11	83±13	90±8	-10±9	12±10	8±6
Ginsenine	1.2	0.01	1.0	5.1	0.04	3.7	87±9	88±9	92±7	-8±8	17±8	10±8
Soyalkaloid A	2.3	0.01	1.1	4.9	0.04	3.2	86±6	89±11	94±6	-8±7	11±9	7±6
Trigonelline	1.4	0.02	1.0	4.1	0.05	2.9	76±10	77±8	73±7	-8±7	19±6	9±5

Tyramine	1.2	0.05	1.1	3.4	0.09	2.8	87±10	89±10	82±8	-10±5	10±5	10±6
Atrazine	0.2	0.02	0.3	1.2	0.05	2.0	91±9	90±13	90±8	-10±9	9±6	11±5
Metolachlor	0.1	0.03	0.3	1.3	0.07	2.4	88±8	86±10	90±8	-7±6	10±6	10±5
Senecionine (internal standard)	5.5	0.06	5.0	3.1	0.12	11	86±6	89±6	90±8	-8±7	10±5	8±4
Prochloraz-d4(surrogate)	1.4	0.02	1.2	3.1	0.08	3.7	89±8	86±10	94±11	9±7	8±4	7±4

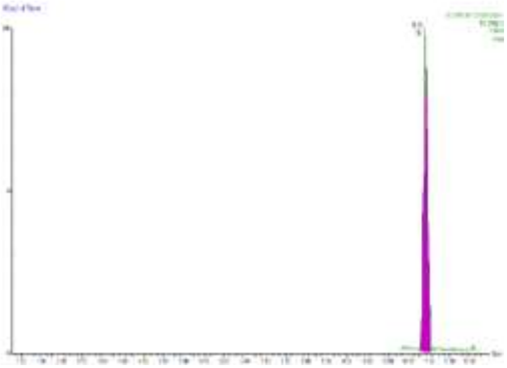
a. Ion suppression(-) and enhancement (no sign)

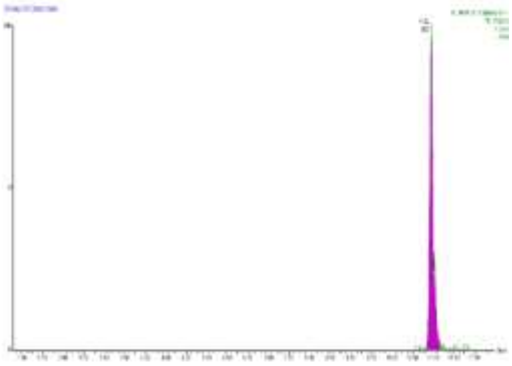
Table S4. **Multiple reaction monitoring (MRM) of phytotoxins and herbicides (atrazine and metolachlor) in standard solutions and samples (water samples from Clear Creek) analyzed during this study.**

	Standard solution (10 ng/L)	Water sample from Clear Creek
Coumestrol		
Daidzein		
Daidzin		

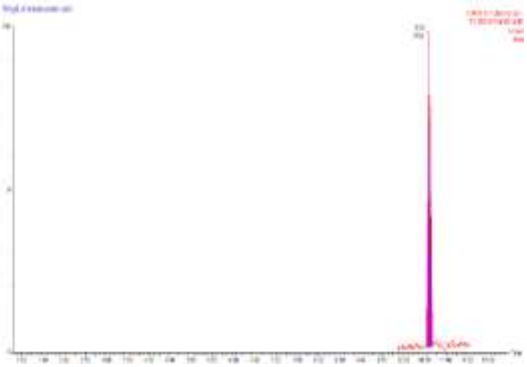
Equol		
Genistein		
Genistin		
Glycitin		

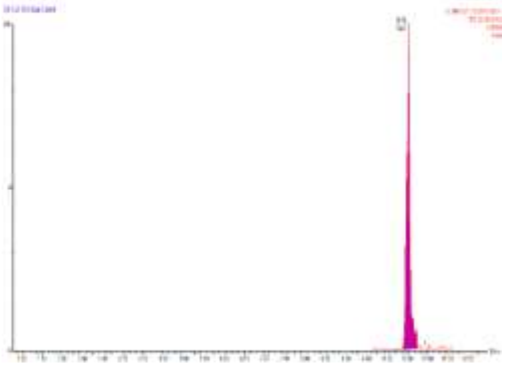
Flazin



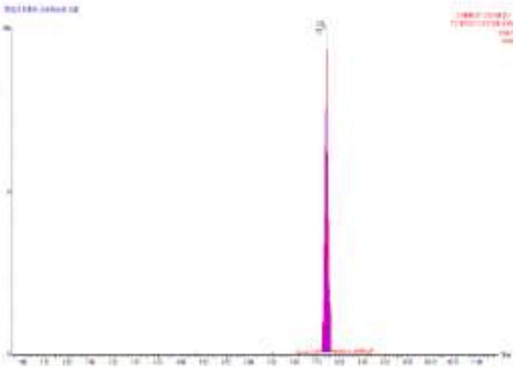


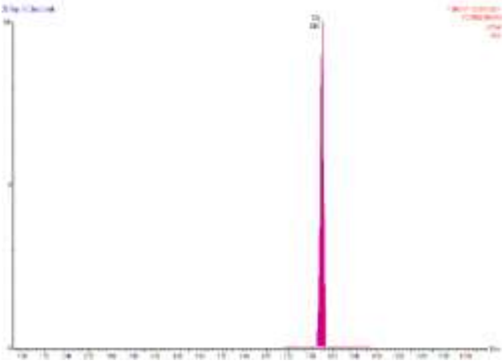
Ginsenenine



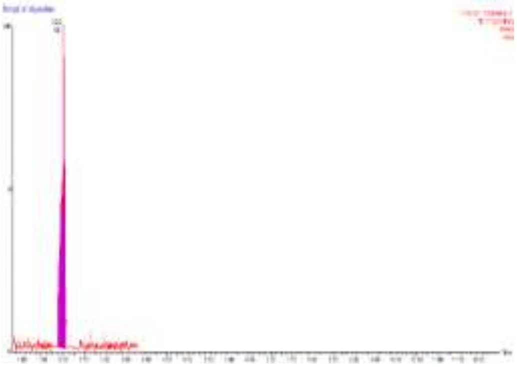


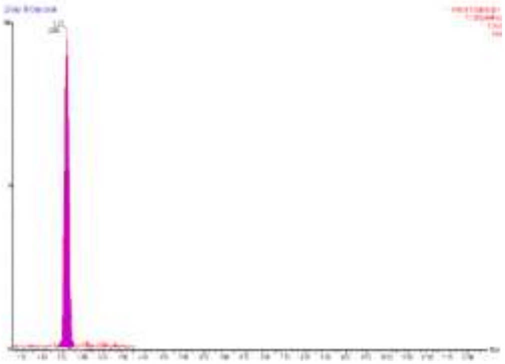
Soyalkaloid A





Trigonelline





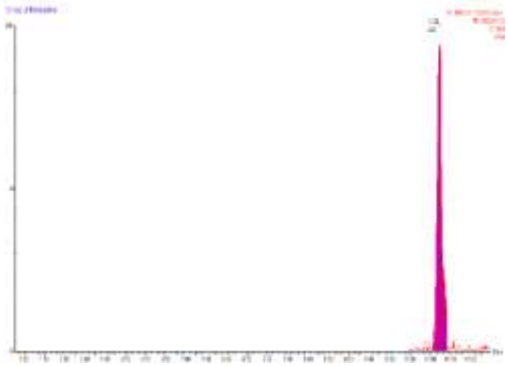
Tyramine		
Atrazine		
Metolachlor		

Table S5. The recovery (%) of phytotoxins and herbicides (atrazine and metolachlor) during their stability test of standards solution and soybean plant tissues extracts stored at room temperature, 5 °C (refrigerator) and -20 °C without exposing to sunlight for 90 days, and dried standards solution loaded on HLB SPE cartridges stored at room temperature without exposing to sunlight for 90 days. [a]

phytotoxins	Standards solution [b]			Soybean plant tissues extract [c]			Standards solution loaded and dried on HLB SPE cartridges [d]
	Room temperature	5 °C	-20 °C	Room temperature	5 °C	-20 °C	
Coumestrol	93±3	95±3	98±2	92±4	93±4	94±4	84±7
Daidzein	94±2	95±2	97±2	91±6	93±3	93±2	90±6
Daidzin	94±4	95±3	95±3	92±3	96±5	94±2	87±5
Equol	95±3	96±3	96±2	ND	ND	ND	82±8
Genistein	94±4	94±1	97±2	90±5	92±5	95±3	88±6
Genistin	95±4	94±2	95±3	90±4	90±4	92±4	92±6
Glycitin	93±5	96±2	96±2	ND	ND	ND	91±8
Flazin	96±3	95±3	98±1	92±5	93±5	93±5	87±5
Ginsenine	-	-	-	93±6	95±3	94±3	-

Soyalkaloid A	-	-	-	94±5	93±4	95±5	-
Trigonelline	94±2	97±2	98±2	94±6	93±4	95±4	85±6
Tyramine	96±2	96±4	97±3	92±5	92±6	93±4	90±5
Atrazine	98±2	99±1	99±1	ND	ND	ND	90±4
Metolachlor	96±2	98±1	99±1	97±3	97±2	98±2	84±5

a. All of the solutions were dried under a gentle stream of nitrogen in glass vials.

b. A mixture of the standards solution (100 ng/L) was used.

c. 0.5 gram of soybean plant tissues extracted as described section 2.3 (Sample Processing) and section S3.2 (Plant and soil extraction) in SI.

d. A mixture of the standards solution (100 ng/L) was loaded on HLB SPE cartridges, as explained in section 2.3 (Sample Processing) and section S3.2 (Plant and soil extraction) in SI.

ND: not detected

- : data not available

Table S6. Natural alkaloids, phytoestrogen, and herbicide concentrations, and streamflow for samples collected across Iowa and Illinois. [ng/L, nanograms per liter; Q, streamflow: m^3/sec; cubic meter per second; nd, not detected; Parentheses indicate concentrations that are between the compound specific limit of detection (LOD) and limit of quantification (LOQ) values].

			Concentration (ng/L)													
Site	**Date**	**Q (m^3/sec)**	**Coumestrol**	**Daidzein**	**Daidzin**	**Equol**	**Genistein**	**Genistin**	**Glycitin**	**Flazin**	**Ginsenine**	**Soyalkaloid A**	**Trigonelline**	**Tyramine**	**Atrazine**	**Metolachlor**
Clear Creek near Oxford, Iowa	09/15/19	1.5	5	nd	nd	nd	9	(1)	(2)	7	7	26	nd	nd	94	228
	09/22/19	1.4	4	8	nd	nd	11	(1)	(2)	4	nd	7	8	nd	87	199
	09/29/19	4.3	11	15	nd	nd	16	2	(1)	11	nd	8	7	6	80	412
	10/03/19	2.9	nd	14	(2)	nd	7	nd	nd	nd	nd	nd	7	4	76	232
	10/05/19	2.2	12	22	3	nd	9	nd	nd	nd	5	nd	nd	11	87	188
	10/21/19	1.0	6	13	nd	nd	3	nd	(1)	6	nd	nd	nd	12	71	73
	11/07/19	0.7	nd	18	(2)	nd	8	nd	(1)	5	(3)	12	6	nd	39	84
	11/21/19	1.3	nd	nd	nd	nd	3	nd	nd	8	9	19	(3)	10	58	55
	11/27/19	0.9	2	11	3	nd	nd	nd	nd	nd	nd	nd	nd	nd	34	41
	11/30/19	1.0	nd	39	5	7	3	nd	nd	nd	nd	nd	nd	8	78	113
	12/17/19	0.5	nd	7	(2)	(2)	7	nd	nd	nd	(3)	3	nd	12	32	61
	12/29/19	2.8	2	nd	nd	nd	3	nd	nd	nd	13	16	8	13	83	209
	01/22/22	1.4	nd	(2)	(2)	nd	nd	nd	nd	4	nd	3	4	(2)	46	63
	02/03/20	1.4	nd	3	nd	19	8	nd	nd	nd	nd	nd	nd	4	22	62
	02/18/20	1.4	4	8	4	9	4	2	nd	4	(3)	(1)	nd	6	100	219

	03/10/20	2.9	3	4	3	68	12	2	nd	8	6	6	(2)	5	258	208
West Branch Wapsinonoc Creek at West Branch, Iowa	09/15/19	0.5	4	nd	nd	nd	9	nd	nd	13	8	15	nd	7	129	163
	09/22/19	0.2	8	10	nd	nd	7	(1)	nd	14	10	12	(3)	10	126	340
	09/29/19	0.4	13	12	nd	nd	8	(1)	nd	10	8	14	4	12	92	199
	10/03/19	0.2	6	18	(2)	nd	9	(1)	nd	10	6	28	4	nd	49	90
	10/05/19	0.2	6	21	3	nd	7	nd	nd	7	nd	nd	nd	nd	119	82
	10/21/19	0.1	9	8	nd	nd	nd	nd	(2)	nd	nd	nd	nd	nd	58	79
	11/07/19	0.0	nd	8	(1)	(3)	nd	nd	(1)	nd	nd	nd	nd	nd	37	69
	11/21/19	0.2	nd	24	nd	nd	nd	(1)	nd	6	nd	nd	(3)	13	51	82
	11/26/19	0.1	7	14	(2)	nd	nd	nd	nd	9	5	4	4	15	46	78
	11/30/19	0.1	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	42	32
	12/17/19	0.1	nd	3	nd	(1)	3	nd	nd	4	nd	6	nd	8	36	35
	12/29/19	0.4	nd	32	4	6	nd	nd	nd	12	8	18	nd	13	78	116
	01/21/20	0.1	nd	3	3	nd	6	2	(1)	8	nd	6	(2)	6	74	71
	02/03/20	0.2	1	(2)	(1)	25	(2)	nd	nd	(3)	nd	nd	nd	4	68	107
	02/18/20	0.2	6	17	(2)	54	9	2	nd	9	(2)	5	(2)	6	37	64
	03/09/20	0.5	5	25	(2)	32	8	(1)	nd	8	(2)	3	(2)	6	113	121
Old Mans Creek near Iowa City, Iowa	09/15/19	1.1	9	nd	nd	nd	10	1	(2)	7	6	37	nd	nd	74	76
	09/23/19	4.3	9	nd	nd	nd	9	nd	nd	14	nd	4	7	nd	150	309
	09/29/19	9.6	nd	nd	nd	nd	6	nd	(1)	10	nd	4	nd	nd	87	147
	10/03/19	6.8	nd	8	(1)	nd	3	nd	nd	9	nd	nd	nd	7	77	100
	10/06/19	12	6	6	(1)	nd	12	2	nd	nd	nd	nd	nd	8	88	76

	10/21/19	2.3	nd	8	nd	nd	nd	nd	nd	nd	nd	nd	6	7	74	59
	11/07/19	1.7	12	14	(2)	5	nd	nd	(2)	nd	nd	5	5	9	34	23
	11/21/19	2.2	17	26	nd	nd	3	2	(1)	10	14	21	6	11	29	50
	11/27/19	2.0	nd	16	nd	5	10	nd	nd	7	nd	nd	nd	nd	88	45
	11/30/19	2.7	13	9	3	nd	8	nd	nd	5	11	13	nd	10	61	88
	12/17/19	1.6	7	nd	(2)	nd	3	nd	(1)	(3)	6	7	nd	11	78	81
	12/29/19	7.7	nd	25	3	8	22	3	nd	7	10	12	5	36	92	119
	01/22/20	3.9	1	4	4	nd	13	2	nd	7	nd	6	(3)	9	56	66
	02/03/20	3.9	nd	4	nd	28	nd	2	nd	nd	(3)	5	nd	7	69	63
	02/18/20	3.9	3	18	2	14	(1)	nd	nd	8	(2)	4	(1)	7	52	226
	03/10/20	12	4	24	3	20	9	nd	nd	14	(2)	7	nd	5	265	272
Iowa River at Wapello, Iowa	10/10/19	801	2	(1)	nd	(2)	nd	nd	nd	nd	nd	nd	nd	nd	48	102
	12/18/19	229	2	nd	(1)	nd	(2)	nd	nd	nd	nd	nd	nd	nd	72	172
	02/12/20	199	1	nd	(2)	nd	nd	(1)	nd	nd	nd	nd	nd	nd	22	57
Mississippi River below Grafton, Illinois	10/07/19	7476	nd	nd	nd	nd	3	nd	nd	nd	nd	nd	nd	nd	20	19
	11/04/19	7278	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	23	26
	12/0319	5437	nd	nd	(2)	nd	3	nd	nd	nd	nd	nd	nd	nd	10	10
	01/05/20	4928	nd	nd	nd	nd	(1)	(1)	nd	nd	nd	nd	nd	nd	7	11
	02/10/20	5522	1	nd	(1)	nd	nd	nd	nd	nd	nd	nd	nd	nd	43	24
Muddy Creek at Coralville, Iowa	09/15/19	3.5	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	13	nd
	09/22/19	2.1	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	21	nd
	09/29/19	1.1	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	4	nd

10/03/19	0.6	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	5	nd
10/05/19	2.3	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	4	nd
10/21/19	1.1	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	8	nd
11/07/19	0.2	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	8	nd
11/21/19	0.8	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	3	nd
11/27/19	0.4	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	4	3
11/30/19	0.4	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	8	nd
12/17/19	0.1	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
12/29/19	1.4	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	11	nd
01/22/20	0.1	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	9	4
02/03/20	0.3	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	3	nd
02/18/20	0.2	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	3	2
03/10/20	1.0	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	6	2

Table S7. Statistical results (p-value and Spearman rho (rs) correlation coefficient) from the correlation of streamflow and compound concentration, and overall data (with streamflow, atrazine (the reference herbicide) and total herbicide concentration), data grouped by sampling site. A p-value <0.05 was considered statistically significant and is bolded and marked with an asterisk.

Streamflow vs	compound	Coumestrol	Daidzein	Daidzin	Equol	Genistein	Genistin	Glycitin	Flazin	Ginsenine	Soyalkaloid A	Trigonelline	Tyramine	Atrazine	Metolachlor
Clear Creek	rs	0.47	-0.11	-0.19	0.04	0.51	0.52	0.12	0.27	0.13	0.16	0.48	-0.01	0.63	0.79
	p-value	0.06	0.69	0.47	0.88	**0.04***	**0.04***	0.66	0.32	0.63	0.55	**0.06***	0.97	**0.01***	**<0.001***
West Branch Wapsinonoc Creek	rs	0.10	0.38	0.07	0.11	0.51	0.36	-0.57	0.62	0.57	0.46	0.23	0.33	0.63	0.76
	p-value	0.71	0.14	0.80	0.68	**0.05***	0.17	**0.02***	**0.01***	**0.02***	0.07	0.39	0.21	**0.01***	**<0.001***
Old Mans Creek	rs	-0.39	-0.06	0.25	0.15	0.31	0.21	-0.54	0.29	-0.26	-0.37	-0.12	-0.12	0.52	0.58
	p-value	0.13	0.82	0.35	0.57	0.25	0.42	**0.03***	0.27	0.32	0.16	0.66	0.67	**0.04***	**0.02***
Iowa River[a]	rs	1.00	0.87	-1.00	-0.50	0.00	-0.87	-	-	-	-	-	-	0.50	0.50
	p-value	0.67	0.33	0.67	0.67	1.00	0.33	-	-	-	-	-	-	0.67	0.67
Mississippi River	rs	0.00	-	-0.34	-	0.15	-0.71	-	-	-	-	-	-	0.60	0.60
	p-value	1.00	-	0.58	-	0.80	0.18	-	-	-	-	-	-	0.28	0.28

Muddy Creek	rs	-	-	-	-	-	-	-	-	-	-	-	-	0.34	-0.48
	p-value	-	-	-	-	-	-	-	-	-	-	-	-	0.20	0.06
Overall data vs streamflow, atrazine and total herbicide concentration		Coumestrol	Daidzein	Daidzin	Equol	Genistein	Genistin	Glycitin	Flazin	Ginsenine	Soyalkaloid A	Trigonelline	Tyramine	Atrazine	Metolachlor
Streamflow	rs	-0.05	-0.12	0.15	0.22	0.25	0.24	-0.06	-0.06	-0.04	-0.02	0.03	-0.02	0.17	0.16
	p-value	0.68	0.33	0.21	**0.07***	**0.04***	**0.04***	0.61	0.62	0.75	0.88	0.79	0.88	0.15	0.17
Atrazine	rs	0.55	0.52	0.33	0.19	0.68	0.32	0.18	0.67	0.47	0.52	0.34	0.47	-	0.88
	p-value	**<0.001***	**<0.001***	**0.01***	0.11	**<0.001***	**0.01***	0.12	**<0.001***	**<0.001***	**<0.001***	**<0.001***	**<0.001***	**-**	**<0.001***
Total herbicides	rs	0.57	0.55	0.34	0.22	0.61	0.30	0.25	0.70	0.48	0.46	0.23	0.56	-	-
	p-value	**<0.001***	**<0.001***	**<0.001***	**0.05***	**<0.001***	**0.01***	**0.03***	**<0.001***	**<0.001***	**<0.001***	**0.04***	**<0.001***	**-**	-

∗ Indicates a statistically significant result.

Table S8. Natural alkaloids, phytoestrogen, and herbicide concentrations in upstream sites collected across Iowa on December 29, 2019. [ng/L, nanograms per liter; nd, not detected; parentheses indicate concentrations that are between the compound specific LOD and LOQ values].

Site	Upstream sites	Coordinates (latitude & longitude) and the distance from the gaging station	Concentration (ng/L)													
			Coumestrol	Daidzein	Daidzin	Equol	Genistein	Genistin	Glycitin	Flazin	Ginsenine	Soyalkaloid A	Trigonelline	Tyramine	Atrazine	Metolachlor
Clear Creek near Oxford, Iowa	Gaging station site	41.718242, -91.740265	2	nd	nd	17	3	nd	nd	3	13	16	8	13	83	209
	Upstream 1	41.711915, -91.798794, 7.3 km	2	nd	nd	4	2	nd	nd	6	7	8	5	9	70	177
	Upstream 2	41.705306, -91.852911, 13.0 km	nd	nd	nd	nd	nd	nd	nd	7	4	7	6	7	65	162
	Upstream 3	41.716657, -91.890791, 16.1 km	nd	nd	nd	nd	nd	nd	nd	2	7	9	5	3	47	86
	Upstream 4	41.713941, -91.929747, 20.2 km	nd	nd	nd	nd	nd	nd	nd	3	4	7	nd	nd	53	86
	Upstream 5	41.714940, -91.968588, 23.7 km	nd	nd	nd	nd	nd	nd	nd	7	nd	nd	nd	nd	54	59

West Branch Wapsinonoc Creek at West Branch, Iowa	Gaging station site	41.606497, -91.615656	nd	25	3	8	22	3	nd	7	10	12	5	36	92	119
	Gaging station site	41.670028, -91.344250	nd	32	4	11	nd	nd	nd	12	8	18	nd	13	78	116
	Upstream 1	41.685093, -91.346531, 1.4 km	nd	13	6	4	nd	nd	nd	4	7	16	nd	22	96	74
	Upstream 2	41.702671, -91.366165, 4.3 km	nd	27	4	nd	nd	nd	nd	12	9	20	nd	8	86	69
Old Mans Creek near Iowa City, Iowa	Upstream 1	41.608519, -91.638557, 2.9 km	nd	16	2	5	10	(1)	nd	7	7	8	6	25	73	72
	Upstream 2	41.604250, -91.709569, 12.0 km	2	9	1	3	11	(1)	nd	7	6	7	6	23	70	60
	Upstream 3	41.601135, -91.751027, 16.6 km	13	6	nd	nd	15	(2)	nd	3	8	10	7	15	48	14
	Upstream 4	41.603295, -91.829931, 27.1 km	1	nd	nd	nd	3	nd	nd	2	4	10	nd	3	42	21
	Upstream 5	41.622762, -91.992910, 43.7 km	nd	nd	nd	nd	3	nd	nd	nd	3	11	nd	nd	33	20

Table S9. Natural alkaloids, phytoestrogen, and herbicide concentrations in upstream sites collected at the effluent site of WWTP roughly 5.1 km upstream from the sampling point at Muddy Creek reference basin. [ng/L, nanograms per liter; nd, not detected].

Date	**Concentration (ng/L)**													
	Coumestrol	**Daidzein**	**Daidzin**	**Equol**	**Genistein**	**Genistin**	**Glycitin**	**Flazin**	**Ginsenine**	**Soyalkaloid A**	**Trigonelline**	**Tyramine**	**Atrazine**	**Metolachlor**
11/08/2019	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	2	nd
01/22/2020	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	6	nd

Table S10. Natural alkaloids, phytoestrogen, and herbicide concentrations in the side ditch site flowing through corn and soybean fields into Old Mans Creek near Iowa City, Iowa (05455100). [ng/L, nanograms per liter; nd, not detected; parentheses indicate concentrations that are between the compound specific LOD and LOQ values].

	Concentration (ng/L)													
Date	**Coumestrol**	**Daidzein**	**Daidzin**	**Equol**	**Genistein**	**Genistin**	**Glycitin**	**Flazin**	**Ginsenine**	**Soyalkaloid A**	**Trigonelline**	**Tyramine**	**Atrazine**	**Metolachlor**
11/21/2019	nd	51	7	nd	(2)	3	nd	32	13	22	13	nd	33	17
02/03/2020	nd	nd	nd	(2)	(1)	nd	nd	nd	nd	nd	nd	5	14	11
02/18/2020	2	3	(1)	(2)	nd	4	nd	(2)	nd	2	(1)	4	15	14
03/10/2020	1	4	(1)	(1)	nd	(2)	nd	(2)	nd	2	nd	4	43	29

Table S11. Natural alkaloids, phytoestrogen, and herbicide concentrations for soybean plant samples collected from the field of upstream from Clear Creek near Oxford in Iowa. [ng/g, nanograms per gram; nd, not detected].

Date	Duplicate	Concentration (ng/g) in dried weight													
		Coumestrol	**Daidzein**	**Daidzin**	**Equol**	**Genistein**	**Genistin**	**Glycitin**	**Flazin**	**Ginsenine**	**Soyalkaloid A**	**Trigonelline**	**Tyramine**	**Atrazine**	**Metolachlor**
10/18/2019	1	$5.7×10^3$	$2.5×10^3$	596	3	$1.9×10^3$	nd	171	43	23	22	$3.1×10^3$	126	nd	3
	2	$8.9×10^3$	$2.9×10^3$	870	4	$2.2×10^3$	nd	195	79	32	14	$3.4×10^3$	81	nd	2
11/19/2019	1	$1.5×10^3$	$2.1×10^3$	24	3	432	nd	9	125	43	12	$1.8×10^3$	77	nd	2
	2	$2.8×10^3$	100	192	2	$1.2×10^3$	nd	31	51	30	10	$15×10^3$	49	nd	1
12/17/2019	1	$2.5×10^3$	673	30	nd	680	nd	4	35	248	12	$1.0×10^3$	426	nd	1
	2	$1.6×10^3$	632	28	nd	602	nd	4	23	167	11	$1.3×10^3$	276	nd	1
1/22/2020	1	915	105	nd	nd	1653	19	11	13	111	5	$1.1×10^3$	33	nd	nd
	2	$1.5×10^3$	84	nd	nd	1411	11	8	10	83	6	882	26	nd	nd

Table S12. Natural alkaloids, phytoestrogen, and herbicide concentrations for soil samples collected from the field of upstream Clear Creek near Oxford in Iowa. [ng/g, nanograms per gram; nd, not detected]. Data are given as mean (n =2).

Date	Duplicate	Soil type	Concentration (ng/g) in fresh weight													
			Coumestrol	Daidzein	Daidzin	Equol	Genistein	Genistin	Glycitin	Flazin	Ginsenine	Soyalkaloid A	Trigonelline	Tyramine	Atrazine	Metolachlor
10/18/2019	1	Surface soil	3.43	2.62	nd	nd	0.86	nd	nd	1.44	0.56	0.56	1.04	2.45	0.73	2.23
11/19/2019			0.28	0.69	nd	nd	0.23	nd	nd	0.15	0.15	0.15	0.27	0.51	0.37	0.30
12/17/2019			0.44	0.49	0.02	nd	1.30	nd	nd	0.11	0.11	0.10	0.74	1.14	0.18	0.23
1/22/2020			0.61	0.39	nd	nd	1.04	nd	nd	0.10	0.08	0.07	0.59	0.91	0.15	0.19
10/18/2019	2	Surface soil	0.23	2.31	nd	0.14	0.76	nd	nd	0.49	0.49	0.49	0.91	1.91	0.95	1.53
11/19/2019			0.01	0.73	nd	nd	0.24	nd	nd	0.40	0.16	0.15	0.29	0.71	0.43	0.62
12/17/2019			0.06	0.64	nd	nd	0.21	nd	nd	0.13	0.12	0.13	0.25	0.63	0.28	0.73
1/22/2020			0.06	0.56	nd	nd	0.19	nd	nd	0.12	0.12	0.11	0.22	0.56	0.26	0.65

10/18/2019	1	Near surface soil	0.28	0.20	nd	0.01	0.07	nd	nd	0.05	0.04	0.04	0.08	0.21	0.10	0.44
11/19/2019			0.07	0.08	nd	nd	0.03	nd	nd	0.04	0.02	0.01	0.03	0.07	0.03	0.15
12/17/2019			0.01	0.07	nd	nd	0.02	nd	nd	0.01	0.01	0.02	0.03	0.06	0.02	0.05
1/22/2020			0.01	0.06	nd	nd	0.02	nd	nd	0.01	0.02	0.02	0.02	0.05	0.02	0.04
10/18/2019	2	Near surface soil	0.02	0.22	0.12	nd	0.07	nd	nd	0.05	0.04	0.05	0.09	0.17	0.15	0.23
11/19/2019			0.07	0.08	nd	nd	0.03	nd	nd	0.02	0.01	0.02	0.03	0.08	0.05	0.06
12/17/2019			0.07	0.07	nd	nd	0.06	nd	nd	0.03	0.02	0.01	0.03	0.28	0.03	0.03
1/22/2020			0.06	0.07	nd	nd	0.07	nd	nd	0.02	0.01	0.01	0.03	0.25	0.02	0.03

Figures:

A

B

C

D

E

F

G

H

I

J

Figure S1. Seasonal views for Clear Creek near Oxford, Iowa (05454220) showing the landscape upstream from the sampling location (left photos, J photo unavailable), and the stream at the sampling location (right photos) on the following dates and streamflow; (A) September 23, 2019 (1.1 m^3/sec); (B) October 6, 2019 (2.4 m^3/sec); (C) October 21, 2019 (1.0 m^3/sec); (D) November 7, 2019 (0.7 m^3/sec); (E) November 17, 2019 (0.7 m^3/sec); (F) December 17, 2019 (0.5 m^3/sec); (G) December 29, 2019 (2.8 m^3/sec); (H) January 22, 2020 (streamflow data unavailable); (I) February 3, 2020 (streamflow data unavailable); and (J) March 10, 2020 (2.6 m^3/sec).

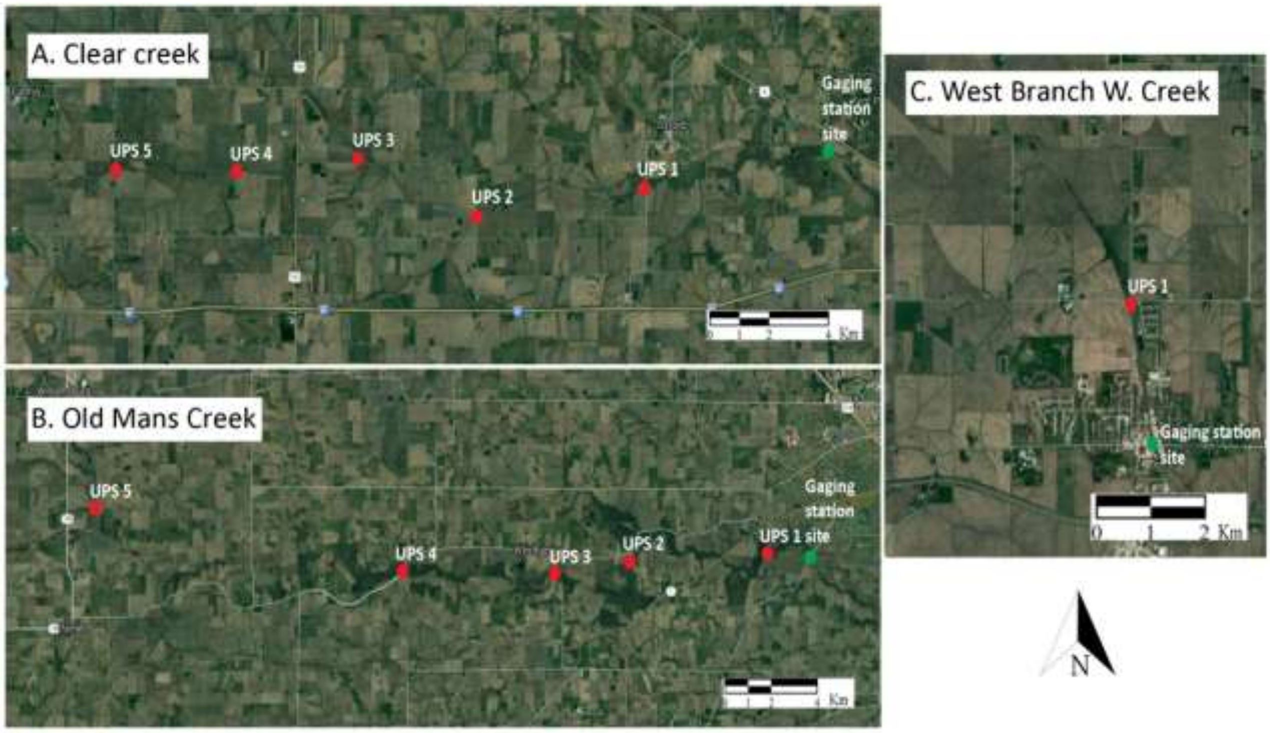


Figure S2. Location of sample collection upstream from (A) Clear Creek near Oxford in Iowa (05454220), (B) Old Mans Creek near Iowa City (05455100), and (C) West Branch Wapsinonoc Creek at West Branch, Iowa (0546494170), December 29, 2019. The green circles are the gaging station sites and the red circles are the upstream sites (UPS 1 to UPS 5) for all basins.

Figure S3. Effluent from wastewater treatment plant approximately 5.1 km upstream from the sampling site at the Muddy Creek reference basin; samples were collected on November 8, 2019 and January 22, 2020.

A

B

Figure S4. The side ditch site at the Old Mans Creek near Iowa City, Iowa (05455100); (A) the side ditch and (B) the ditch in relation to the field flowing through corn and soybean fields into the creek; samples were collected on November 21, 2019, and February 3, February 18 and March 10, 2020.


A


B


C

D

E

Figure S5. Temporal variations of the field adjacent to Clear Creek upstream from Clear Creek near Oxford, IA (05454220), samples collected on (A) October 18, 2019, (B) November 19, 2019, (C) December 17, 2019, and (D) January 22, 2020. The soybean field was harvested on (E) November 7, 2019.

A

B

C

Figure S6. Demonstration of soil sampling using (A) the 12.7 cm metal pipe, and (B and C) soil sample collection in 500-mL polypropylene jars. The soil type of the field is Colo silt loam [12].

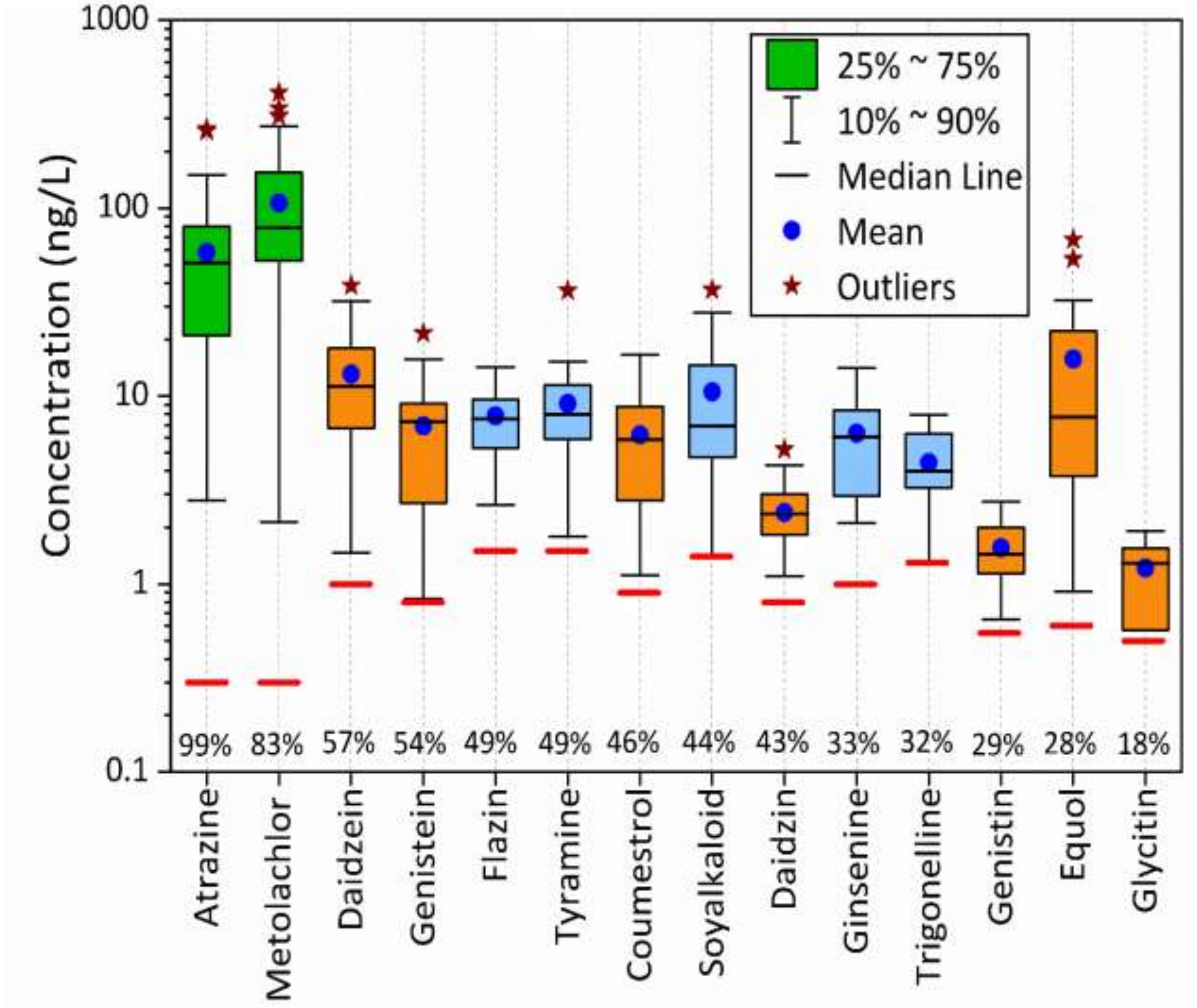


Figure S7. Distribution of phytotoxins and herbicides concentration in the samples (n=72) at all stream sampling sites of Clear Creek (n=16), West Branch Wapsinonoc Creek (n=16), Old Mans Creek (n=16), Iowa River (n=3), and Mississippi River (n=5), collected from September 2019 to March 2020. Blue point, red star, and percentiles (listed along the x-axis) represent site specific mean, outliers, and detection frequencies, respectively. The red line indicates the method LOD in ng/L. Only concentrations above the LOD are shown in the figure, but all measurements were included in the calculation of detection frequencies provided along the x-axis. Note that y-axis in a logarithmic scale. Targeted compounds grouped with color; green for herbicides, blue for alkaloids, and orange for phytoestrogens.

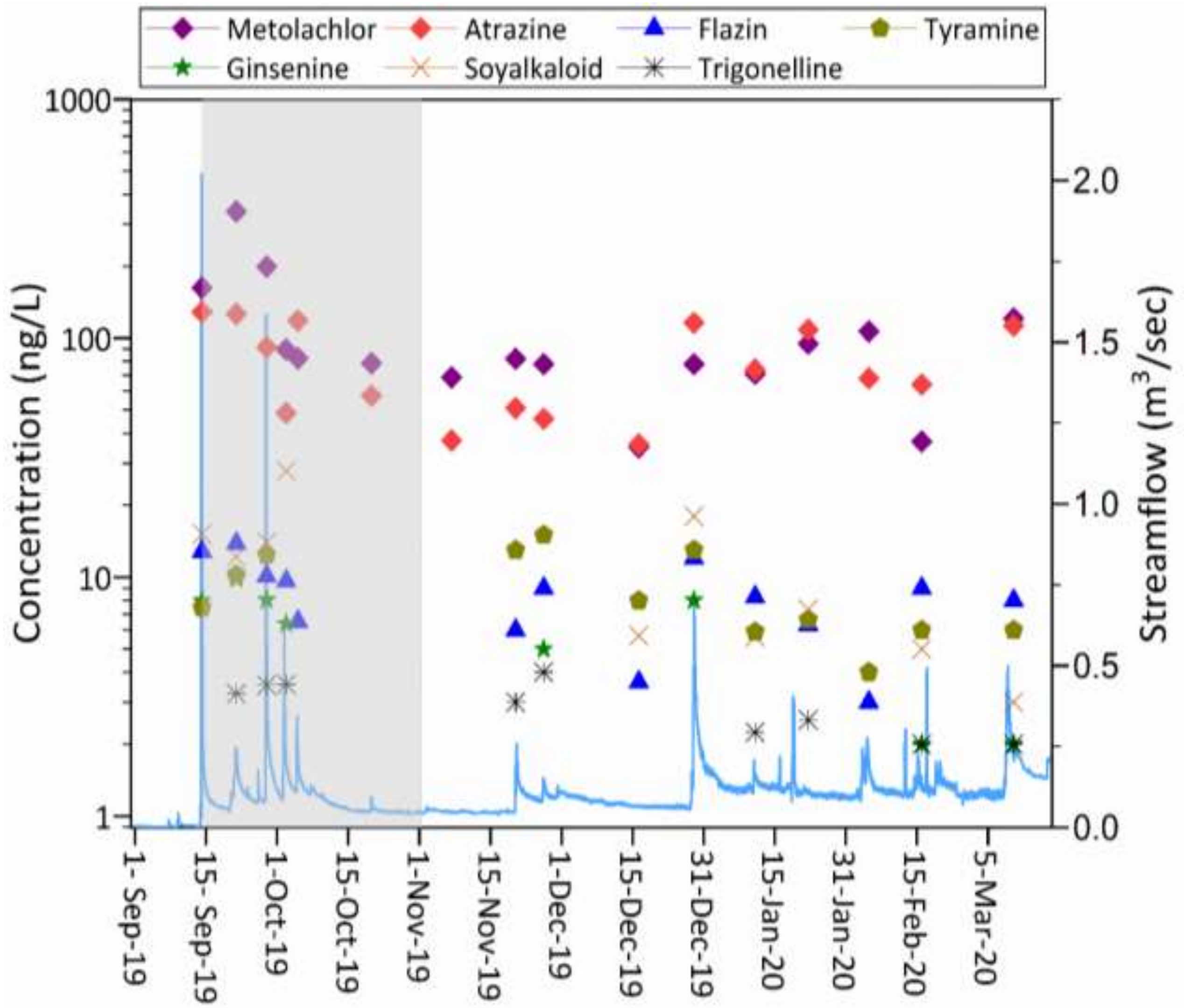


Figure S8. Concentrations of alkaloids, herbicides (atrazine and metolachlor), and streamflow for the samples (n=16) collected at West Branch Wapsinonoc Creek at West Branch, (0546494170), September 2019 through March 2020.[13] Note that the y-axes (left) is in a logarithmic scale, and the shaded area is the harvest season in Iowa.

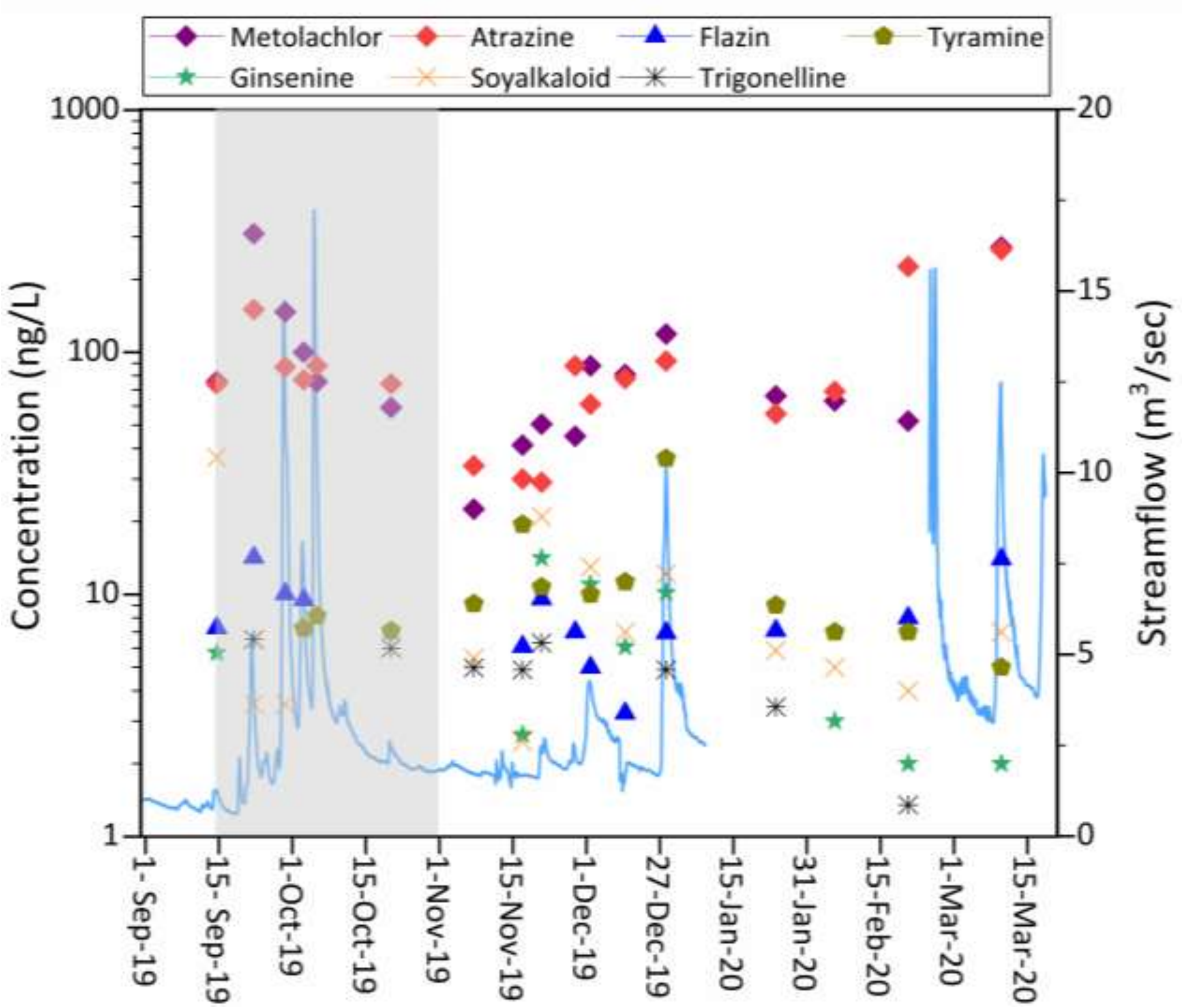


Figure S9. Concentrations of alkaloids, herbicides (atrazine and metolachlor), and streamflow for the samples (n=16) collected at Old Mans Creek near Iowa City (05455100), September 2019 through March 2020.[13] In January and February, streamflow data were unavailable due to frozen conditions. Note that the y-axes (left) is in a logarithmic scale, and the shaded area is the harvest season in Iowa.

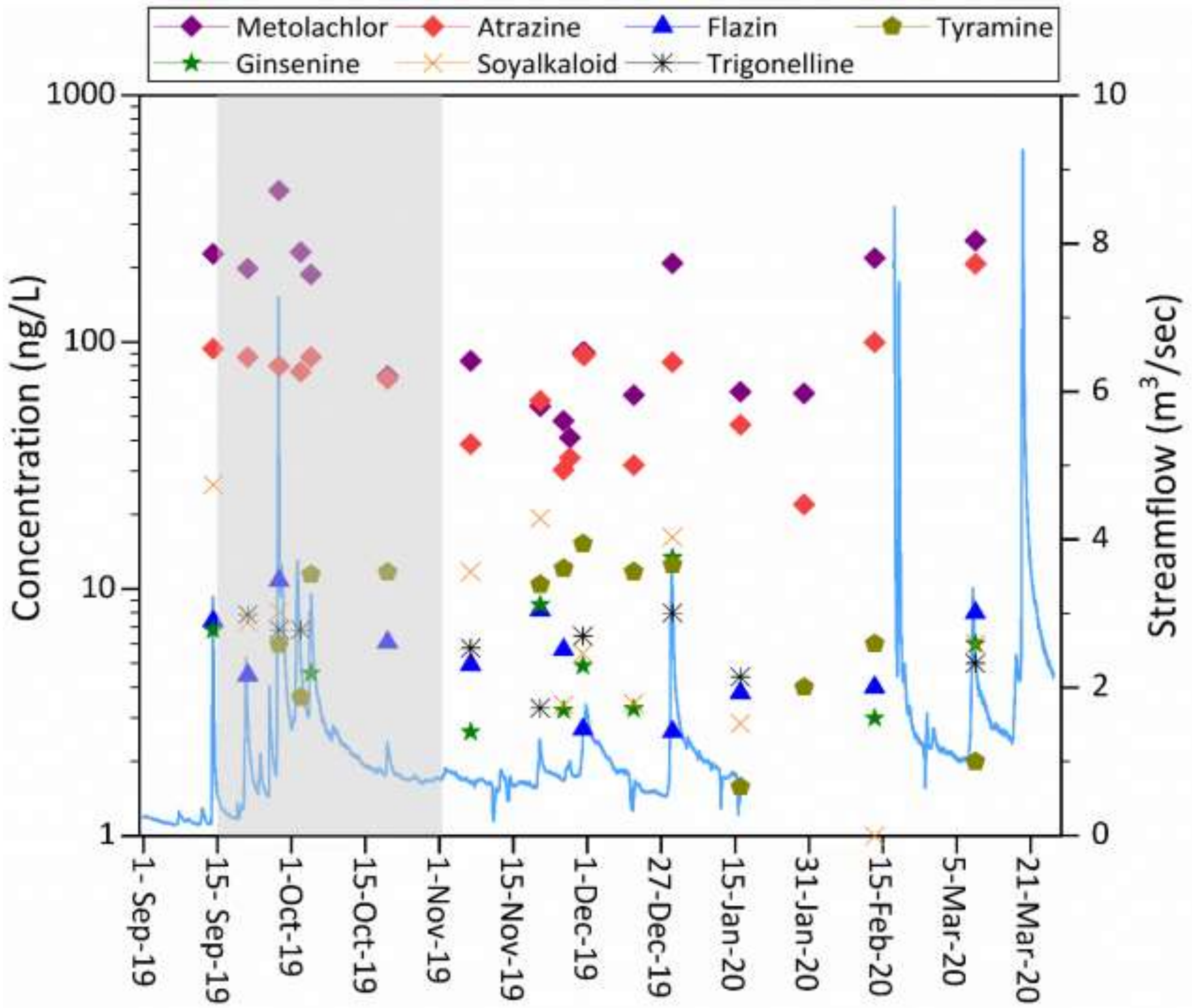


Figure S10. Concentrations of phytoestrogens, herbicides (atrazine and metolachlor), and streamflow for the samples (n=16) collected at Clear Creek, near Oxford, Iowa (05454220), September 2019 through March 2020.[13] In January and February, streamflow data were unavailable due to frozen condition. Note that the y-axes (left) is in a logarithmic scale, and the shaded area is the harvest season in Iowa.

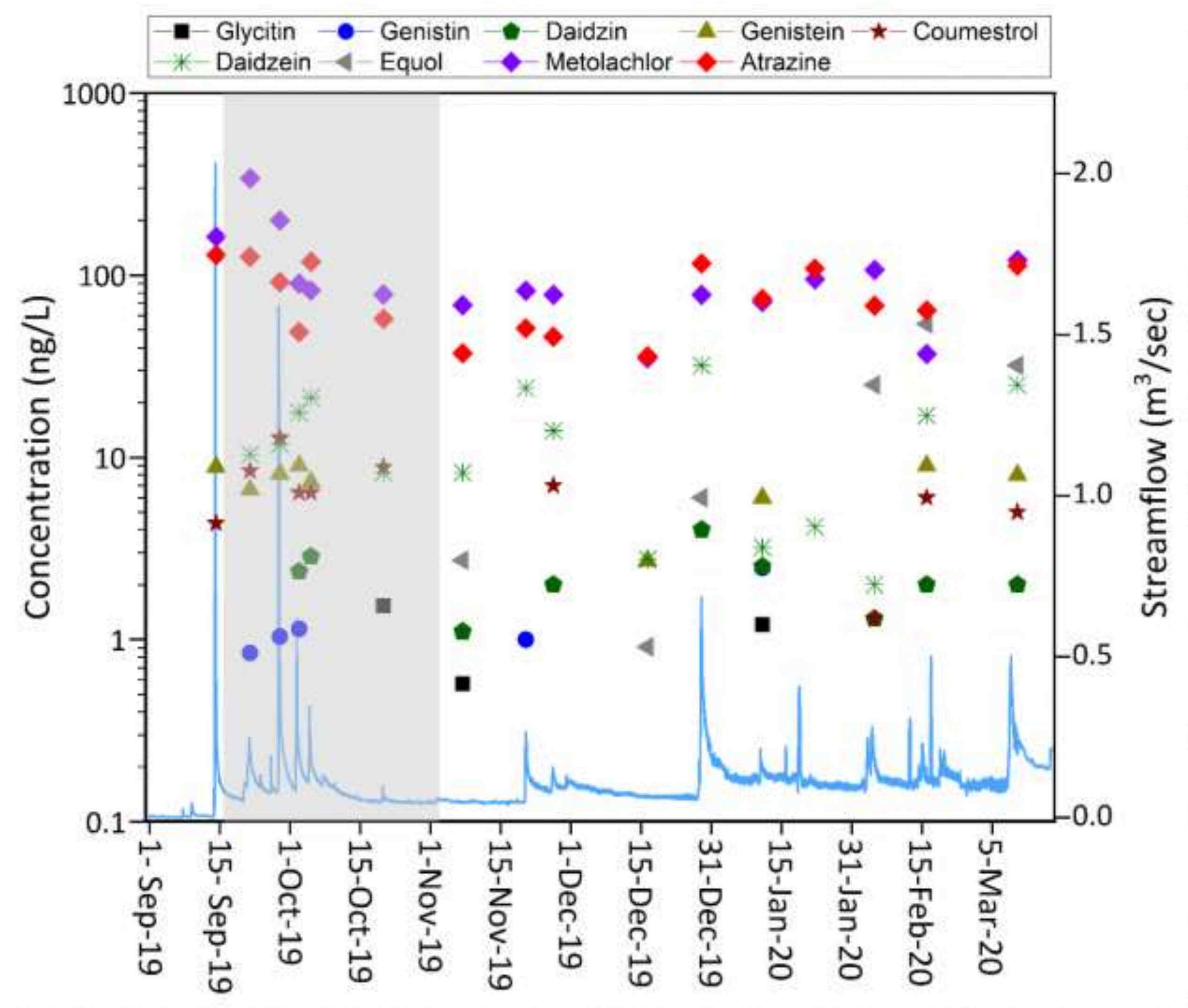


Figure S11. Concentrations of phytoestrogens, herbicides (atrazine and metolachlor), and streamflow for the samples (n=16) collected at West Branch Wapsinonoc Creek at West Branch, Iowa (0546494170), September 2019 through March 2020.[13] Note that the y-axes (left) is in a logarithmic scale, and the shaded area is the harvest season in Iowa.

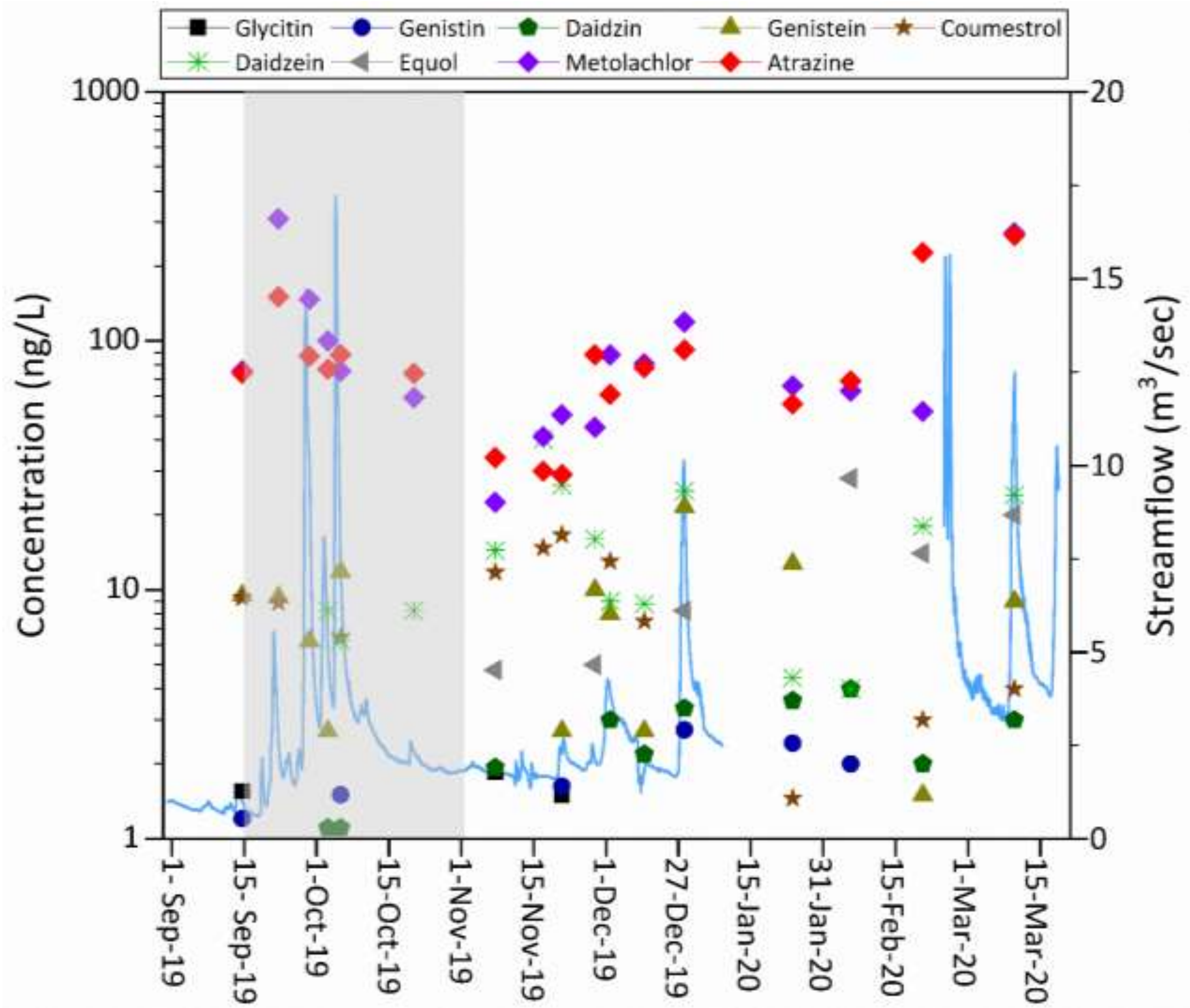


Figure S12. Concentrations of phytoestrogens, herbicides (atrazine and metolachlor), and streamflow for the samples (n=16) collected at Old Mans Creek near Iowa City, Iowa (05455100), September 2019 through March 2020.[13] In January and February, streamflow data were unavailable due to frozen conditions. Note that the y-axes (left) is in a logarithmic scale, and the shaded area is the harvest season in Iowa.

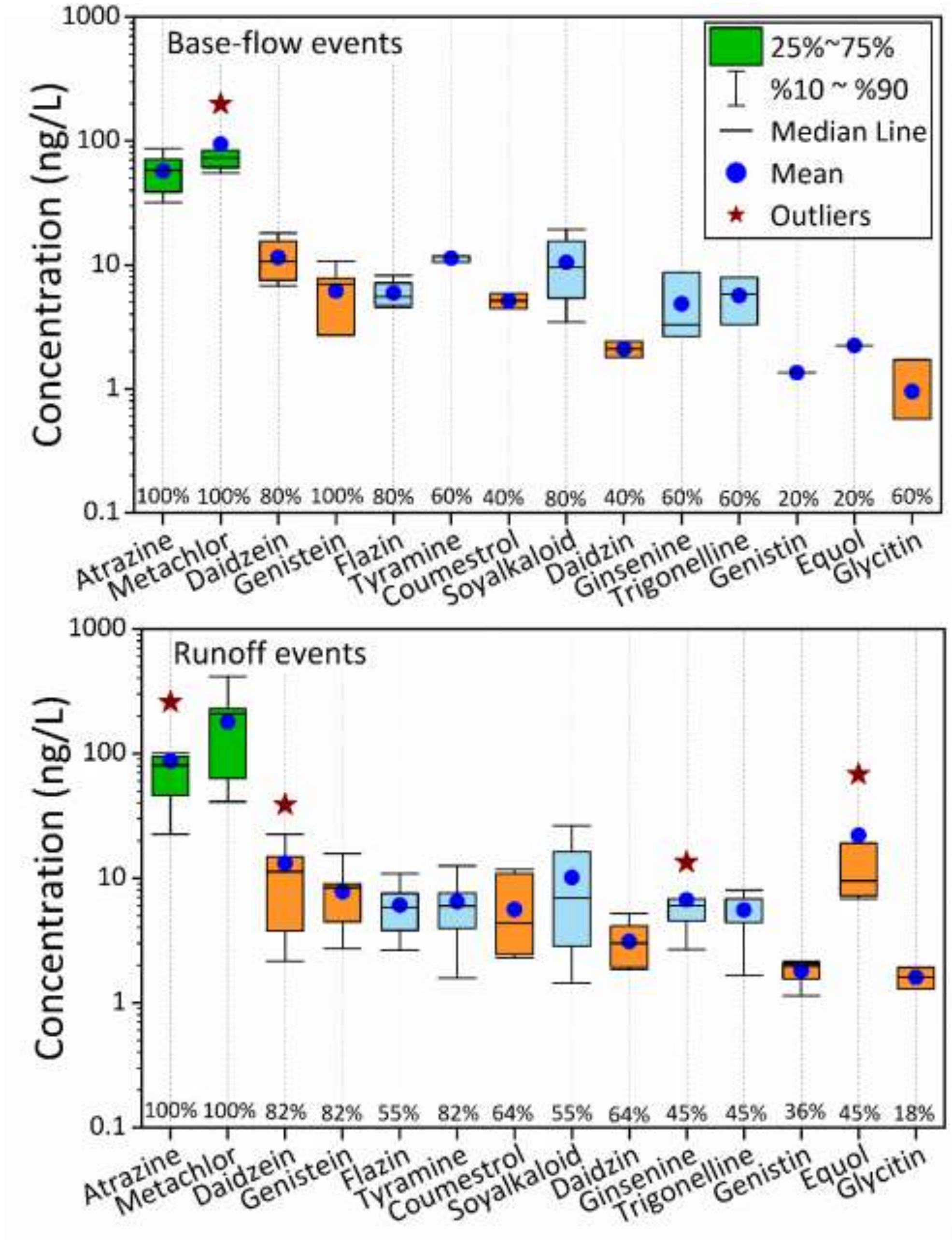


Figure S13. Concentrations of phytotoxins and herbicides (atrazine and metolachlor) for the samples collected during base-flow (n=5) and runoff events (n=11) at Clear Creek, near Oxford, Iowa (05454220), September 2019 through March 2020. Blue point, red star and percentiles (listed along the x-axes) represent site specific medians, outliers, and detection frequencies, respectively. Note that y-axes in a logarithmic scale. Targeted compounds are grouped by color; green for herbicides, blue for alkaloids, and orange for phytoestrogens. Only concentrations above the LOD are shown in the figure, but all measurements were included in the calculation of detection frequencies provided along the x-axes.

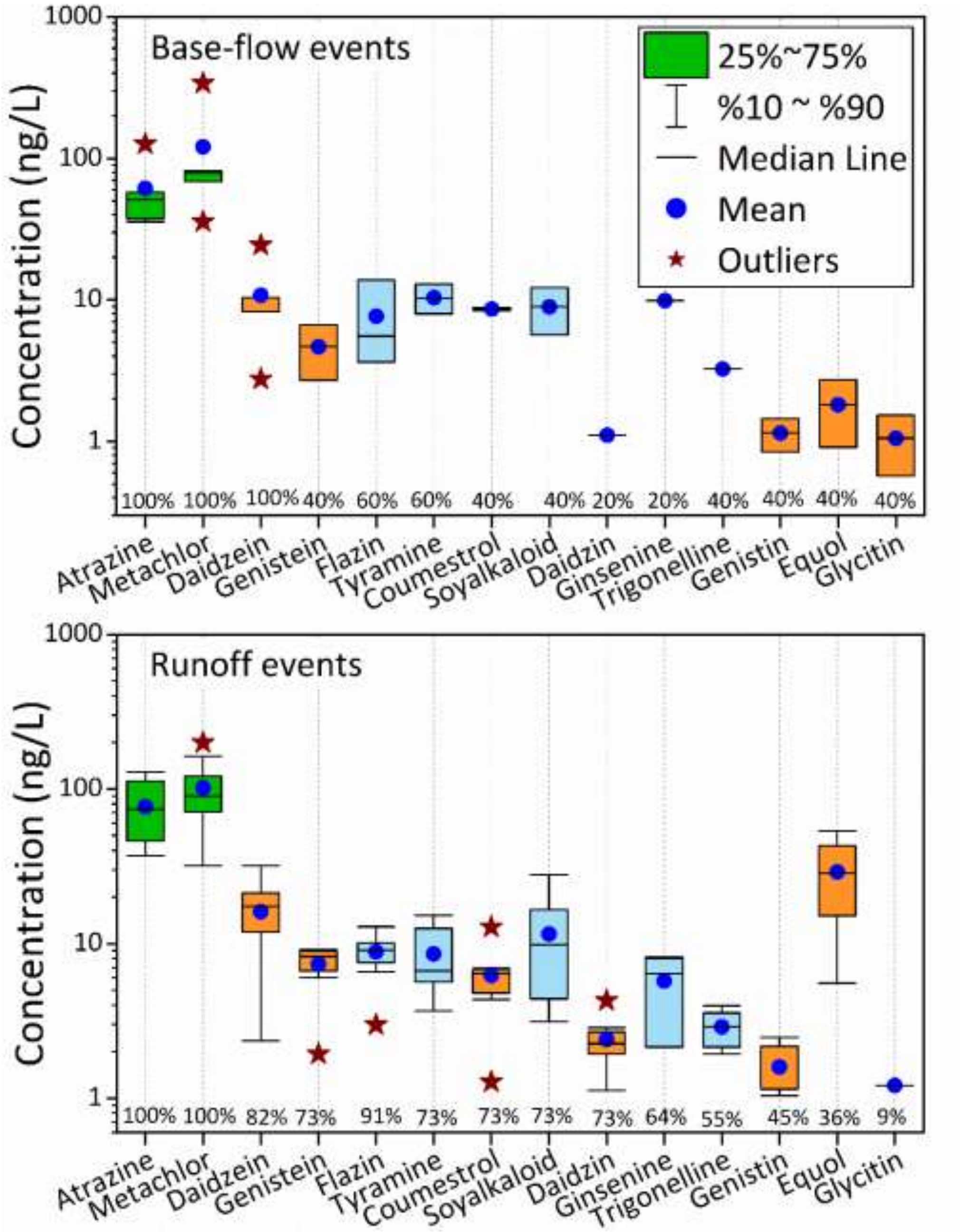


Figure S14. Concentrations of phytotoxins and herbicides (atrazine and metolachlor) for the samples collected during base-flow (n=5) and runoff events (n=11) at West Branch Wapsinonoc Creek at West Branch, Iowa (0546494170), September 2019 through March 2020. Blue point, red star and percentiles (listed along the x-axes) represent site specific medians, outliers, and detection frequencies, respectively. Note that y-axes in a logarithmic scale. Targeted compounds are grouped by color; green for herbicides, blue for alkaloids, and orange for phytoestrogens. Only concentrations above the LOD are shown in the figure, but all measurements were included in the calculation of detection frequencies provided along the x-axes.

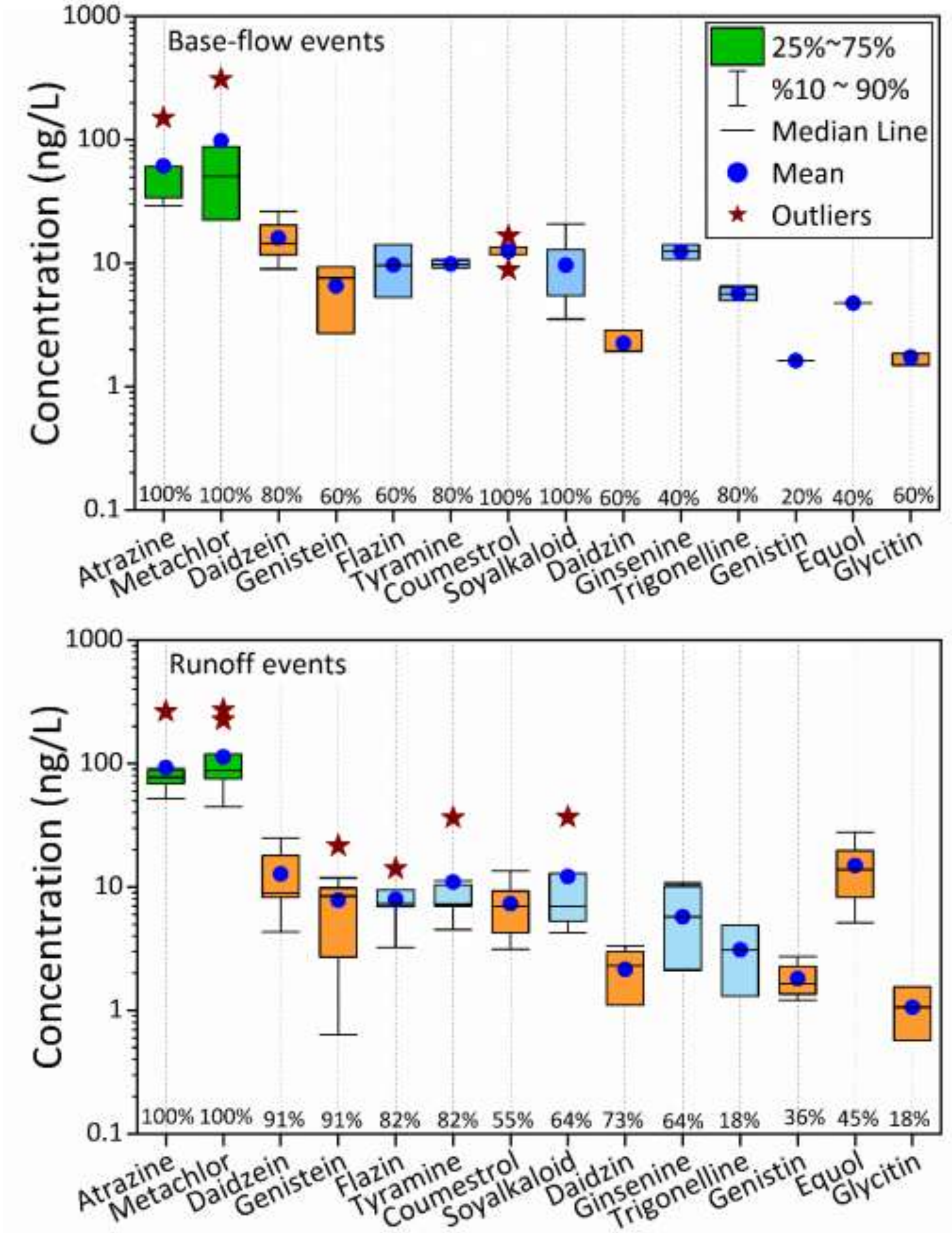


Figure S15. Concentrations of phytotoxins and herbicides (atrazine and metolachlor) for the samples collected during base-flow (n=5) and runoff events (n=11) at Old Mans Creek near Iowa City, Iowa (05455100), September 2019 through March 2020. Blue point, red star and percentiles (listed along the x-axes) represent site specific medians, outliers, and detection frequencies, respectively. Note that primary y-axes in a logarithmic scale. Targeted compounds are grouped by color; green for herbicides, blue for alkaloids, and orange for phytoestrogens. Only concentrations above the LOD are shown in the figure, but all measurements were included in the calculation of detection frequencies provided along the x-axes.

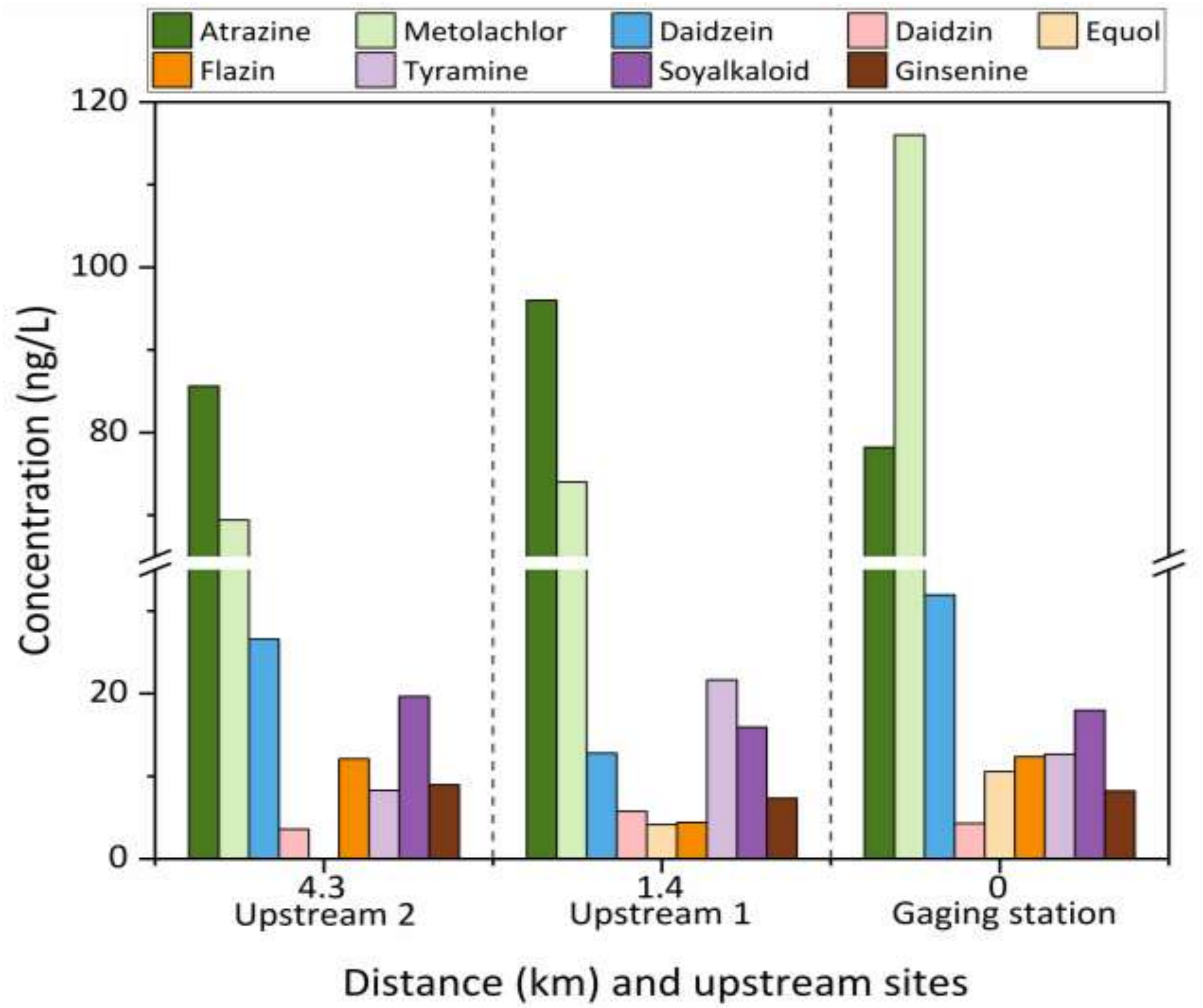


Figure S16. Concentrations of alkaloids, phytoestrogens, and herbicides (atrazine, and metolachlor) for the samples (n=2) collected at West Branch Wapsinonoc Creek at West Branch, Iowa (0546494170), and upstream sites 1 and 2, December 29, 2019. Coordinates (latitude & longitude) and the distance of upstream sites from gaging station location of 41.670028, -91.344250, upstream site 1: 41.685093, -91.346531, 1.4 km; upstream site 2: 41.702671, -91.366165, 4.3 km. Note the split scale of the y-axes.

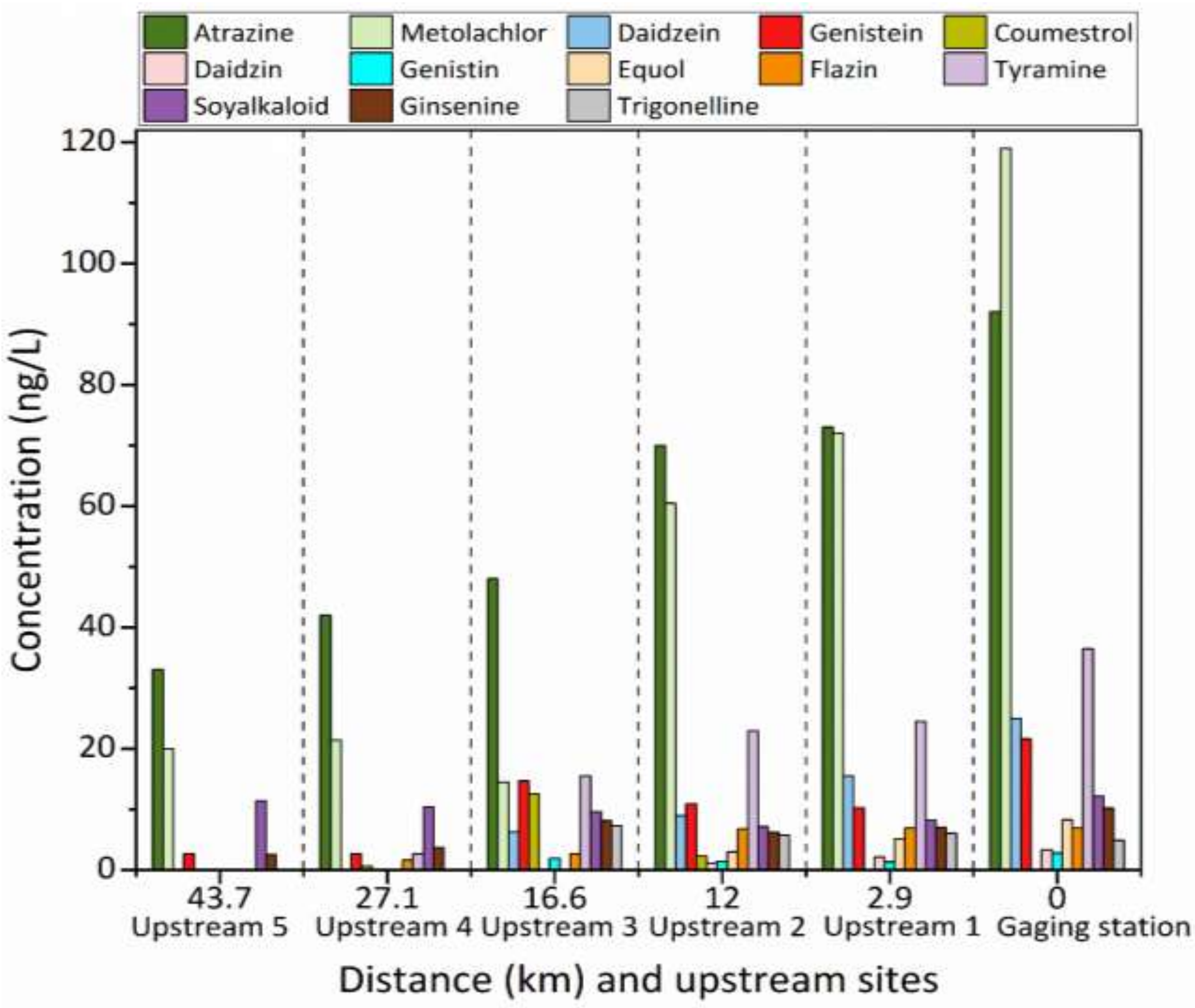


Figure S17. Concentrations of alkaloids, phytoestrogens, and herbicides (atrazine, and metolachlor) for the samples (n=5) collected at the Old Mans Creek near Iowa City, Iowa (05455100), and upstream sites 1-5, December 29, 2019. Coordinates (latitude & longitude) and the distance of upstream sites from the gaging station location of 41.606497, -91.615656; upstream site 1: 41.608519, -91.638557, 2.9 km; upstream site 2: 41.604250, -91.709569, 12.0 km; upstream site 3: 41.601135, -91.751027, 16.6 km; upstream site 4: 41.603295, -91.829931, 27.1 km; upstream site 5: 41.622762, -91.992910, 43.7 km.

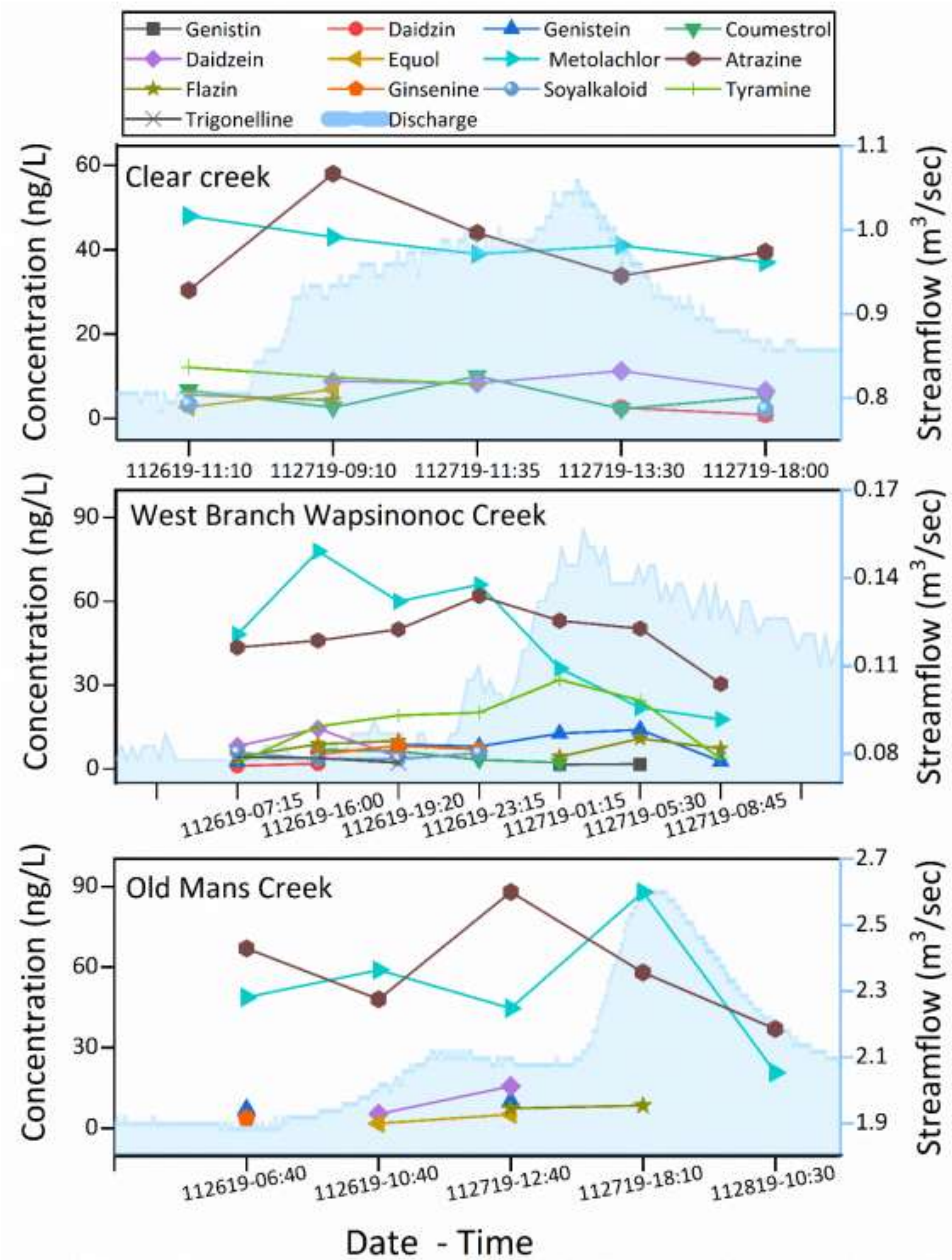


Figure S18. Concentrations of phytotoxins, and herbicides (atrazine and metolachlor) for the samples collected during the storm events, and streamflow at Clear Creeknear Oxford, Iowa (n=5), West Branch Wapsinonoc Creek at West Branch, Iowa (n=7), and Old Mans Creek (n=5) near Iowa City, Iowa, November 2019.

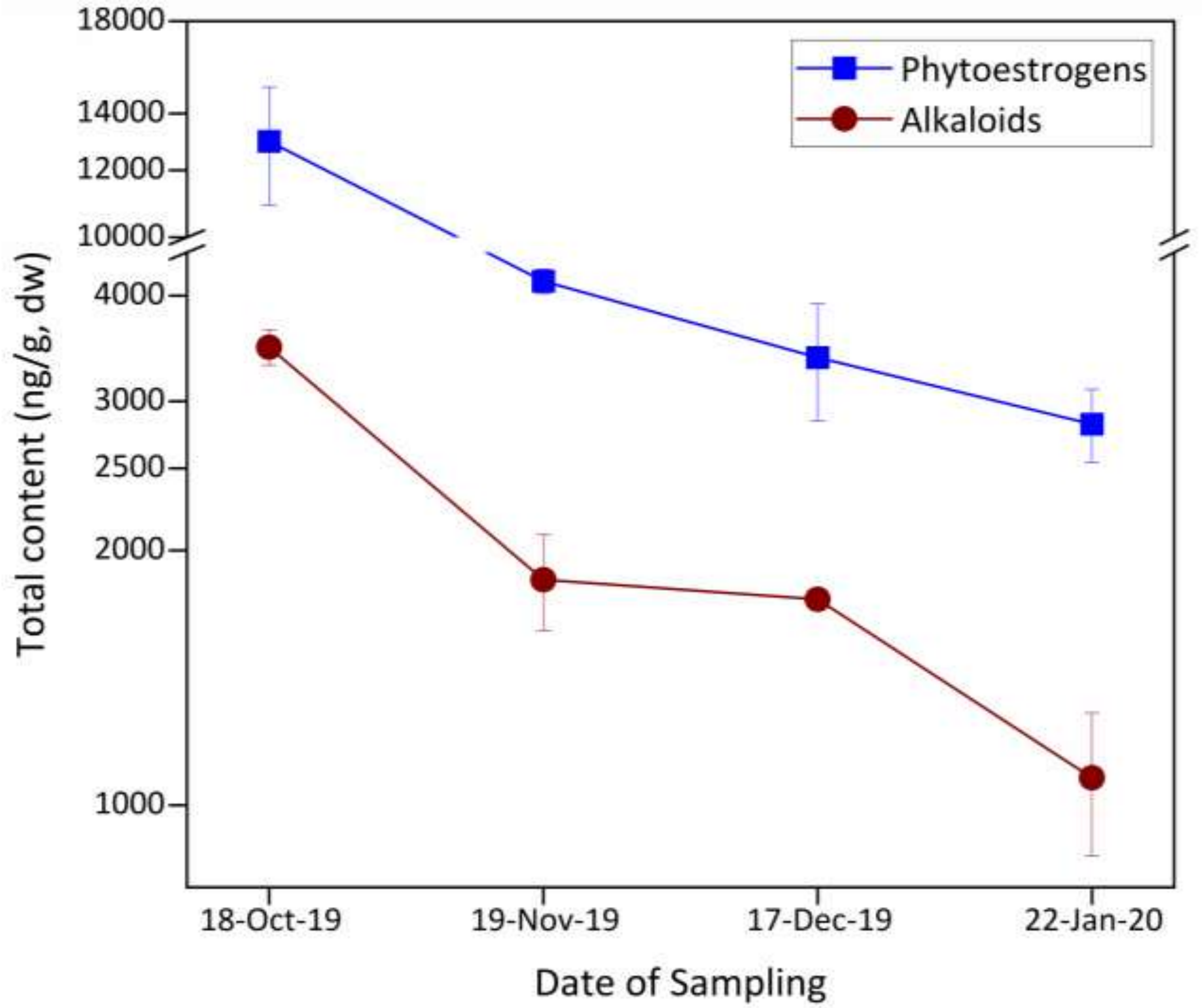


Figure S19. Alkaloid and phytoestrogen concentrations (ng/g) in plant tissues sampled from a selected agricultural field in the Clear Creek Basin near Oxford, Iowa, collected monthly on October 18, November 19, December 17, 2019, and January 22, 2020. Data points are given as mean (n =2) and error bars indicate standard deviations. Note the split scale of the y-axes.

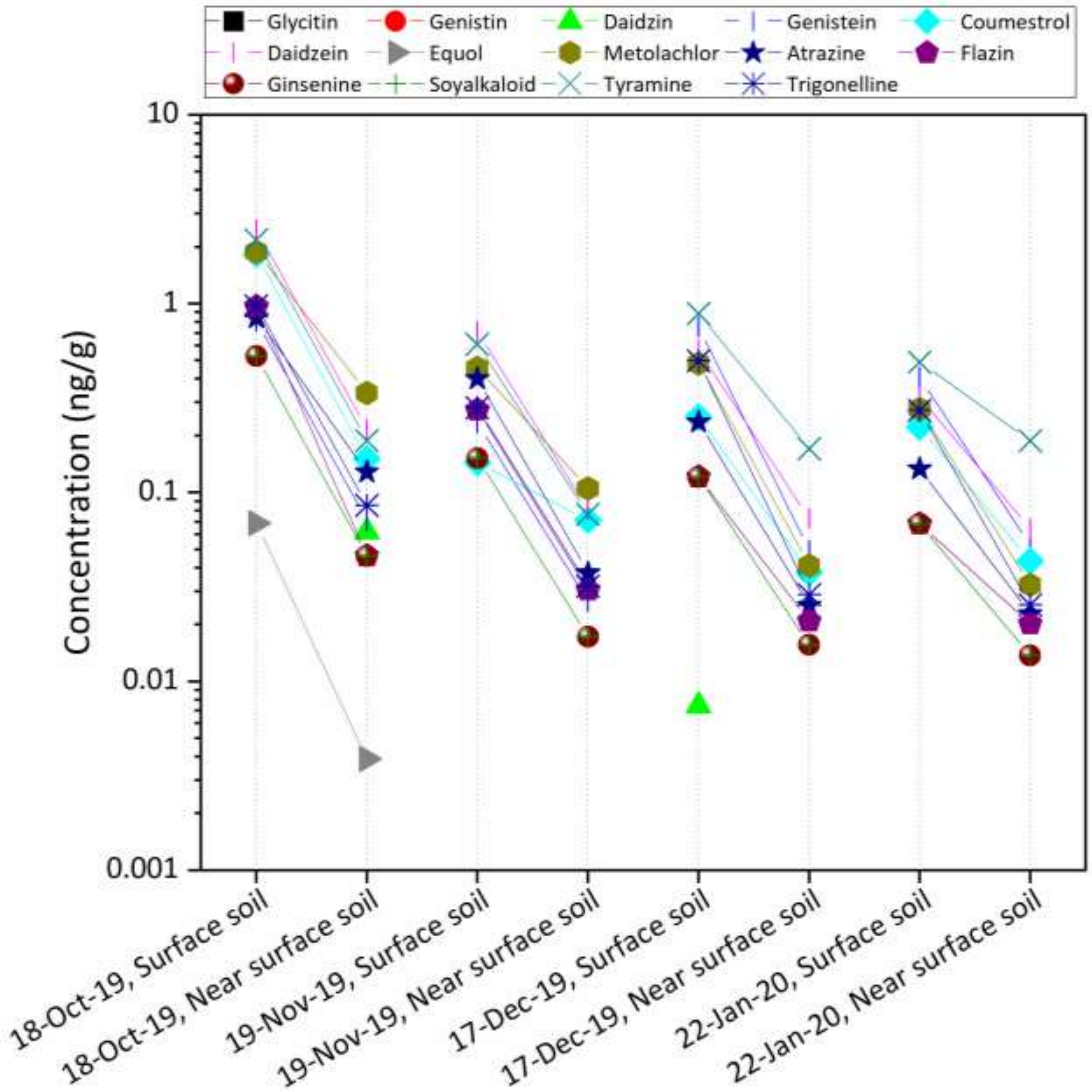


Figure S20. Alkaloid and phytoestrogen concentrations (ng/g) in soils sampled from a selected agricultural field in the Clear Creek Basin near Oxford, Iowa, collected monthly on October 18, November 19, and December 17, 2019 and in January 22, 2020. Data points are given as mean of (n =2). Note that the y-axes is in a logarithmic scale.

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