

MOBILIZATION OF ENDOCRINE-DISRUPTING CHEMICALS AND ESTROGENIC ACTIVITY  
IN SIMULATED RAINFALL RUNOFF FROM LAND-APPLIED BIOSOLIDS

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**Abstract**—Municipal biosolids are commonly applied to land as soil amendment or fertilizer as a form of beneficial reuse of what could otherwise be viewed as waste. Balanced against this benefit are potential risks to groundwater and surface water quality from constituents that may be mobilized during storm events. The objective of the present study was to characterize the mobilization of selected endocrine-disrupting compounds, heavy metals, and total estrogenic activity in rainfall runoff from land-applied biosolids. Rainfall simulations were conducted on soil plots amended with biosolids. Surface runoff and leachate was collected and analyzed for the endocrine-disrupting compounds bisphenol A, 17 $\alpha$ -ethynylestradiol, triclocarban, triclosan, octylphenol, and nonylphenol; a suite of 16 metals; and estrogenic activity via the estrogen receptor-mediated chemical activated luciferase gene expression (ER-CALUX) bioassay. Triclocarban (2.3–17.3 ng/L), triclosan (<51–309 ng/L), and octylphenol (<4.9–203 ng/L) were commonly detected. Chromium (2.0–22  $\mu$ g/L), Co (2.5–10  $\mu$ g/L), Ni (28–235  $\mu$ g/L), Cu (14–110  $\mu$ g/L), As (1.2–2.7  $\mu$ g/L), and Se (0.29–12  $\mu$ g/L) were quantifiable over background levels. Triclosan, Ni, and Cu were detected at levels that might pose some risk to aquatic life, though levels of metals in the biosolids were well below the maximum allowable regulatory limits. The ER-CALUX results were mostly explained by background bisphenol A contamination and octylphenol in runoff, although unknown contributors or matrix effects were also found. Environ. Toxicol. Chem. 2011;30:2220–2228. © 2011 SETAC

**Keywords**—Emerging contaminants    Personal care products    Rainfall simulation    Metals    ER-CALUX

## INTRODUCTION

In the United States, 3.4 million dry tons of biosolids are used as soil amendment or fertilizer each year, 61% of the biosolids that are generated during municipal wastewater treatment [1]. Biosolids contain high levels of nutrients and organic matter that can be a valuable resource to agricultural sites and sites in need of remediation, and state and federal agencies have long promoted the practice of land application [2]. The beneficial reuse of biosolids must be evaluated relative to potential risks, which include, among other concerns, impacts to water quality because of mobilization of nutrients, heavy metals, pathogens, and organic compounds. Disposal and reuse of biosolids in the United States is subject to 40 CFR 503, which includes regulations governing acceptable land and climate characteristics, maximum application rates, and limits on the heavy metal and pathogen content of the biosolids ([http://ecfr.gpoaccess.gov/cgi/t/text/text-idx?c=ecfr&tpl=/ecfrbrowse/Title40/40cfr503\\_main\\_02.tpl](http://ecfr.gpoaccess.gov/cgi/t/text/text-idx?c=ecfr&tpl=/ecfrbrowse/Title40/40cfr503_main_02.tpl)).

Heavy metals in biosolids have historically been the primary concern related to their beneficial reuse. Several studies that have investigated the movement of metals from land-applied biosolids have described a higher-than-expected mobility of metals in column studies and have linked this phenomenon to transport associated with dissolved organic carbon (DOC) [3–5]. More recently, concerns have arisen over levels of several groups of organic chemicals in biosolids, including endocrine-disrupting chemicals and pharmaceuticals and personal care products. Although the effects these chemicals may have in the environment is uncertain, the potential risks have led

to a few studies that have examined their mobilization from land-applied biosolids. These studies included analysis of pharmaceuticals and personal care products in tile drainage [6,7] and surface runoff [8,9] after application of liquid municipal biosolids and dewatered municipal biosolids. In general, concentrations found in tile drainage and surface runoff were far lower than those found in treated wastewater effluent, and, with few exceptions, are well below the lowest known environmental endpoints. Cell-based bioassays have been used to characterize endocrine-disrupting potential in municipal biosolids [10,11] but have not, to our knowledge, been used previously to characterize the movement of this potential in runoff from land-applied biosolids. In the present study, the chemical-activated luciferase gene expression (ER-CALUX) assay, which uses light to measure binding to the estrogen receptor, is applied to rainfall runoff and leachate.

The objective of the present study is to characterize the mobilization of selected endocrine disrupting chemicals, heavy metals, and estrogenic activity in runoff from land-applied biosolids during controlled rainfall simulations. The study examines whether the rate of loss of these constituents in surface runoff changes throughout the storm and whether overall levels could pose a threat to receiving waters. Furthermore, results of the present study will be used to determine whether current regulations governing maximum allowable levels of metals in biosolids are sufficiently protective to address concerns related to pharmaceuticals and personal care products.

## MATERIALS AND METHODS

*Chemicals*

Acetonitrile, ethyl acetate, methanol, acetone, hydrochloric, nitric, and acetic acid were all obtained from Fisher Scientific and were the highest purity available. Bisphenol A (BPA),

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17 $\alpha$ -ethynylestradiol, 4-*t*-octylphenol (OP), *n*-nonylphenol (NP), triclosan (TCS), triclocarban (TCC), and zearalanone were obtained from Sigma-Aldrich. Deuterated triclocarban (TCC-d7), <sup>13</sup>C-labeled triclocarban (<sup>13</sup>C<sub>6</sub>-TCC), <sup>13</sup>C-labeled bisphenol A (<sup>13</sup>C<sub>12</sub>-BPA), and <sup>13</sup>C-labeled nonylphenol (<sup>13</sup>C<sub>6</sub>-NP) were obtained from Cambridge Isotope laboratories. 17 $\beta$ -Estradiol (E2) and phenol red-free Dulbecco's modified Eagle medium were purchased from Sigma; cell culture reagents and media were obtained from Gibco/BRL. Properties of chemicals analyzed are shown in Table 1.

#### Batch desorption experiments

Batch desorption experiments were performed to examine how the water-extractable biosolids concentrations compared with the solvent-extractable concentrations, to compare against concentrations found in runoff from the rainfall simulations (described later), and to compare the water-extractable concentrations of analytes in the biosolids sample used in the rainfall simulations with other biosolids samples from publicly owned treatment works (POTWs) in California. Six different biosolids samples from six POTWs in California (including the biosolids used in the rainfall simulations) and one commercial biosolids fertilizer product were analyzed. Descriptions of the facilities from which these biosolids samples originated can be found in T. Ogunyoku and Young (University of California Davis, Davis, CA, USA, unpublished data). Approximately 1 to 2 g biosolids and approximately 40 ml Milli-Q water were added to centrifuge tubes. The biosolids were then broken up by means of crushing and stirring with mini-spatulas, and the tubes were tumbled at room temperature in the dark for 1 h to simulate approximate rainfall simulation contact times. The tubes were centrifuged for 30 min at 3,390 g, then decanted into new vials. A total of 5 ml was removed and acidified for metals analysis as described later, and the remaining water was extracted via solid-phase extraction and analyzed via liquid chromatography/mass spectrometry (LC/MS) for organics as described later. Results were corrected by recovery of surrogates, which were spiked into the solution immediately before extraction, and method blank concentrations were subtracted.

#### Rainfall simulator and test-plot design and operation

Two identical rainfall simulators were placed side-by-side over each of three test-plots. Rainfall simulators were constructed largely according to Battany and Grismer [12]. Briefly, each simulator consisted of a 1 m  $\times$  1 m acrylic reservoir with 900 hypodermic syringe needles (23-gauge) as drop formers suspended 1.7 m above plot surfaces by aluminum and polyvinyl chloride tubing. Plastic sheeting was attached to each simulator to prevent wind from blowing drops off course. Water was supplied to each from a polyethylene tank filled with well

water by a centrifugal pump. A needle valve on each simulator provided flow adjustment control. As water in the tank was used, a valve opened and the tank was refilled by fresh well water filtered through a sediment filter. Water in the tanks was continuously cycled through a carbon filter attached to each tank by the pumps to remove chlorine and other contaminants from the water. Before each simulated rain event, steel pans were inserted between the simulator and the plot to allow the rainfall rate to reach steady state. Rainfall was directed off these pans into a graduated cylinder and timed to calibrate the rainfall rate, which was always adjusted to 60 mm/h. This intensity was meant to approximate the maximum 30-min rainfall recorded at most rainfall stations in the Sacramento River Basin. Storm simulation duration was variable because the simulations were stopped when 24 L of runoff had been collected. Drops were an estimated 2.5 mm in diameter, and in falling 1.7 m, reached approximately 65% of their terminal velocity at the time of impact on the soil surface [12,13].

Three replicate plots were constructed. The plots consisted of a 2 m long  $\times$  1 m wide  $\times$  0.38 m deep box constructed out of 1.6-cm (5/8") plywood. Each box was lined with 0.15-mm (6 mil) plastic sheeting adhered by silicone and fastened across the top edge by duct tape. A makeshift tile-drain consisting of 1.3-cm (1/2") polyvinyl chloride tubing with 0.64-cm (1/4") holes drilled at 2.5-cm (1") intervals was placed on the bottom of the box with a drain exiting at the down-slope end of the box. The bottom layer of fill was 6.4 cm (2.5") of all-purpose pea gravel prerinse to remove clay particles. The middle layer of fill was an agricultural soil 19 cm (7.5") thick, compacted with a 15 cm  $\times$  15 cm (6"  $\times$  6") soil tamper approximately every 5 cm (2"). The top layer was 7.6 cm (3") of loose soil. The soil was a sandy loam with the following properties: pH 8.0; electrical conductivity 0.60 dS/m; cation exchange capacity 20.7 mEq/100 g; organic matter 0.64%; organic carbon 0.37%; CaCO<sub>3</sub> 1.3%; and 60:26:14 sand:silt:clay. This soil is a typical agricultural soil found in the region, although it contained more sand and less clay than soils that have previously been analyzed in nearby agricultural fields. Care was taken to ensure minimal leakage out of the plastic sheeting so that all water not running off would exit through the tile drain. One end of each plot was elevated using a pallet-jack to achieve a 3.5- to 4-degree slope. Although most fields in California's Central Valley are very flat, this slope is within typical ranges found in agricultural fields. A collection flume and cover to block rainfall from directly entering the flume similar to that used in Battany and Grismer [12] were attached to the downslope end of the plot. The flume directed water through Teflon tubing into collection bottles.

An initial control storm (CS) and three successive treatment storm events (TS1, TS2, and TS3) after biosolids application were simulated on each of the three replicate plots between

Table 1. Chemical properties

Constituent	Chemical Abstracts Service no.	Molecular weight g/mol	pK <sub>a</sub>	Solubility <sup>a</sup> mg/L	Log K <sub>ow</sub> <sup>a</sup>	EEF CALUX <sup>b</sup>
Bisphenol A (BPA)	80-05-7	228.3	9.6 [25]	85 [25]	3.32-4.16 [25]	7.80 $\times$ 10 <sup>-6</sup> [26]
17 $\alpha$ -Ethinylestradiol (EE2)	57-63-6	296.4	10.4 [27]	4.8 [28]	3.67-4.15 [28]	1.2 [26]
Triclocarban (TCC)	101-20-2	315.6	12.7 [29]	0.02-1.55 [7]	4.9 [29]	0 [30]
4- <i>t</i> -Octylphenol (OP)	140-66-9	206.3	10.2 [21]	12.6 [21]	4.12 [21]	1.40 $\times$ 10 <sup>-6</sup> [26]
<i>n</i> -Nonylphenol (NP)	104-40-5	220.4	10.7 [21]	5.43 [21]	4.48 [21]	2.30 $\times$ 10 <sup>-5</sup> [26]
Triclosan (TCS)	3380-34-5	289.54	8.1 [19]	1.97-10 [7]	4.8 [29]	0 [30]

<sup>a</sup> Measured or estimated at 20°C, pH 7.

<sup>b</sup> Molar based estradiol equivalent factor (EEF), the estrogenic potency relative to estradiol, using the estrogen receptor-chemical activated luciferase gene expression (ER-CALUX) bioassay.

March 23 and April 24, 2009. The control storm was simulated 5 d before application of biosolids. Biosolids were applied to plots (day 0) at a rate equivalent to approximately 10 tons/acre ( $2.25 \text{ kg/m}^2$ , dry wt) and incorporated into the top 3 to 6" (7–15 cm) soil less than 24 h later (day 1). The application rate is the maximum typically applied to corn, and a moderate rate for soybeans. Treatment storms TS1, TS2, and TS3 were conducted at days 3, 9, and 24, respectively. Soil was undisturbed and allowed to dry between the treatment storms. The top three to six inches (7–15 cm) of soil were loose and disturbed for CS and TS1 but compacted by the storm events for the start of TS2 and TS3. Approximately 16 mm (0.6 inches) of natural rain fell on the plots between TS2 and TS3 (days 11–14); however, the plots were not inclined, and the relatively light intensity of the rainfall meant that no runoff and very little leaching occurred.

Biosolids were obtained from a POTW in the Central Valley of California. The POTW, which provides sewerage to residential, commercial, and industrial users, as well as state correctional facilities, is a conventional activated sludge facility, and sludge undergoes anaerobic digestion and is dewatered on a belt filter press. Biosolids were collected directly after the belt press and had an initial solids content of 15%. Typically, the facility allows biosolids to dry in the sun for several months before being used or disposed, and solids contents at that time are at least 70%. To simulate these conditions, biosolids were predried in a large outdoor oven at  $85^\circ\text{C}$  for 18 h to achieve a solids content of 30%. After spreading, high winds and low humidity further dried biosolids, and solids content was approximately 60% at the time of incorporation into the soil.

#### *Analysis of biosolids*

Biosolids samples were prepared and analyzed for TCC and TCS according to methods by T. Ogunyoku and Young (unpublished data). This method had been previously developed for TCC and TCS only, so no attempt was made to measure levels of BPA,  $17\alpha$ -ethynylestradiol, NP, or OP in biosolids. Biosolids samples (1 g) were dried in an oven at  $70^\circ\text{C}$  for 24 h and homogenized. Samples were spiked with the surrogate standard (TCC; d7) and allowed to dry, extracted with 15 ml 1:1 acetone:methanol on a shaker table for 24 h at  $55^\circ\text{C}$ , centrifuged for 30 min at 3,390 g, and the supernatant filtered to  $0.2 \mu\text{m}$  using polytetrafluoroethylene syringe filters. A 300- $\mu\text{l}$  aliquot of the sample was transferred to a vial and diluted with 250  $\mu\text{l}$  pure methanol and 50  $\mu\text{l}$  of  $2 \mu\text{g/ml}$   $^{13}\text{C}_6$ -TCC (internal standard) in methanol, and analyzed by LC/MS. A Phenomenex C18 Prodigy (5  $\mu\text{m}$ , 100  $\text{\AA}$  pore size;  $2.0 \times 100 \text{ mm}$ ) with a guard column ( $2.0 \times 4.0 \text{ mm}$ ) was used at  $40^\circ\text{C}$  with an injection volume of 10  $\mu\text{l}$ . A gradient method consisting of 90:10 MilliQ water:acetonitrile with 10 mM acetic acid and 50:50 methanol:acetonitrile with 35 mM acetic acid at a constant flow rate of 0.500 ml/min was used for analysis of the sample. Detection was achieved using an Agilent 1100 series LC/mass spectrometry detection ion trap with electrospray ionization in negative ion mode and multiple reaction monitoring. The criteria used for positive identification of TCC were the retention time  $\pm 0.1 \text{ min}$ , the parent ion ( $m/z$  313), and transition ion ( $m/z$  160). Triclosan identification criteria were retention time  $\pm 0.1 \text{ min}$ , the parent ion ( $m/z$  287), and spectra matching. Triclocarban product ion and TCS parent ion were used for quantification.

Metals were extracted using a modified version of U.S. Environmental Protection Agency (U.S. EPA) method 3050 [14]. Briefly, 5 ml trace metals grade nitric acid was added to

approximately 500 mg predried biosolids in test tubes. The tubes were capped and left for 24 h, then sonicated for 1 h at  $50^\circ\text{C}$ . Slowly, 5 ml 1:1 30% hydrogen peroxide was added, and the tubes were allowed to sit for 1 h. After another 1-h sonication, the solution was diluted to 50 ml with Milli-Q water. Samples were analyzed for metals using an Agilent 7500i inductively coupled plasma mass spectrometer. Dilute nitric acid in ultrapure water rinses were analyzed every 20 to 25 samples to quantify machine drift. Counts-per-second of 10 elements were detected for each sample. Elements measured were Cr, Co, Ni, Cu, Zn, As, Se, Ag, Cd, and Pb. Instrument detection limits (3 standard deviations [SDs], U.S. EPA 6020) were approximately 0.01 ng/ml. Sample concentrations always exceeded method detection limits, which varied by metal, but were all less than or equal to  $0.2 \mu\text{g/L}$  ( $0.02 \text{ mg/kg}$ ). Concentrations for each element were adjusted to a baseline zero point by subtracting the average background levels determined in all analyzed rinses throughout the sample run.

#### *Analysis of runoff*

Six runoff samples from each storm simulation were collected in 4-L amber bottles. Leachate samples were collected in amber bottles and periodically dumped into a large glass reservoir. A single 2.5-L sample of leachate was taken from the composite reservoir at the end of each simulation. Total suspended solids (TSS) concentration was measured in all samples, using Standard Method 2540D [15]. Subsamples of 50 ml were centrifuged,  $0.45 \mu\text{m}$  filtered, and analyzed for DOC (method detection limit =  $0.5 \text{ mg/L}$ ) for one replicate set of samples from each storm simulation. Approximately 425 ml of each sample was centrifuged for 45 min at 3,390 g in polypropylene centrifuge bottles. From each sample, 5 ml was removed and acidified with 50  $\mu\text{l}$  nitric acid for metals analysis via inductively coupled plasma mass spectrometry. The remaining supernatant was acidified to pH 2 with hydrochloric acid and extracted by solid-phase extraction within 24 h. The extraction was carried out on Waters OASIS HLB 6-cc disposable cartridges. Each cartridge was conditioned with 5 ml 75:25 ethyl acetate:acetone mixture followed by 5 ml methanol and then 5 ml acidified (pH 2 with HCl) Milli-Q water. Samples of approximately 400 ml were loaded at a rate of 2 ml/min and then dried for 10 min. Cartridges were eluted with 8 ml 75:25 ethyl acetate:acetone. Eluates were evaporated to dryness under a gentle stream of nitrogen at  $65^\circ\text{C}$ . Finally, extracts were redissolved in 150  $\mu\text{l}$  dimethylsulfoxide for analysis via the ER-CALUX bioassay and LC/MS. For TS1, separate samples were prepared for LC/MS and ER-CALUX, and surrogate compounds  $^{13}\text{C}_{12}$ -BPA, TCC d7, and  $^{13}\text{C}_6$ -NP were spiked into samples that were to be run on the LC/MS before extraction to calculate average recovery.

Extracts were analyzed for organics by LC/MS/MS. Injection volume was 50  $\mu\text{l}$ , and separation was achieved on an Ascentis C18  $25 \text{ cm} \times 4.6 \text{ mm}$ , 5  $\mu\text{m}$  (Supelco) column at  $30^\circ\text{C}$ . The binary mobile phase consisted of 0.9 ml/min (A, Milli-Q; B, acetonitrile), each with 0.2% acetic acid. The gradient was as follows: 40% B from 0 to 5 min; linear gradient to 75% B at 19 min; linear gradient to 95% B at 21 min; linear gradient to 100% B at 25 min; 100% B until 27 min; and linear gradient to 40% B at 33 min. Detection was achieved using an Agilent 1100 series LC/MSD ion trap with electrospray ionization in negative ion mode and multiple reaction monitoring. From 20 to 33 min, postcolumn injection of 0.1 ml/min of 50 mM ammonium hydroxide was used to amplify the signal of OP and NP.

The requirements for detection were the presence of the fragment ion above the instrument detection limit and elution within the expected retention time window ( $\pm 0.1$  min). The sample analyte responses for all three storm simulations were corrected by the average recovery of their respective surrogates from TS1 samples. Calibration was via external standards, and solvent blanks and a mid-level standard were included to ensure there was no carryover, degradation, or significant change in instrument response. Method detection limits were determined via extraction and analysis of seven replicate, low-level Milli-Q water samples spiked with target compounds, and subsequent multiplication of the standard deviation of the response by the associated Student's *t* value (per U.S. EPA guidance), and were as follows: BPA 5.2 ng/L; 17 $\alpha$ -ethynylestradiol 10 ng/L; TCC 1.0 ng/L; TCS 51 ng/L; OP 4.9 ng/L; and NP 8.8 ng/L. All data were analyzed using Bruker Daltonik DataAnalysis version 2.1 software.

Recombinant human ovarian cancer cells (BG1Luc4E<sub>2</sub>, ER- $\alpha$ -positive) were grown and maintained as described in Rogers and Denison [16]. These cells contain a stably integrated, ER-responsive firefly luciferase reporter plasmid, pGudLuc7ERE. Cells were maintained in estrogen-stripped media for 5 d before they were plated into white, clear-bottomed 96-well tissue culture dishes at 75,000 cells/well and allowed to attach for 24 h. Cells were then incubated with carrier solvent (dimethyl-sulfoxide: 1% final solvent concentration), E2 (1 nM), and runoff sample extracts for 24 h at 37°C. For luciferase measurement, sample wells were washed twice with phosphate-buffered saline, followed by the addition of cell lysis buffer (Promega); the plates were then shaken for 20 min at room temperature to

allow cell lysis. Luciferase activity in each well was measured with an Orion microplate luminometer (Berthold) with automatic injection of Promega stabilized luciferase reagent. Luciferase activity in each well is expressed relative to that maximally induced by 1 nM E2.

After the runoff sample preparation steps, metals were analyzed by the same methods used for metals in biosolids extracts.

## RESULTS

### Batch desorption experiments

Results for the biosolids that were also used in the rainfall simulations are shown in Table 2. Results for all seven of the biosolids tested are shown in Table 3. The water-extractable concentrations of metals in the rainfall simulator biosolids were generally the highest among the POTW samples. The commercial fertilizer product Milorganite had the highest levels of leachable metals among all of the samples. The water-extractable concentrations of OP, TCS, and TCC of these biosolids were toward the low, middle, and high end of the range of results found for the seven biosolids samples analyzed (Table 3). Estrogenic activity as measured by ER-CALUX was lowest for the rainfall simulator biosolids among the seven samples.

### Rainfall simulations

Duration of storms ranged from 29.5 (TS3) to 83 min (CS), whereas volume of water leached during the simulation ranged from 0.5 (TS3) to 17.5 L (CS). The average duration across

Table 2. Levels of constituents measured in biosolids, runoff, and leachate<sup>a,b</sup>

Constituent	Biosolids concentrations			Runoff and leachate event mean concentrations								Units
	Total extractable <sup>c</sup> (mg/kg)	Water extractable (mg/kg)	Ceiling concentrations <sup>d</sup> (mg/kg)	Control storm (CS)		Treatment storm 1 (TS1)		Treatment storm 2 (TS2)		Treatment storm 3 (TS3)		
				Runoff	Leachate	Runoff	Leachate	Runoff	Leachate	Runoff	Leachate	
Cr	53.6	0.13	-	2.8 (A)	6.7	3.4 (A)	22	3.3 (A)	2.0	0.07 (B)	9.0	$\mu\text{g/L}$
Co	5.1	0.16	-	0.69 (A)	0.46	2.5 (B)	10	8.8 (C)	4.1	2.8 (B)	8.6	$\mu\text{g/L}$
Ni	57.6	1.66	420	6.4 (A)	6.8	39.6 (B)	235	90.9 (C)	46	28.1 (B)	51	$\mu\text{g/L}$
Cu	362.8	0.84	4300	0.25 (A)	2.2	19 (BC)	110	26.8 (B)	14	11.4 (C)	27	$\mu\text{g/L}$
Zn	538.0	0.98	7500	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ	$\mu\text{g/L}$
As	4.8	0.15	75	0.5 (A)	1.6	2.7 (B)	2.5	2.8 (B)	2.1	1.7 (C)	1.2	$\mu\text{g/L}$
Se	6.1	0.10	100	0.09 (A)	1.8	0.94 (B)	12	1.6 (C)	0.73	0.29 (A)	2.3	$\mu\text{g/L}$
Ag	3.4	0.00027	-	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ	$\mu\text{g/L}$
Cd	1.6	0.0015	85	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ	$\mu\text{g/L}$
Pb	7.8	0.012	840	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ	$\mu\text{g/L}$
TCC	17.6	0.11	-	ND	ND	13.1 (AB)	2.7	6.3 (A)	2.3	17.3 (B)	5.2	$\text{ng/L}$
TCS	15.9	0.12	-	ND	ND	ND	ND	282.1 (A)	ND	309.6 (A)	ND	$\text{ng/L}$
OP	NM	0.012	-	ND	ND	41.5 (A)	ND	82.7 (A)	ND	203.2 (B)	38	$\text{ng/L}$
NP	NM	ND	-	ND	ND	20 <sup>e</sup>	ND	38 <sup>f</sup>	ND	ND	ND	$\text{ng/L}$
EE2	NM	0.047	-	ND	ND	ND	ND	ND	ND	ND	ND	$\text{ng/L}$
BPA	NM	NQ	-	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ	$\text{ng/L}$
ER-CALUX	NM	37.5	-	17 (A)	30	43 (B)	15	55 (B)	42	50 (B)	19	% 1 nM E2
TSS	-	-	-	4,094 (A)	2,170	4,543 (A)	2,258	1,579 (B)	2,734	1,199 (B)	85	$\text{mg/L}$
DOC	-	-	-	1.0	3.4	24	207	83	42	18	34	$\text{mg/L}$

NM = not measured; ND = not detectable (i.e., below method detection limits); NQ = not quantifiable (i.e., not significantly different from field/laboratory blank results); OP = 4-*t*-octylphenol; NP = *n*-nonylphenol; EE2 = 17 $\alpha$ -ethynylestradiol; BPA = bisphenol A; ER-CALUX = estrogen receptor-mediated chemical activated luciferase gene expression; TSS = total suspended solids; DOC = dissolved organic carbon.

<sup>a</sup> Organic chemical and ER-CALUX abbreviations shown in Table 1. Method detection limits as follows: BPA 5.2 ng/L; EE2 10 ng/L; TCC 1.0 ng/L; TCS 51 ng/L; OP 4.9 ng/L; and NP 8.8 ng/L.

<sup>b</sup> (A), (B), and (C) represent runoff event mean concentration (EMC) results that are significantly different ( $p < 0.05$ ) between storms.

<sup>c</sup> Acid digestion for metals, solvent extraction for organics.

<sup>d</sup> Code of Federal Regulations Title 40 part 503.13 Table 1 Land Application Ceiling Concentrations.

<sup>e</sup>  $n = 2$ , ND omitted.

<sup>f</sup>  $n = 3$ , ND omitted.

Table 3. Batch experiment 1-h leachable concentrations of metals, organics, and ER-CALUX activity in biosolids samples from various publicly owned treatment works (POTWs) and a commercial biosolids product<sup>a,b</sup>

Constituent	POTW rainfall simulators	POTW 1	POTW 2	POTW 3	POTW 4	POTW 5	Milorganite
Cr	127	38.0	5.69	9.66	19.6	10.9	471
Co	157	44.0	14.2	17.8	19.5	20.3	466
Ni	1660	464	66.1	113	409	144	2,377
Cu	841	469	48.5	162	86.1	40.4	14,012
Zn	977	584	217	355	120	177	4,081
As	147	58.5	43.8	42.8	67.0	44.3	421
Se	104	13.4	1.66	2.87	6.34	6.06	158
Ag	0.27	ND	ND	ND	11.1	ND	40.36
Cd	1.49	ND	ND	ND	ND	ND	6.19
Pb	12	8.21	ND	ND	ND	ND	13.35
TCC	105	93.5	5.79	12.2	24.2	7.38	9.77
TCS	120	ND	127	182	ND	ND	ND
OP	11.9	ND	2.38	58.1	153	37.7	ND
NP	ND	ND	24.5	36.5	13.8	ND	ND
EE2	47.2	ND	20.9	ND	25.1	ND	ND
BPA	ND	ND	ND	124	ND	ND	ND
ER-CALUX	22.2	41.9	43.9	48.9	42.3	44.4	29.5

<sup>a</sup> POTW = publicly owned treatment works; TCC = triclocarban; TCS = triclosan; OP = 4-*t*-octylphenol; NP = *n*-nonylphenol; EE2 = 17 $\alpha$ -ethynylestradiol; BPA = bisphenol A; ER-CALUX = estrogen receptor-mediated chemical activated luciferase gene expression.

<sup>b</sup>  $\mu\text{g}/\text{kg}$ , except ER-CALUX in % 1 nM E2.

replicates for CS, TS1, TS2, and TS3 were 67.1 (SD = 16.7), 55.3 (SD = 4.9), 32.6 (SD = 0.8), and 33.3 (SD = 3.3) min, respectively. Surface runoff flow rate generally increased throughout each storm and approached a steady-state value of between 0.8 and 1.45 L/min near the end of each storm. The top 3 inches (7.6 cm) of soil was loose (uncompacted) before CS and TS1, but it had compacted and settled because of previous storms before TS2 and TS3. This accounts for some of the disparity in hydraulics between CS/TS1 and TS2/TS3. Runoff timing and TSS concentrations are shown in Figure 1.

Dissolved organic carbon averaged 1 mg/L (standard error of the mean [SEM] = 0.19) in all fractions of CS runoff, and was 3.4 mg/L in CS leachate. In the one replicate of TS1 runoff fractions analyzed, DOC decreased from 33.5 to 15.3 mg/L throughout the storm and averaged 23.9 mg/L (SEM = 3.1), but was 206.9 mg/L in leachate. An increase in TS2 was seen, in which DOC in the first runoff fraction was 128 mg/L and

decreased throughout the storm to 46.6 mg/L, but was 41.8 mg/L in leachate. Levels in TS3 were similar to those in TS1, decreasing from 29.4 mg/L to 10.2 mg/L at the end of the storm, and was 33.9 mg/L in leachate. Total suspended solids varied from an average of 1.1 g/L (SEM = 0.23) in the final fraction of TS3 to 10.0 g/L (SEM = 1.3) in the initial fraction of TS1.

#### Organics

The biosolids used in the rainfall simulations contained 17.6 mg/kg TCC and 15.9 mg/kg TCS. Median levels in biosolids from U.S. POTWs are 22 mg/kg for TCC and 4 mg/kg for TCS [17]. The total contents of other organic chemicals were not measured in biosolids.

17 $\alpha$ -Ethinylestradiol was never detected in any of the samples. Nonylphenol was detected in less than 10% of samples and averaged 27.5 ng/L (SEM = 7.5) when detected. Bisphenol

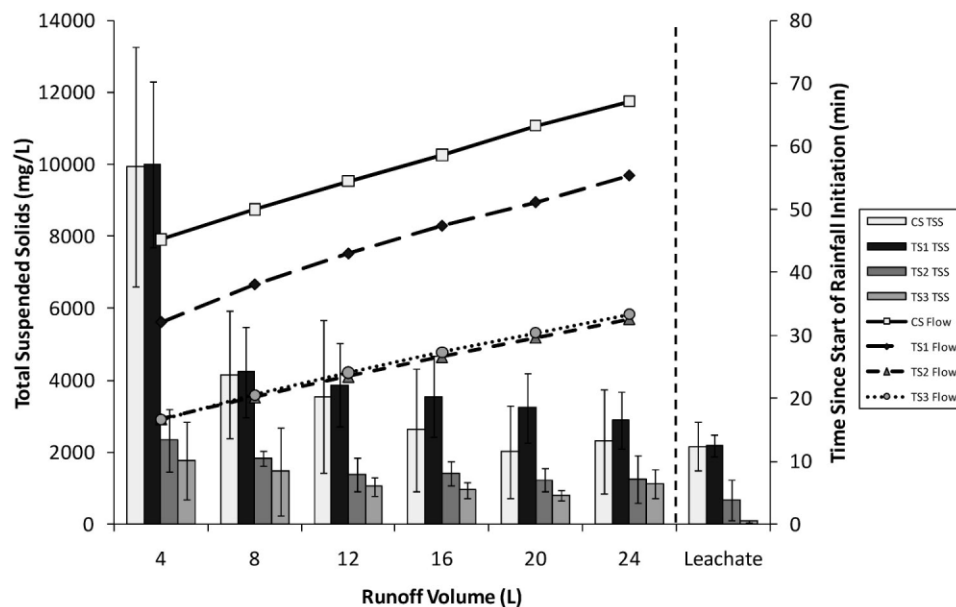


Fig. 1. Total suspended solids (TSS) and timing of runoff fractions versus runoff volume. Error bars for TSS measurements represent  $\pm 1$  standard deviation.

A in runoff was always detectable but was unable to be quantified because of large amounts in the field blanks. Octylphenol and TCC were both detected in every surface runoff sample except one (OP) from every treatment storm. Triclosan was also detected in every surface runoff sample from TS2 and TS3 but was not able to be detected in TS1 because of high matrix interference. Triclosan was never detected in any leachate sample. Octylphenol was not detectable in leachate for TS1 and TS2 but averaged 37.8 ng/L (SEM = 8.0) in TS3, approximately 18% of the surface runoff concentration. Triclocarban was generally detected in leachate, gradually increasing from TS1 to TS2 to TS3, and averaged 3.2 ng/L (SEM = 0.47).

For TCC, TCS, and OP, total mass lost in runoff appeared to be linearly correlated with runoff volume (Fig. 2). The magnitude of runoff losses showed an interesting trend with subsequent storms. For TCC, event mean concentrations (EMCs; total mass lost divided by total runoff volume) in runoff from least to greatest were TS2, TS1, then TS3. However, EMCs for TS2 and TS1 and for TS2 and TS3 were not significantly different ( $p < 0.05$ ). For OP, the EMC trend from least to greatest was in chronological order: TS1, TS2, then TS3, and all were significantly different from each other. Triclosan EMCs were not significantly different.

#### ER-CALUX

The ER-CALUX response showed a significant increase from CS to the treatment storms. Control storm response averaged 17% in runoff fractions and 29% in leachate, whereas

averages across treatment storms varied from 43 to 55% in runoff fractions and 13 to 43% in leachate (Table 2). Results of treatment storms did not differ significantly from each other, except the leachate fraction from TS2, which exhibited significantly greater response than TS1 and TS3. For each individual simulated storm event, runoff samples taken at different times within each event did not differ significantly from each other; therefore, no time-dependent trends were detected in response.

#### Metals

Three metals, Pb, Ag, and Cd, were negligible in all runoff samples. Zinc could not be quantified because of large amounts in field blanks. The only metals that showed significantly elevated runoff concentrations were Cr, Co, Ni, Cu, As, and Se; results are shown in Table 2. Cobalt, Ni, Cu, and Se showed the same trend: concentrations increased from TS1 to TS2, then decreased to below TS1 levels in TS3. Chromium and As concentrations remained approximately the same in TS1 and TS2, then decreased in TS3. All reported concentrations are corrected by subtracting average field blank concentrations.

#### DISCUSSION

Water-extractable concentrations of TCC and TCS were similar and bore a similar relationship to their solvent-extractable concentrations as well. The OP had an order of magnitude lower water-extractable concentrations, although in rainfall runoff its concentration was much higher than TCC. 17 $\alpha$ -

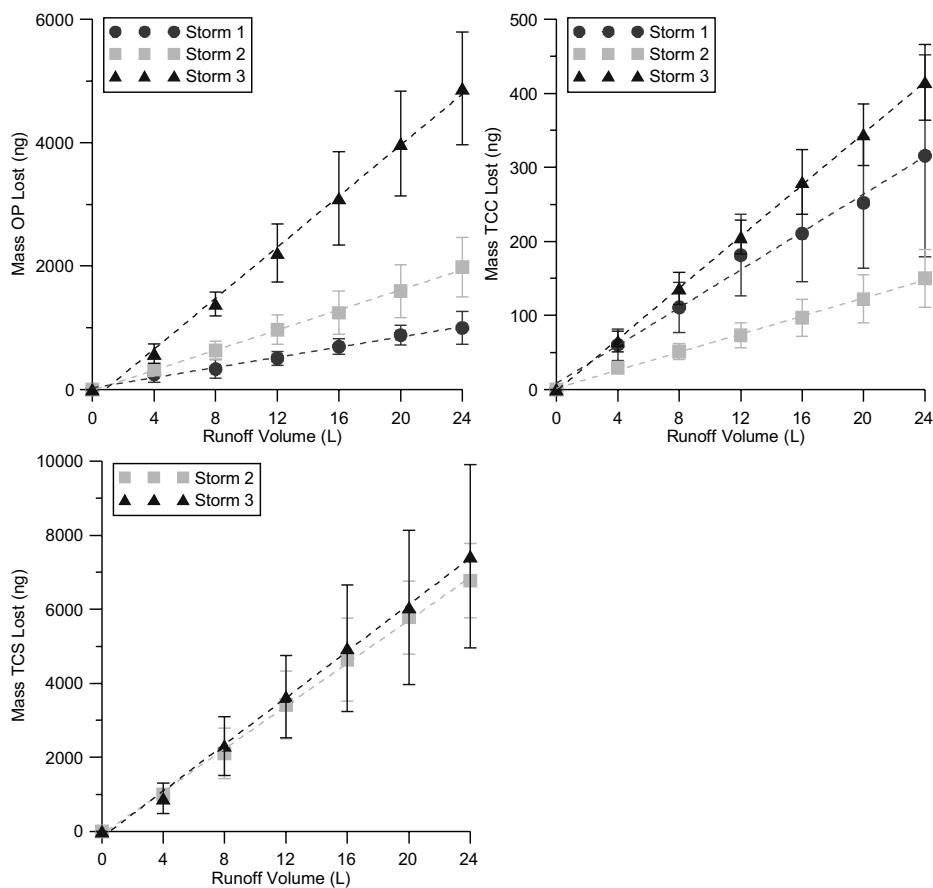


Fig. 2. Loss of 4-*t*-octylphenol (OP), triclocarban (TCC), and triclosan (TCS) with increasing runoff fraction. TCS was not quantifiable during Storm 1 because of high matrix interference. Error bars represent  $\pm 1$  standard deviation.

Ethinylestradiol was detected at low levels in the water-extractable portion, although it was never detectable in runoff samples.

Runoff and leachate flow behaved as expected, with runoff being lower and infiltration being higher when soil was loose and uncompacted, and runoff becoming higher and infiltration lower after a storm had compacted the soil. Total suspended solid levels also behaved as expected, with values being highest when soil was loose and early in storms. The DOC showed a dramatic increase in TS1 when compared with CS because of the addition of the biosolids, which contain high amounts of organic carbon. The increase in DOC from TS1 to TS2 in rainfall runoff can be attributed to one of two factors: as microbial activity increased in the biosolids after TS1, organic carbon was liberated from the biosolids matrix and could be more easily mobilized in runoff (which has less contact time with the biosolids than does leachate) in TS2, or the compacted nature of the soil in TS2 limited infiltration flow, and thus a greater portion of the mobilized DOC was transported in the runoff. The total mass of DOC lost from the plots in runoff and leachate was greatest for TS1, followed by TS2, then TS3. After TS2, the combination of microbial use of organic carbon and loss to runoff/infiltration are likely to have caused the decrease in TS3.

Concentrations of TCS found in runoff in the present study are within the range of concentrations found in similar studies (Table 4), but were slightly higher than most other reported values. This is likely attributable to a combination of factors, including lower soil organic carbon content, a higher concentration in the biosolids, and a higher soil pH. Concentrations of TCC in runoff were five times greater than the next highest concentration found in similar studies [9]. Again, this can be attributed to the lower soil organic carbon content and the significantly greater concentration of TCC in the biosolids than in previous runoff studies, although the levels here were typical of those found in U.S. biosolids [17]. The difference in concentrations of TCC and TCS in runoff in the present study is likely attributable to the difference in their  $pK_a$  values. Although the reported  $\log K_{OW}$ s for the two compounds are similar (Table 1), the  $pK_a$  of TCC is 12.7 [18], whereas TCS is 8.1 [19]. The soil pH was 8.0, and runoff pH varied from 7.8 to 8.0. This indicates that nearly half of the TCS present in the biosolids was ionized and is thus much more likely to partition into the runoff than the neutral TCC. The ratio of TCS/TCC in runoff in the present study varied from 18 to 45, which is similar to the ratios of approximately 32 to 40 found by others [9].

The reason for the difference between losses of TCC and OP with subsequent storms is not clear. An increasing trend from TS2 to TS3 was shown for both, however. Observation showed that the biosolids were breaking up into smaller pieces as they were impacted by raindrops and as they dried out after simu-

lated storms. The increased specific surface area exposed to runoff would likely lead to this common trend between TCC and OP. Additionally, as organic carbon was degraded by microorganisms or lost through runoff in previous storms, less was available for TCC and OP to sorb to, and thus, mobilization may have increased for this reason. Octylphenol ethoxylates present in the biosolids may have been transformed into OP over time, and thus the amount of OP available to mobilization increased with each additional storm.

Concentrations of dissolved organic compounds showed no relationship to measured DOC, which indicates that organics lost from the plots and measured were either truly dissolved or bound to suspended solids that were extracted with the dissolved fraction. Samples were not filtered, so the maximum particle size remaining in suspension after centrifugation was calculated to be approximately 3.6  $\mu\text{m}$  (for reference, an estimated 50% of 2.5- $\mu\text{m}$  particles and 25% of 1.9- $\mu\text{m}$  particles were removed via centrifugation). Dissolved organic compounds also showed no relationship to TSS within the samples. A limitation of this study was that organics bound to suspended solids were not analyzed. Because organics measured in this study strongly bind to particulates, likely total mass of compounds lost from the site would correlate to TSS measurements, although this should be confirmed by future studies.

Concentrations of TCC, TCS, and OP in runoff were below or toward the low range of typical levels in treated wastewater effluent (100–6,000 ng/L for TCC [20], 27–2,700 ng/L for TCS [20], and 20–1,700 ng/L for OP [21]). Concentrations of TCC and OP were below their most sensitive known environmental endpoints (60 ng/L no-observed-effect concentration for TCC, *Mysidopsis bahia* reproduction [20]; 1  $\mu\text{g/L}$  no-observed-effect concentration for OP, *Oncorhynchus mykiss* vitellogenin synthesis [22]), whereas TCS was slightly above its most sensitive value found in the literature (200 ng/L no-observed-effect concentration, *Selenastrum capricornutum* growth [20]).

The ER-CALUX response in CS of approximately 17% can largely be attributed to background BPA. Literature dose-response values for BPA indicate a response of 33% for 1,000 nM BPA (228  $\mu\text{g/L}$ ) [23], and our own dose-response tests indicate a typical response of 28% for 3,400  $\mu\text{g/L}$ . Some of the increase in response during treatment storms is attributable to detected concentrations of OP. The literature indicates a response of 15% to 1,000 nM OP (206  $\mu\text{g/L}$ ) [24], and our own dose-response tests indicate typical responses of 18% to the same concentration. Taking this into account, between 5 and 15% of response is unaccounted for by chemical measurements. This could be because of matrix enhancement effects or chemicals not analyzed, or possibly a combination of the two. For example, steroid hormones such as estradiol and estrone can be present in biosolids and elicit an estrogenic response [11], but

Table 4. Comparison of present study TCS and TCC results with other studies<sup>a</sup>

Study	Soil organic carbon (%)	Soil pH	Type of biosolids	Type of sample	Rainfall rate (mm/h)	TCS biosolids (mg/kg)	TCS water (ng/L)	TCC biosolids (mg/kg)	TCC water (ng/L)
Lapen et al. [6]	0.5–2.1	6.6–6.8	LMB	Tile drainage	Varied	3.8	3680 <sup>b</sup>	NA	NA
Edwards et al. [7]	0.4–2.0	7.0–7.5	DMB	Tile drainage	Varied	14	230 <sup>b</sup>	8.0	<5
Topp et al. [8]	1.97	7.5	LMB	Surface runoff	210	NR	258 <sup>c</sup>	NA	NA
Sabourin et al. [9]	1.97	7.5	DMB	Surface runoff	186	7.0	110 <sup>b</sup>	8.2	3.4
Present study	0.37	8	DMB	Surface runoff	60	16	310 <sup>c</sup>	18	17.3

<sup>a</sup> TCS = triclosan; TCC = triclocarban; LMB = liquid municipal biosolids; DMB = dewatered municipal biosolids; NR = not reported.

<sup>b</sup> Maximum detected concentration in a grab sample.

<sup>c</sup> Maximum event mean concentration (EMC).

these were not analyzed in the present study. As with organics, discussed earlier, ER-CALUX response showed no relationship to DOC, suggesting that measured responses were largely associated with freely dissolved organics. Finally, no significant decrease of ER-CALUX activity was apparent in the runoff from three storms over the three-week study period. As discussed, concentrations of OP increased throughout this period, although the specific mechanism for this increase is unknown. The conclusion that can be drawn from this result is that mobilization of estrogenic activity from land-applied biosolids is not limited to the first storm after biosolid application and may not even reach its maximum value in that storm. This complex process clearly requires further study.

Nickel concentrations were highly correlated linearly with DOC ( $r = 0.986$ ). Copper and other metals were not as tightly correlated but showed strong relationships to DOC nonetheless.

Results for Ni, Cu, and As are shown in Figure 3. These results confirm findings from previous studies on the mobility of metals from biosolids being correlated with DOC [3,5].

Concentrations of metals were thus highest in the leachate from TS1 and the runoff from TS2, because of the higher levels of DOC measured in those samples. As mentioned, the increase in DOC in TS2 runoff may have been attributable to either increased liberation from the biosolid matrix after TS1 or to reduced infiltration (relative to TS1) in the compacted soil of TS2. In either case, because biosolids are a source of organic carbon in the settings in which they are applied, and infiltration will generally be highest in the first storm after incorporation of the biosolids into the soil, these results have implications for when maximum concentrations of metals would be expected in leachate and runoff. In many settings, metals transported in leachate will be highest in the first storm after biosolids

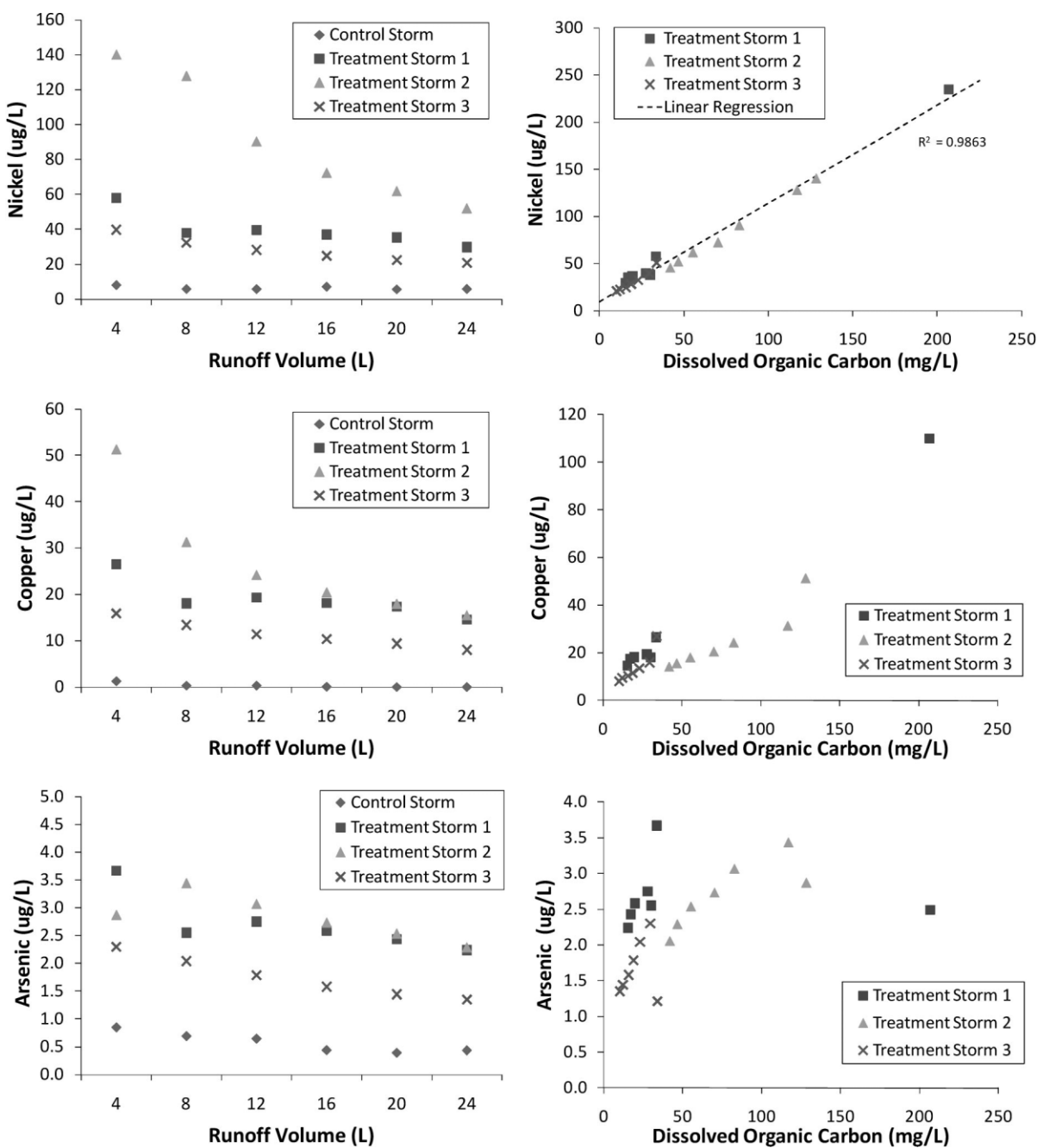


Fig. 3. Mean Ni, Cu, and As concentrations in runoff fractions versus runoff volume, and in runoff and leachate fractions versus dissolved organic carbon.



application and incorporation, whereas maximum concentrations in runoff may be seen in subsequent storms, eventually diminishing as DOC concentrations decrease.

Concentrations of Ni in runoff were below the California Toxics Rule (CTR; <http://www.epa.gov/region9/water/ctr/>) maximum freshwater criterion of 591  $\mu\text{g/L}$  (based on the study water hardness of 131  $\text{mg/L}$ ), and Cu concentrations were above the CTR maximum freshwater criterion of 18  $\mu\text{g/L}$  for TS1 and TS2, but below for TS3. Had rainwater been used for the simulations, hardness would have likely been much lower, which would have lowered the criteria and resulted in more exceedances.

Although levels of metals in biosolids were generally far below maximum allowable limits, possible environmental risk in rainfall runoff was demonstrated for Cu, Ni, and TCS. In a field setting, setbacks and erosion control (i.e., berms) can mitigate this risk, but the findings of this study nonetheless indicate that current limits on metal concentrations in biosolids may not be sufficiently protective with respect to either metal or TCS in runoff.

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