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EVALUATING THE EFFECTS OF TRICLOSAN ON 3 FIELD CROPS GROWN IN 4 FORMULATIONS OF BIOSOLIDS

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Abstract: A growing body of evidence suggests that amending soil with biosolids can be an integral component of sustainable agriculture. Despite strong evidence supporting its beneficial use in agriculture, there are concerns that chemicals, such as pharmaceuticals and personal care products, could present a risk to terrestrial ecosystems and human health. Triclosan is one of the most commonly detected compounds in biosolids. To date, laboratory studies indicate that triclosan likely poses a de minimis risk to field crops; however, these studies were either conducted under unrealistic exposure conditions or only assessed 1 or 2 formulations of biosolids. The purpose of the present study was to characterize the effects of triclosan on field crops in soils amended with 4 different formulations of biosolids (liquid, dewatered, compost, and alkaline-hydrolyzed), containing both background and spiked triclosan concentrations, following best management practices used in the province of Ontario. Three crop species (corn, soybean, and spring wheat) were evaluated using several plant growth endpoints (e.g., root wet mass, shoot length, shoot wet/dry mass) in 70-d to 90-d potted soil tests. The results indicated no adverse impact of triclosan on any crop-biosolids combination. Conversely, amending soil with biosolids either enhanced or had no negative effect, on the growth of plants. Results of the present study suggest little risk of triclosan to crops in agricultural fields amended with biosolids. *Environ Toxicol Chem* 2016;9999:1–13. © 2016 SETAC

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INTRODUCTION

The land application of municipal biosolids as a fertilizer for agricultural fields is becoming an increasingly important method of managing human waste. To date, industry, government, and academia report with greater confidence that biosolids offer organic nutrients favorable for crop production and soil tilth, contribute to nutrient recycling, and promote sustainable agricultural practices [1–6]. However, public concerns about the fate and potential risks of contaminants contained in biosolids continue to linger, with many calling for a cessation of the practice of land application until the risks are better understood.

Biosolids are produced from isolating the solid portion of wastewater influent received in a municipal wastewater treatment plant (WWTP). The remaining sludge is further stabilized to become biosolids, which can take liquid, dewatered, compost, or alkaline-hydrolyzed forms depending on the method and degree of additional processing [3,7,8]. Liquid municipal biosolids (LMBs) are manufactured by removing excess water through anaerobic or aerobic digestion to produce a product with 3% to 7% solids content [7]. Dewatered municipal biosolids (DMBs) are manufactured by removing water through belt pressing or centrifugation to produce a product with 15% to 40% solids content [7]. Compost municipal biosolids (CMBs) are manufactured through a static or dynamic stabilization process of moisture redistribution and oxygen introduction to produce a low-odor product with 21% to 50% solids content [7,9,10]. Alkaline-hydrolyzed municipal biosolids (AMBs) are manufactured through a high-shear mixing process that involves heat and alkali to produce a homogenous liquid product with 14% to 17% liquids content [11]. Regardless of the type of biosolids produced, land application must follow strict compliance with regulations [12,13] that govern allowable limits on pathogenic microorganisms, concentrations of metals, and nutrient content, and that follow various best management application practices [7].

Although biosolids are rich in nutrients and organic matter, they also contain a variety of contaminants, including pharmaceuticals and personal care products (PPCPs) that may be introduced to soils when biosolids are applied to land. Many PPCPs that are only partially degraded during waste treatment have been detected in biosolids [2,12,14–16]. Moreover, the presence of PPCPs in biosolids continues to generate concerns from the public about potential risks to human or environmental health [2,17–21]. One of the most commonly cited concerns pertaining to PPCPs in biosolids is the potential impact of antimicrobial compounds. Among antimicrobial compounds, triclosan is the most commonly detected in biosolids and has attracted considerable public and scientific attention [12,13].

Triclosan (2,4,4,′-trichloro-2′-hydroxydiphenyl ether; CAS 3380-34-5) is an antimicrobial agent used in health, veterinary, personal care, and household products that include soaps, shower gels, toothpastes, household detergents, plastics, and even toys [3,22–24]. Increasingly, triclosan is being assessed for toxicity to humans and the environment. Most pressing among the health effects surrounding triclosan is the putative potential for endocrine disruption and concerns about antibiotic resistance [23,25]. Although triclosan has low acute toxicity, significant amounts of this antimicrobial compound enter WWTPs and have been detected in surface waters, soil, and sewage sludge [3,24,26–28]. In two nationwide surveys

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conducted by the Canadian Council of Ministers of the Environment and the US Environmental Protection Agency, 97% and 92% of biosolids samples from WWTPs contained triclosan at concentrations ranging from $<102\ ng\ g^{-1}$ to $30\,600\ ng\ g^{-1}$ dry weight and $334\ ng\ g^{-1}$ to $133\,000\ ng\ g^{-1}$ dry weight, respectively [3,12,21]. The frequent and pervasive occurrence of triclosan in biosolids reflects its physicochemical properties (Table 1) that favor sorption to soil particles and organic matter, and thus resistance to degradation [28]. In the case of amending biosolids to agricultural fields, triclosan typically exhibits little movement within the soil, adsorbs to lipid-rich root structures, and resists translocation from roots to shoots in plants [28–30].

A number of studies have evaluated the potential risks of triclosan to field crops from biosolids-amended soil, and found negligible uptake of triclosan in plant tissues, above and below ground [19,31–33]. However, the majority of these studies focused on 1 formulation of biosolids, typically dewatered biosolids. Few studies have investigated how other formulations of biosolids, which may vary in characteristics such as moisture and solids content, pH, contaminant concentrations, and pathogen loads, and which are commonly applied in agricultural landscapes, affect the potential for triclosan toxicity to plants [32]. Whether the findings from studies focusing on dewatered biosolids apply to plant species grown in other formulations of biosolids remains largely unknown.

In the present study, we evaluated the effects of 4 formulations of biosolids (liquid, dewatered, compost, and alkaline-hydrolyzed), containing background concentrations of triclosan and triclosan spiked at various concentrations, on plant emergence and growth of 3 crop species (corn, soybean, and spring wheat). The pot studies were conducted under greenhouse conditions with the biosolids applied to the soil following emulated best management practices used in the province of Ontario, Canada. A stronger understanding of the potential effects of chemicals in general, and of antimicrobials such as triclosan, in various biosolids formulations on crops to which it is typically applied, will provide stakeholders with a stronger basis to improve current sustainable biosolids management practices [17,34–36].

MATERIALS AND METHODS

Soil and biosolids

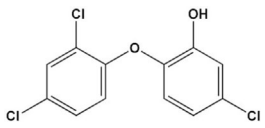
Loam soil was taken from an agriculture field in Guelph, Ontario, Canada (latitude: 43.577997; longitude: –80.224128; altitude: 346 m) to which biosolids and pesticides had not been applied for at least 10 yr. Biosolids from 4 municipal WWTPs across Canada were tested in the present study: LMBs, DMB, CMB, and AMB. Physical and chemical properties of the soil and biosolids are shown in Tables 2 and 3 [3].

Experimental design

The experimental design (Table 4) was adapted from Prosser et al. [37]. Corn (*Zea mays* var. *saccharata*), soybean (*Glycine max*), and spring wheat (*Triticum aestivum*) plants were selected for the present study, and seeded in biosolids-amended soil spiked with triclosan at increasing concentrations. Corn (variety HZ982GT, Syngenta) was grown in 4-L pots (24.8 cm × 19.2 cm; Stuewe & Sons), whereas soybean (variety S20-Z9, Syngenta) and spring wheat (variety 5604, Syngenta) were grown in 3-L pots (20.3 cm × 14.3 cm; ITML).

The Nutrient Management Software Program (NMAN3) was used to determine the biosolids rate of amendment for each pot [3,38,39]. The NMAN3 software was designed by the Ontario Ministry of Agriculture, Food and Rural Affairs to calculate a suitable rate for biosolids amendment to soil after data were entered on crop type, properties of soil (pH, nutrient content) and biosolids (pH, nutrient content, concentration of metals, and pathogenic microorganisms), and field characteristics [39]. The rates of amendment calculated by NMAN3 were 9 t, 15 t, 15 t, and 15 t dry weight/ha for LMB, DMB, CMB, and AMB, respectively. To emulate the amount of biosolids that would typically be applied to an agricultural field, the rates of amendment were used to calculate the total amount of biosolids that would be added to each pot, accounting for surface area and depth. These total amounts corresponded to 511.63 g, 324.11 g, and 324.11 g wet weight of LMB/pot for corn, soybean, and spring wheat plants, respectively; 252.55 g, 159.99 g, and 159.99 g wet weight of DMB/pot for corn, soybean, and spring wheat plants, respectively; 148.81 g, 94.27 g, and 94.27 g wet weight of CMB/pot for corn, soybean, and spring wheat plants, respectively; and, 82.23 g, 52.09 g, and 52.09 g wet weight of AMB/pot for corn, soybean, and spring wheat plants, respectively. Biosolids were spiked with triclosan at 6 different concentrations (i.e., BS1–BS6), mixed thoroughly, and left for 24 h to allow for equilibration and solvent evaporation. Methanol was used as a solvent carrier for triclosan. Treatment BS1 was not spiked with triclosan, to determine the effects of triclosan inherently present in each biosolids formulations. Treatments BS2 to BS6 were spiked with triclosan to produce nominal concentrations of 25 000 ng/g, 75 000 ng/g, 150 000 ng/g, 300 000 ng/g, and 600 000 ng/g dry weight, respectively. Treatments BS2 to BS4 are representative of concentrations typically found in municipal biosolids produced in Canada and the United States [40]. Treatments BS5 and BS6 were added as theoretically plausible but unlikely realistic exposure scenarios [13]. A soil control (no biosolids) and solvent control (no biosolids plus solvent) were included in all experiments with the purpose of distinguishing between the efficacy of biosolids-amended soil and soil only and to assess potential effects of the solvent carrier.

Table 1. Physicochemical properties of triclosan

Compound	Application	Structure	Solubility (25 °C)	Acid/base	p*K*a	Log K_{OW}	$t_{1/2}$ in soil (days)
Triclosan	Antimicrobial		4.6 mg/L[a]	Weak acid	7.9[b]	4.8[c]	12.7[d]–83[e]

[a]Halden and Paull [57].
[b]Loftsson et al. [58].
[c]Zhao et al. [59].
[d]Xu et al. [60].
[e]Cha and Cupples [61].

Table 2. Physical and chemical composition of field soil used in laboratory studies at start of experiment[a]

Properties	Units	Soil
Texture		Loam
Organic matter	% dry	3.3
pH		7.9
Ammonium-N	mg/kg dry	10.2
Nitrate-N	mg/kg dry	4.8
Phosphorus	mg/L soil dry	14[b]
Magnesium	mg/L soil dry	300[c]
Potassium	mg/L soil dry	100[c]
Inorganic carbon	%	2.59
Organic carbon	%	1.81
Total carbon	%	4.40
Cation exchange capacity	cmol+/kg	14.1
Water-holding capacity	%	49.1
Arsenic	μg/g dry	3.2
Cadmium	μg/g dry	0.34
Chromium	μg/g dry	25
Cobalt	μg/g dry	5.3
Copper	μg/g dry	11
Lead	μg/g dry	28
Molybdenum	μg/g dry	1.3
Nickel	μg/g dry	14
Zinc	μg/g dry	130
Mercury	μg/g dry	0.05

[a]Soil was collected from the same source as Prosser et al. [3]; results are reported from Prosser et al. [3].
[b]Sodium bicarbonate extraction.
[c]Ammonium acetate extraction.

Biosolids were mixed with loam soil to a depth of 15 cm in each pot for 3 min using a gloved hand. This procedure was used to emulate the incorporation of biosolids into soil during or after application in an agricultural field, which is common practice for biosolids amendment in Ontario [37]. After soil amendment, all treatments were left for 48 h to allow for equilibration of triclosan and evaporation of solvent before inoculation with arbuscular mycorrhizal fungi (AMF) and seed planting.

Soil control, solvent control, and treatments BS1 to BS6 were inoculated with AMF (Micronized Endomycorrhizal Inoculant, BioOrganics). The inoculant was a powder that contained a minimum of 10 spores/cm^3 of *Glomus aggregatum*, *Glomus etunicatum*, *Glomus intraradices*, and *Glomus mosseae*, and 2 spores/cm^3 of *Glomus clarum*, *Glomus monosporus*, *Gigaspora margarita*, and *Paraglomus brasilianum*. The inoculant was evenly mixed into the soil at a depth of 8 cm for 3 min using a gloved hand. For spring wheat tests grown in DMB, CMB, and AMB, an additional soil control with no-AMF and biosolids control with no-AMF was added in the experimental design to evaluate whether AMF inoculation might affect plant growth.

Corn experiments included 10 replicate pots/treatment, and 3 additional replicate pots for each treatment were reserved for triclosan analysis in the soil. All treatments received approximately 25.2 g of AMF inoculants/replicate pot. Each pot received 3 seeds sown at a depth of 50 mm. After seeding, pots received 500 mL of deionized water.

Soybean experiments included 5 replicate pots/treatment, and 3 additional replicate pots for each treatment were reserved for triclosan analysis in the soil. All treatments received approximately 18.9 g of AMF inoculants/replicate pot. Each pot received 7 seeds sown at a depth of 50 mm. After seeding, pots received 250 mL of deionized water.

Spring wheat experiments included 5 replicate pots/treatment, and 3 additional replicate pots for each treatment were reserved for triclosan analysis in the soil. All treatments except the no-AMF controls received approximately 18.9 g of AMF inoculants/replicate pot. Each pot received 8 seeds sown at a depth of 30 mm. After seeding, pots received 250 mL of deionized water.

All tests followed a completely randomized design. Once every week the pots were randomly repositioned using a random number table. Soybean and spring wheat were grown in a growth chamber (23 ± 1 °C day, 20 ± 1 °C night, 16:8-h day: night, 60 ± 10% relative humidity, and 299 ± 87 μmol photons/m^2 s), and corn was grown in a greenhouse (19–31 °C, 32–93% relative humidity). Each day, all pots were irrigated with the same volume of water. Depending on the plant species and soil moisture, pots were watered daily with 100 mL to 500 mL of deionized water. Corn, soybean, and spring wheat plants were grown for 85 d, 80 d, and 70 d, respectively. After emergence, corn, soybean, and spring wheat were randomly thinned, using a random number generator, to 1, 5, and 5 plants/pot, respectively. The percentage of emergence was recorded. Shoot length was measured from the soil surface to the highest point of the plant. Plants were carefully removed from the pots and rinsed in deionized water to remove adhering soil particles. Plant shoots were excised from the roots, and wet mass was immediately recorded after weighing. Plants that bore fruits were further excised to separate the fruit from the shoot, and fruit wet masses were weighed and recorded. In each treatment group, 5 roots were randomly selected and separately stored in 70% ethanol at 4 °C for AMF analysis at a later date. The remaining plant shoots, roots, and fruits were dried to constant weight in an oven at 70 °C for 7 d, and weighed to determine dry mass.

Chemical analyses for emerging substances of concern

Soil replicates from treatment BS1 were randomly sampled at the end of each experiment in triplicate. From each experiment, a composite sample was created from the triplicates. The soil samples were submitted to the Ministry of the Environment and Climate Change, Ontario, Canada, for analysis of contaminant composition in each formulation of biosolids [38]. A suite of emerging substances of concern was analyzed, including pharmaceuticals, drug metabolites, hormones, antibacterials, synthetic musks, a sweetener, caffeine, and markers of nanomaterials [38]. The Ministry of the Environment and Climate Change performed liquid chromatography/mass spectrometry (Supplemental Data, Table S1) and liquid chromatography tandem mass spectrometry on biosolids-amended soil (Supplemental Data, Table S2).

Sample preparation and triclosan chemical analysis

Triclosan in the biosolids-amended soil was measured following the method of Chu and Metcalfe [26], as modified by Prosser et al. [3]. Triplicate samples were collected from each treatment group at the beginning and end of all plant experiments and stored in a freezer until further analysis. Spring wheat and corn were selected for triclosan chemical analysis. Duplicate subsamples of soil (~10 g wet wt) were collected from each treatment group for spring wheat, and a reduced sample analysis for corn (i.e., BS2, BS4, and BS6), at the beginning and end of each plant experiment. Samples were placed in a cellulose extraction thimble and spiked with 25 ng of internal standard, $^{13}C_{12}$–triclosan (Wellington Laboratories). Each thimble was placed in a Soxhlet apparatus, and triclosan was extracted using 250 mL of dichloromethane (all solvents were high-performance liquid chromatography [HPLC] grade; Caledon Laboratory Chemicals) for 24 h. Dichloromethane was

Table 3. Physical and chemical composition of biosolids obtained from municipal wastewater treatment plants used in laboratory studies at start of experiment

Properties	Units	Liquid municipal biosolids	Dewatered municipal biosolids	Compost municipal biosolids	Alkaline-hydrolyzed municipal biosolids
Dry matter	% dry	9.32	23.75	44.80	13.58
Ammonium–N	mg/kg dry	19 300	22 300	123	25 000
Nitrate–N	mg/kg dry	9.76	8.74	1.32	8.76
pH		7.6	7.7	7.8	8.4
Electrical conductivity (total salts)	mS/cm	4.06	9.12	1.95	11.2
Magnesium, extractable	mg/kg dry	3060	524	1300	646
Potassium, extractable	mg/kg dry	3970	710	6740	21 700
Sodium, extractable	mg/kg dry	1660	822	459	19 900
Calcium, extractable	mg/kg dry	8830	6480	9240	7020
Calcium, total	% dry	4.00	3.55	4.81	3.37
Magnesium, total	% dry	1.06	0.559	1.05	0.571
Phosphorus, total	% dry	4.51	3.01	1.73	3.55
Potassium, total	% dry	0.462	0.103	0.795	2.14
Sodium, total	% dry	0.203	0.109	0.0632	2.20
Inorganic carbon	% dry	0.415	0.609	0.628	0.356
Organic carbon	% dry	28.4	32.8	28.3	30.6
Total carbon	% dry	28.8	33.4	28.9	31.0
Total Kjeldahl nitrogen	% dry	6.27	5.80	2.18	5.40
Arsenic	μg/g dry	2.1	2.6	3.6	3.1
Cadmium	μg/g dry	1.5	0.85	0.80	0.27
Chromium	μg/g dry	46	87	78	69
Cobalt	μg/g dry	3.0	4.9	3.2	4.1
Copper	μg/g dry	460	1200	210	470
Lead	μg/g dry	33	55	18	26
Molybdenum	μg/g dry	12	6.7	5.0	7.0
Nickel	μg/g dry	25	33	26	24
Selenium	μg/g dry	6.8	5.0	1.4	3.1
Zinc	μg/g dry	870	820	310	620
Mercury	μg/g dry	1.2	0.65	0.24	0.49

removed by rotary evaporation, and triclosan extracts remaining in the round-bottomed flask were reconstituted in 6 mL of hexane. Prior to purifying extracts by solid phase extraction, Supelco Select HLB columns (12 mL, 500 mg; Sigma Aldrich) were conditioned by rinsing with 3 mL each of methanol, acetone, dichloromethane, and hexane at a flow rate of 2 mL/min. Then each extract was loaded onto the column, and the column was washed with 2×3 mL of hexane, 2×2 mL of dichloromethane, and 2×2 mL of deionized water. The column was dried for 12 min using a 70-kPa vacuum. Analytes and internal standards of triclosan were eluted from columns into 10-mL test tubes using 3×3 mL of 50:50 acetone/methanol (v/v). The 9 mL of 50:50 acetone/methanol in each test tube was evaporated using a steady stream of air; analytes and internal standards were reconstituted in 1 mL of acetonitrile. Vials were stored at 4 °C prior to analysis.

Instrumental analysis was completed following the method described by Prosser et al. [3], which was modified by the method described by Chu and Metcalfe [26]. Analytes were analyzed using an Agilent 1100 Series HPLC and Applied Biosystems MDS Sciex API 4000 triple quadrupole mass spectrometer (AB Sciex). Analyst 1.5.1 software (AB Sciex) was used to compile analyte data. A Phenomenex Synergi Polar-RP column (4 μm, 150 mm × 4.60 mm; Canadian Life Sciences) was used to separate analytes. Mobile phase A was 0.1% formic acid in water, and mobile phase B was acetonitrile. The total flow rate was 300 μL/min, and the injection volume was 5 μL. The gradient elution began with 30% A and 70% B for 2 min, modified to 10% A and 90% B in 3 min, and continued for 5 min, and readjusted back to 30% A and 70% B in 2 min, and continued for 13 min. The ionization method was electrospray ionization operated in negative ion mode, with multiple reaction monitoring using N_2 as the collision gas and a dwell time of 0.5 s. The m/z values of triclosan and $^{13}C_{12}$-triclosan for the precursor ions were 287 and 299, respectively. The m/z values of triclosan and $^{13}C_{12}$-triclosan for the product ions were 35 and 35, respectively.

As the internal standard, $^{13}C_{12}$-triclosan was used to control for matrix effects and potential losses during processing. A 5-point calibration curve, with a coefficient of determination (r^2) of $\geq$0.99, was constructed to determine the expected range of analyte concentrations in the sample against the fixed concentrations of the internal standard. A procedural blank was run with each set of samples to trace contamination in the sample preparation and cleanup. The consistency of analyte recovery was accounted for by running spiked samples with each set of samples. Because no biosolids sample could be acquired that did not contain triclosan, peat moss was used a surrogate. The average recovery rates of triclosan in soil and biosolids were $91 \pm 9\%$ and $87 \pm 8\%$, respectively [3]. The method detection limits for triclosan in soil and biosolids were 1.7 ng/g and 2.1 ng/g dry weight, respectively, and the limits of quantitation for triclosan in soil and biosolids were 5.9 ng/g and 7.4 ng/g dry weight, respectively [3].

Data analysis

All analyses were conducted using measured concentrations and tested for normality and equality of variance using the Shapiro–Wilk and Levene's tests, respectively. When normality was met, a one-way analysis of variance (ANOVA; $\alpha = 0.05$) was performed to evaluate if there was a significant difference in percentage of emergence, wet and dry shoot mass, wet and dry root mass, and shoot height of plants among treatments. When normality was not met, a Kruskal–Wallis one-way ANOVA on ranks ($\alpha = 0.05$) was performed. If a significant difference between treatments was identified by the ANOVA, a post hoc Tukey's test ($\alpha = 0.05$) was performed to compare all treatment means. Linear regression ($\alpha = 0.05$) was performed to model

Table 4. Experimental design for 3 field crops grown in 4 formulations of biosolids at recommended rates of application with nominal concentration of triclosan spiked in biosolids prior to amendment with soil

	Biosolids amendment rate (g wet wt/pot)						
Crop type	Liquid municipal biosolids	Dewatered municipal biosolids	Compost municipal biosolids	Alkaline-hydrolyzed municipal biosolids	Treatment	Biosolids amendment	Expected concentration in amendment (ng/g soil dry wt)
Corn	–	–	–	–	Control		–
	–	–	–	–	Solvent control		–
	511.6	252.6	148.8	82.2	BS1	×	0
	511.6	252.6	148.8	82.2	BS2	×	25 000
	511.6	252.6	148.8	82.2	BS3	×	75 000
	511.6	252.6	148.8	82.2	BS4	×	150 000
	511.6	252.6	148.8	82.2	BS5	×	300 000
	511.6	252.6	148.8	82.2	BS6	×	600 000
Soybean	–	–	–	–	Control		–
	–	–	–	–	Solvent control		–
	324.1	160.0	94.3	52.1	BS1	×	0
	324.1	160.0	94.3	52.1	BS2	×	25 000
	324.1	160.0	94.3	52.1	BS3	×	75 000
	324.1	160.0	94.3	52.1	BS4	×	150 000
	324.1	160.0	94.3	52.1	BS5	×	300 000
	324.1	160.0	94.3	52.1	BS6	×	600 000
Spring wheat	–	–	–	–	Control		–
	–	–	–	–	Control–NO AMF		–
	–	–	–	–	Solvent control		
	324.1	160.0	94.3	52.1	Biosolids–NO AMF	×	0
	324.1	160.0	94.3	52.1	BS1	×	0
	324.1	160.0	94.3	52.1	BS2	×	25 000
	324.1	160.0	94.3	52.1	BS3	×	75 000
	324.1	160.0	94.3	52.1	BS4	×	150 000
	324.1	160.0	94.3	52.1	BS5	×	300 000
	324.1	160.0	94.3	52.1	BS6	×	600 000

AMF = arbuscular mycorrhizal fungi.

the relationship between the plant-health endpoints) and the triclosan concentrations. Statistical analysis was performed using Sigma Stat (Ver 3.5, Systat Software).

RESULTS

Triclosan and emerging substances of concern concentrations in soil

The nominal and measured concentrations of triclosan in biosolids-amended soil at the beginning and end of the experiments were determined for corn and spring wheat (Supplemental Data, Tables S3 and S4). There was a decline in the concentrations of triclosan in treatments BS2 to BS6 at the end of both the corn and spring wheat experiments. The percentage difference between measured concentrations of triclosan at the beginning and end of corn and spring wheat experiments indicates that exposure predominantly occurred during the early stages of plant development. The percentage difference between mean measured concentrations of triclosan at the beginning and end of the experiments increased with each treatment (BS2–BS6). For example, the greatest percentage differences were observed in treatment BS6, whereas the lowest percentage differences were observed in treatment BS2. The concentrations of triclosan in soil samples from the BS1 treatment of the corn and spring wheat tests were below the Ministry of the Environment and Climate Change detection limits (Supplemental Data, Table S2), which corresponds with our measurements of triclosan in the soil of the BS1 treatment (Supplemental Data, Table S3). All other emerging substances of concerns analyzed by the Ministry of the Environment and Climate Change were below the detection limit (Supplemental Data, Table S2) [38].

Zea mays var. *saccharata*

There was no significant concentration–response relationship between triclosan and any endpoint in all experiments with corn (Figures 1 and 2 and Supplemental Data, Figures S1–S4). There was a significant increase in mean shoot length ($p < 0.001$; Figure 1), mean shoot mass (wet: $p < 0.001$; dry: $p < 0.001$; Figure 2 and Supplemental Data, Figure S2), and mean root mass (wet: $p < 0.001$; Supplemental Data, Figure S1) in LMB treatments relative to the controls. There was a significant increase in mean shoot mass (wet: $p < 0.001$; dry: $p = 0.006$; Figure 2 and Supplemental Data, Figure S2) of DMB treatments relative to the controls, but no significant differences in other plant growth endpoints. There were no significant differences in any of the plant growth endpoints in CMB treatments relative to the controls; however, in contrast to the other biosolids formulations, corn grown in all CMB treatments did not produce ears. There was a significant increase in mean shoot mass (wet: $p = 0.034$; dry: $p = 0.040$; Figure 2 and Supplemental Data, Figure 2) in AMB treatments relative to the controls. Corn did not produce ears in the control and solvent treatments across all formulations of biosolids.

Glycine max

There was no significant concentration–response relationship between triclosan and any endpoint in all experiments with

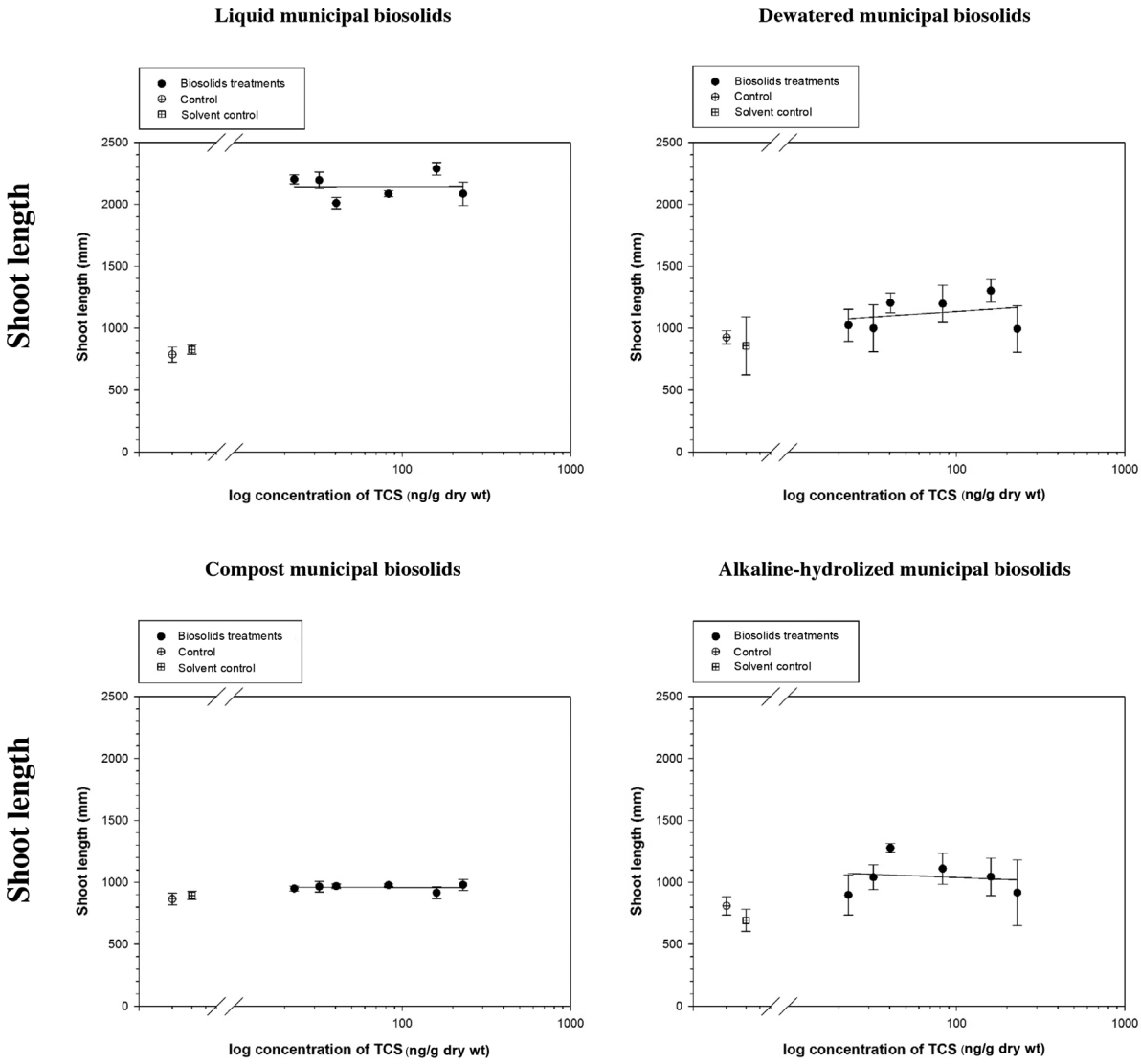


Figure 1. Mean shoot length for corn grown in soil without biosolids and triclosan (TCS; control and solvent control), and biosolids-amended soils with increasing triclosan (BS1–BS6). Liquid municipal biosolids: $y = 1527.273 + (3.938x)$; $r^2 = 0.269$; $p = 0.188$. Dewatered municipal biosolids: $y = 1009.104 + (0.744x)$; $r^2 = 0.158$; $p = 0.330$. Compost municipal biosolids: $y = 923.195 + (0.222x)$; $r^2 = 0.182$; $p = 0.292$. Alkaline-hydrolyzed municipal biosolids: $y = 941.796 + (0.446x)$; $r^2 = 0.0400$; $p = 0.635$.

soybean (Figures 3 and 4 and Supplemental Data, Figures S5–S8). Relative to the controls, there was a significant increase in mean fruit mass (wet wt: $p = 0.001$; dry wt: $p = 0.006$; Supplemental Data, Figures S7 and S8) in LMB treatments, but no significant differences in other plant growth endpoints. There was a significant increase in mean shoot length ($p < 0.001$; Figure 3), mean shoot mass (wet wt: $p < 0.001$; dry wt: $p < 0.001$; Figure 4 and Supplemental Data, Figure S6), and mean fruit mass (wet wt: $p < 0.001$; dry wt: $p < 0.001$; Supplemental Data, Figures S7 and S8) in DMB treatments relative to the controls. There was a significant decrease in mean root mass (wet wt: $p = 0.002$; Supplemental Data, Figure S5) in DMB treatments relative to the controls. There were no significant differences in the plant growth endpoints for CMB and AMB treatments relative to the controls. Linear regression indicated a statistically significant decrease in mean root mass (wet wt: $p = 0.04$, $r^2 = 0.531$) in LMB treatments, and increased mean shoot wet mass (wet wt: $p = 0.046$, $r^2 = 0.511$) in DMB treatments relative to the controls. Although occasional statistical significances were observed between treatments and control, overall there was little evidence of a concentration– or dose–response relationship, as indicated by the very low *r*-squared values in mean root mass in LMB treatments, and mean shoot wet mass in DMB treatments. Low *r*-squared values show that this was not a concentration response. Considering all plant-health endpoints that were analyzed, the overwhelming conclusion is that triclosan did not cause an adverse impact on growth.

Triticum aestivum

There was no significant concentration–response relationship between triclosan and any endpoint in all experiments with spring wheat (Figures 5 and 6 and Supplemental Data, Figures S9 and S10). There was a significant increase in mean root mass (wet wt: $p = 0.006$; Supplemental Data, Figure S9), and mean shoot mass (wet wt: $p < 0.001$; dry wt: $p < 0.001$; Figure 6 and Supplemental Data, Figure S10) in LMB treatments relative to the controls, but no significant differences were observed for any other plant growth endpoint. Similarly, there was a significant increase in mean root mass (wet wt: $p = 0.003$; Supplemental Data, Figure S9), and mean shoot mass (wet wt: $p = 0.004$; dry wt: $p = 0.044$; Figure 6 and Supplemental Data, Figure S10) of DMB treatments relative to the controls, but no significant differences in other plant growth endpoints. There

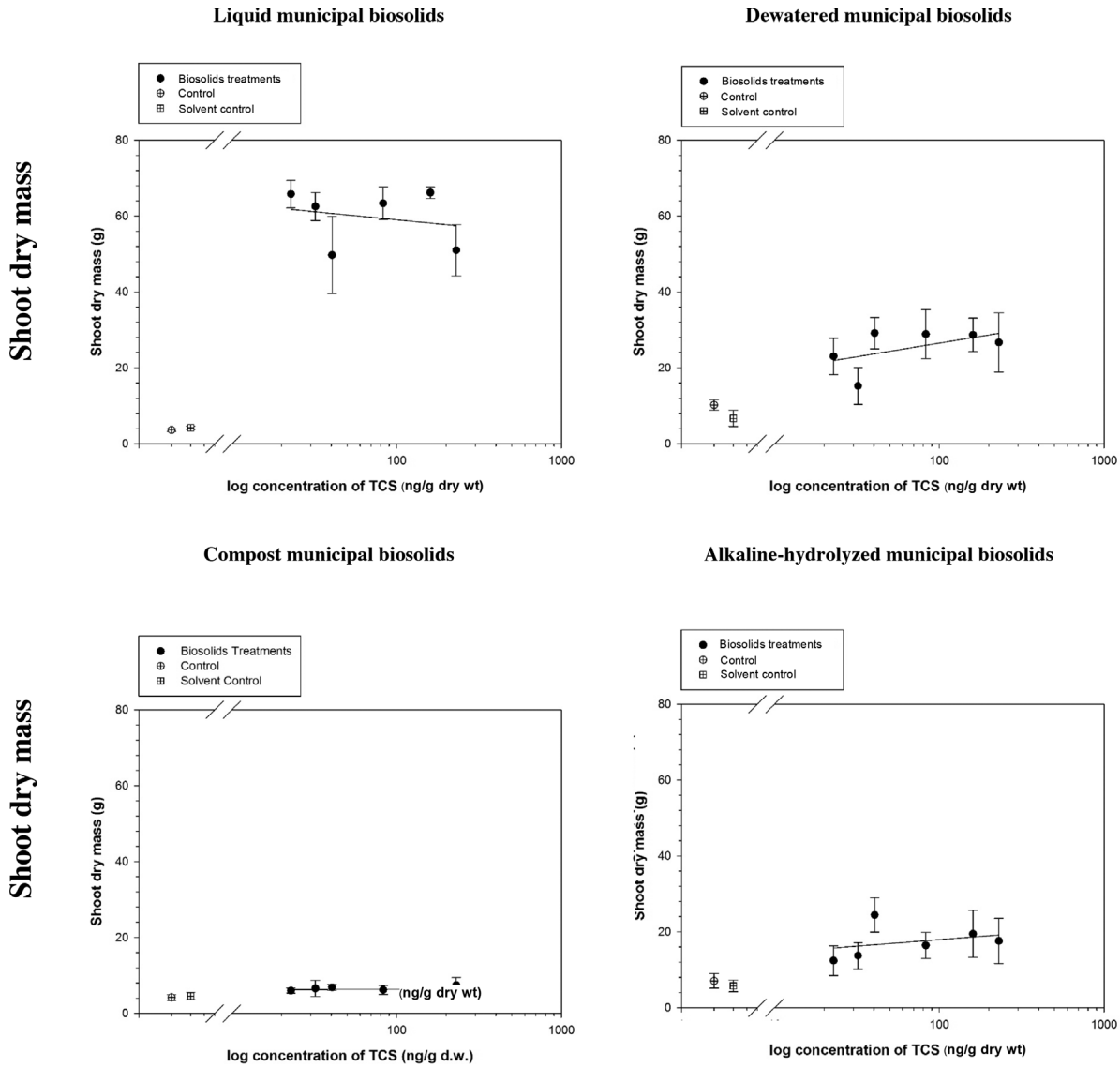


Figure 2. Mean shoot dry mass for corn grown in soil without biosolids and triclosan (TCS; control and solvent control), and biosolids-amended soils with increasing triclosan (BS1–BS6). Liquid municipal biosolids: $y = 35.447 + (0.144x)$; $r^2 = 0.200$; $p = 0.267$. Dewatered municipal biosolids: $y = 16.101 + (0.0690x)$; $r^2 = 0.391$; $p = 0.097$. Compost municipal biosolids: $y = 5.346 + (0.00665x)$; $r^2 = 0.222$; $p = 0.239$. Alkaline-hydrolyzed municipal biosolids: $y = 11.739 + (0.0399x)$; $r^2 = 0.276$; $p = 0.181$.

was a significant increase in mean shoot mass (wet wt: $p < 0.001$; dry wt: $p < 0.001$; Figure 6 and Supplemental Data, Figure S10) of CMB treatments and AMB treatments (wet wt: $p < 0.001$; dry wt: $p < 0.001$; Figure 6 and Supplemental Data, Figure S10) relative to the controls, but no significant differences in other plant growth endpoints. The absence of AMF inoculation in the no-AMF soil control and no-AMF biosolids control had no significant effect on plant growth.

DISCUSSION

The results from the present study demonstrate the following 3 findings among all plant experiments across all formulations of biosolids: 1) no dose–response relationship was established between increasing concentrations of triclosan and an adverse effect on seed emergence and plant growth; 2) significant positive effects on plant growth, when present, came as a result of amending biosolids into soil despite the presence of native and spiked triclosan; and 3) plant growth in the soil controls were, for the most part, significantly less than the biosolids treatments. Although the mean root wet mass of soybean grown in LMB treatments, and the mean shoot wet mass of soybean grown in DMB treatments were significantly less than the soil controls, these trends were not observed with the other plant and biosolids-type combinations, and there was no dose–response relationship with the concentration of triclosan. Overall, either a positive effect was seen on plant growth endpoints, or there was no change relative to the soil controls. Our results corroborate a growing body of evidence suggesting that the land application of municipal biosolids presents a low risk for triclosan toxicity on plant emergence and growth [3,4,6,28,33,40,41]. It is also important to note that the indifferent or positive influence from biosolids on plant growth occurred despite the presence of a wide range of emerging substances of concern constituents at concentrations that would typically be applied to agricultural lands (Supplemental Data, Table S2). Our results therefore support the conclusion of Prosser et al. [20] that there is likely minimal risk to plant health from triclosan via land-applied biosolids.

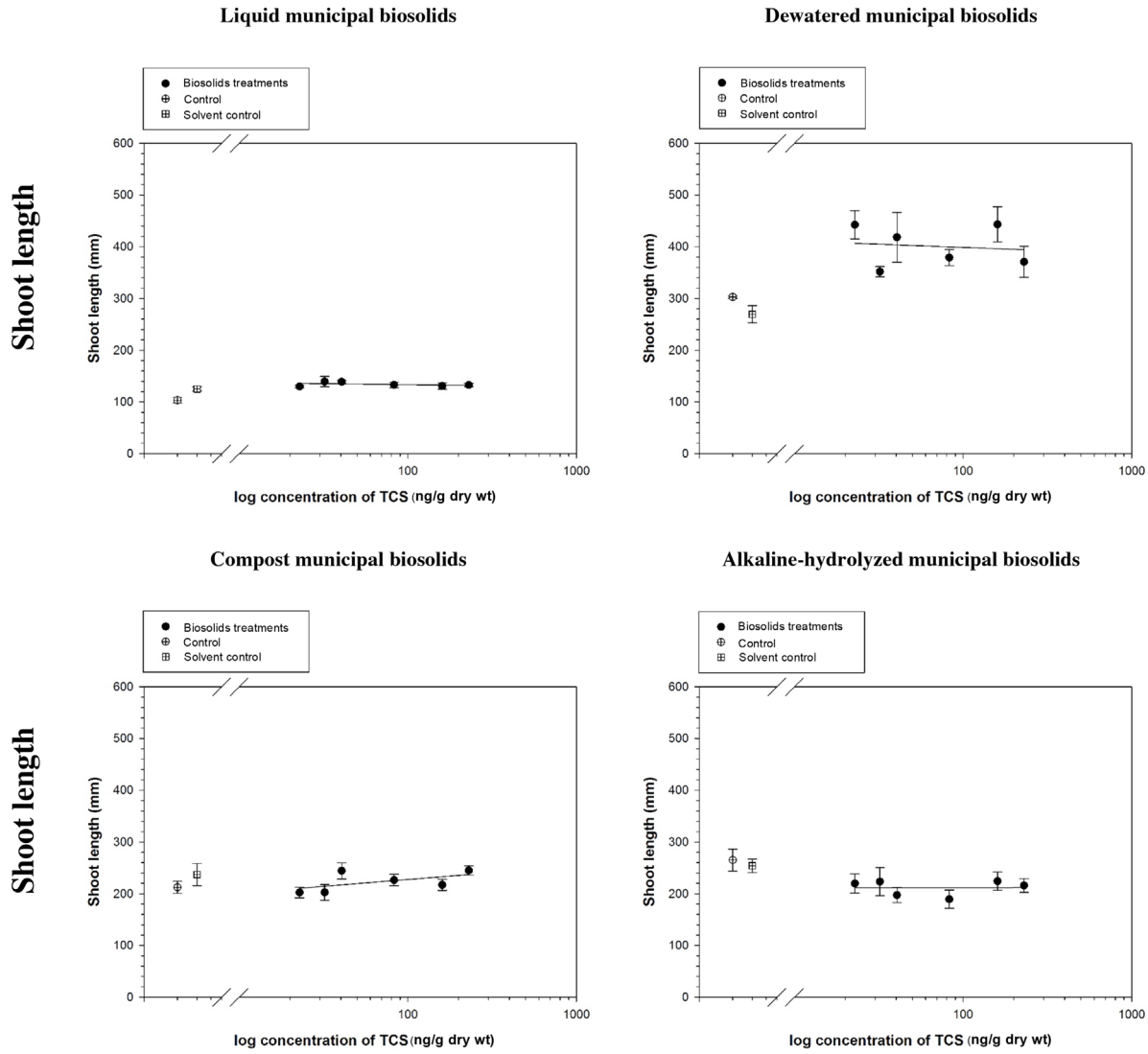


Figure 3. Mean shoot length for soybean grown in soil without biosolids and triclosan (TCS; control and solvent control), and biosolids-amended soils with increasing triclosan (BS1–BS6). Liquid municipal biosolids: $y = 125.949 + (0.0436x)$; $r^2 = 0.0984$; $p = 0.449$. Dewatered municipal biosolids: $y = 351.340 + (0.293x)$; $r^2 = 0.146$; $p = 0.351$. Compost municipal biosolids: $y = 217.449 + (0.0824x)$; $r^2 = 0.151$; $p = 0.341$. Alkaline-hydrolyzed municipal biosolids: $y = 232.036–(0.117x)$; $r^2 = 0.141$; $p = 0.360$.

Although triclosan is known to be a persistent antimicrobial agent, numerous factors could affect its availability for plant uptake in biosolids-amended soil [1,4,40,42,43]. In the present study the concentration of triclosan decreased in all spiked treatments over the course of the experiment (Supplemental Data, Tables S3 and S4). This may be a result of dissipation and/or degradation of soil triclosan. The dissolved portion of an organic compound (e.g., triclosan) is thought to be the only fraction that is available for plant uptake in the rhizosphere, and it is prone to leaching out of the soil [42]. Loss via leaching out of the soil during irrigation can decrease triclosan availability to plants, and may have been the main pathway observed in the present study because triclosan likely existed in a soluble anionic form at the soil pH measured (i.e., triclosan p*K*a = 7.9, soil pH = 7.9) [42]. At pH values ≥ the p*K*a of triclosan, triclosan has increased solubility and mobility in soil [3,44,45]. Wu et al. [45], for example, used the batch equilibrium method for adsorption/desorption of chemicals in soil and found that increasing the soil pH from 4 to 8 decreased triclosan sorption. A co-solute experiment (triclosan and triclocarban) was also included in their study and confirmed a decrease in sorption of triclosan [45]. In the presence of other chemicals at low concentrations, as is the case in biosolids, triclosan may be more amenable to leaching because of competition for available sites to sorb [45]. This observation may offer insight into the lack of sorption and subsequent lack of adverse effect by native and spiked triclosan across all experiments in the present study. Loss of triclosan from the soil pots in the present study may also have been the result of microbial degradation. In 1 study conducted by Liu et al. [46] on the potential for constructed wetlands to remove triclosan, results suggested that an abundance of alpha- and gamma-*Proteobacteria* could be important in the microbial degradation of triclosan. Furthermore, the extent to which triclosan is available for plant uptake may have also been affected by its half-life, which ranges from 12.7 d to 83 d in soil (Table 1). In the present study, plant experiments ranged between 70 d and 85 d, so it is probable that some of the native and spiked triclosan degraded. Spiking of biosolids with triclosan may also affect triclosan availability in biosolids-amended soil [43]. Langdon et al. [43] investigated whether spiking experiments accurately predicted the degradation of triclosan in biosolids-amended soil. Results from analyses on 2

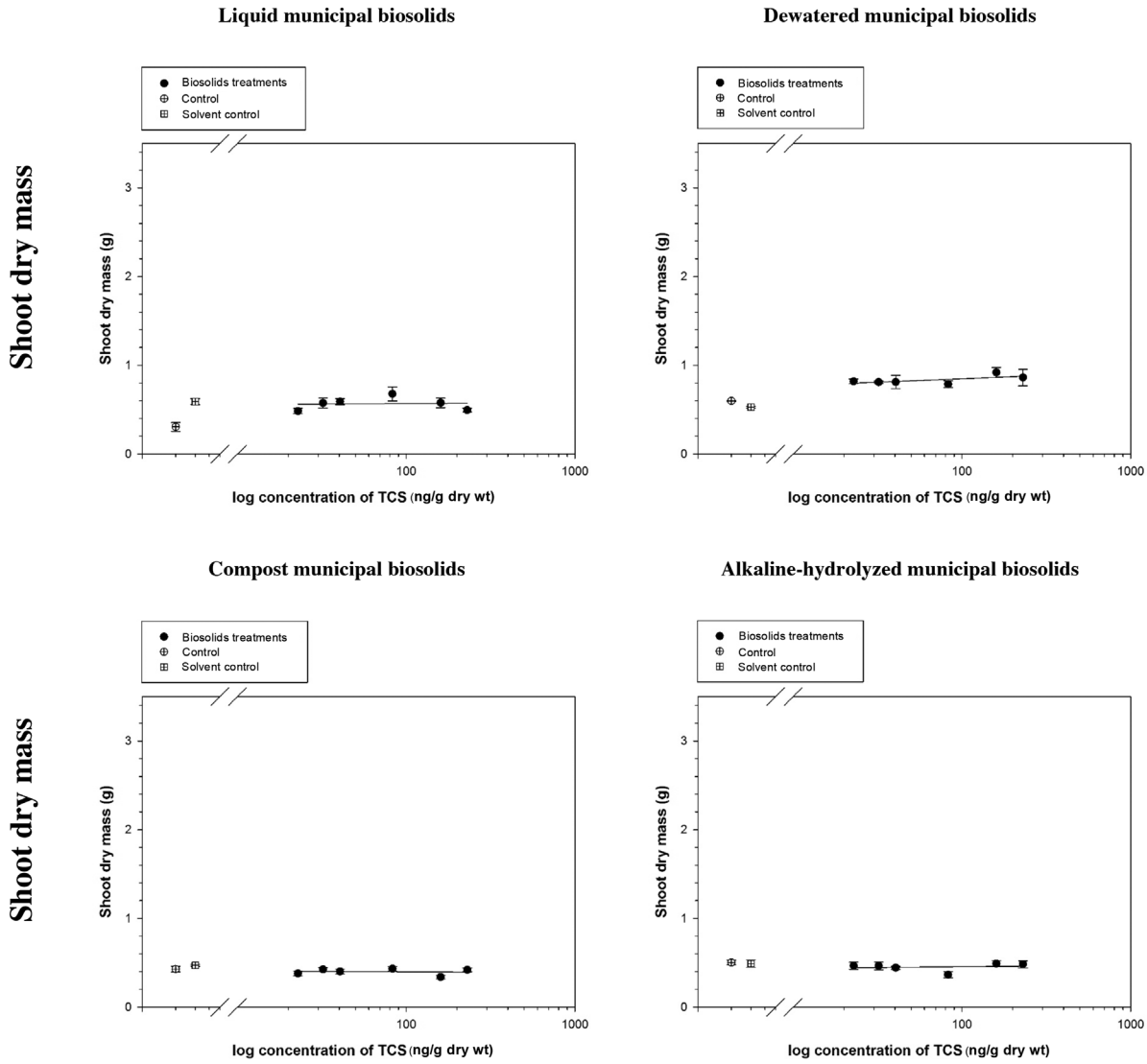


Figure 4. Mean shoot dry mass for soybean grown in soil without biosolids and triclosan (TCS; control and solvent control), and biosolids-amended soils with increasing triclosan (BS1–BS6). Liquid municipal biosolids: $y = 0.521 + (0.000219x)$; $r^2 = 0.0263$; $p = 0.701$. Dewatered municipal biosolids: $y = 0.689 + (0.00108x)$; $r^2 = 0.444$; $p = 0.071$. Compost municipal biosolids: $y = 0.425–(0.000186x)$; $r^2 = 0.149$; $p = 0.345$. Alkaline-hydrolyzed municipal biosolids: $y = 0.462 + (0.00000507x)$; $r^2 = 0.0000919$; $p = 0.982$.

formulations of DMB biosolids (1 centrifuged and the other solar dried) over a period of 224 d showed that native triclosan sorbed more strongly onto the biosolids matrices compared with spiked triclosan, and was more susceptible to microbial degradation and/or leaching [43].

The results of the present study are consistent with other studies that have shown little effect from triclosan in biosolids-amended soil on plants and organisms [20,33,47]. Prosser et al. [20], for example, analyzed whether the exposure of triclosan could adversely affect the seed emergence and growth of soybean, lettuce (*Lactuca sativa*), spring wheat, and corn grown in pots containing soil amended with DMB at rates of 29 t/ha, 26.5 t/ha, 21 t/ha, and 32 t/ha, respectively. Triclosan was spiked across treatments to produce nominal concentrations reflective of an exposure scenario in Ontario, Canada, with the exception of the highest treatment (i.e., 307 000 ng/g dry wt), which was 200% greater than the largest reported value of triclosan in biosolids from North America [20]. Across all species, triclosan had little to no effect on seed emergence and growth [20]. Pannu et al. [33] acquired soil from a field that received a single application of biosolids (228 Mg/ha) after 2 yr of equilibration; lettuce, radish (*Raphanus sativus*), and bahia grass (*Paspalum notatum*) were grown under laboratory conditions, and treatments were spiked to produce nominal concentrations of 0.99 mg triclosan/kg soil, 5.9 mg triclosan/kg soil, and 11 mg triclosan/kg soil, to establish a dose–response relationship. The results indicated no evidence of toxicity to plants from the unrealistically high concentration of triclosan present in the single application of biosolids (228 Mg/ha) [33]. As in the present study, plants experienced an increase in yield biomass relative to the control treatment [33]. In another study, Pannu et al. [47] investigated the toxicity and bioaccumulation of triclosan in biosolids on macro- (earthworms) and micro-organisms, and found that when biosolids were applied at an agronomic rate (~22 Mg/ha) with typical concentrations of triclosan (10–20 mg/kg), no toxicity was observed in earthworms or microbial functions (respiration, nitrification, or ammonification). These studies [20,33] represent worst-case scenarios for triclosan exposure to plants and organisms. The frequency of applying biosolids, and the amount that can be applied, is strictly regulated in most jurisdictions in North America. In the province of Ontario, for example, application to

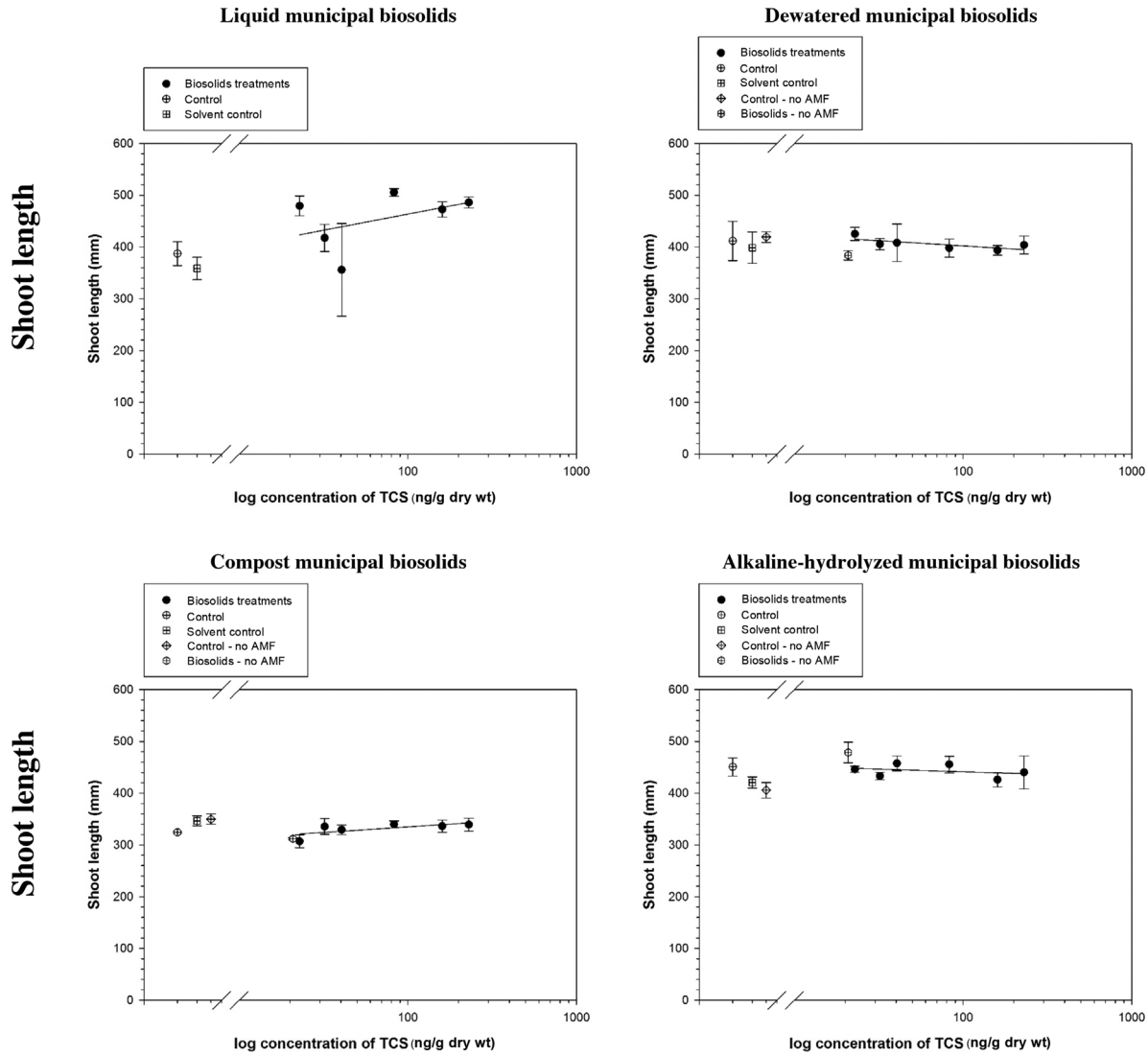


Figure 5. Mean shoot length for spring wheat grown in soil without biosolids and triclosan (TCS; control and solvent control), soil without arbuscular mycorrhizal fungi (AMF; CTNA), biosolids without AMF (BSNA), and biosolids-amended soils with increasing triclosan (BS1–BS6). Liquid municipal biosolids: $y = 399.938 + (0.459x)$; $r^2 = 0.389$; $p = 0.098$. Dewatered municipal biosolids: $y = 407.503–(0.0445x)$; $r^2 = 0.0787$; $p = 0.432$. Compost municipal biosolids: $y = 329.610 + (0.0398x)$; $r^2 = 0.0476$; $p = 0.545$. Alkaline-hydrolyzed municipal biosolids: $y = 442.072–(0.00935x)$; $r^2 = 0.00118$; $p = 0.925$.

agricultural land is typically once every 5 yr and cannot exceed 22 tonnes per hectare over the 5-yr period. Regulated waiting periods for both harvest and animal grazing, which provide time for degradation of emerging substances of concerns such as triclosan, are mandated. Thus, the potential for triclosan toxicity to crop plants appears to be negligible, as has been concluded by others [3,4,20,33,41].

For the present study, amending municipal biosolids either had a stimulatory effect on crop yield, as observed in the LMB, DMB, and AMB formulations, or had little or no effect, as demonstrated by the CMB formulation. Among the 3 formulations of biosolids that had a stimulatory effect on crop yield, LMB resulted in the greatest plant growth relative to the other formulations. The enhanced performance of the LMB relative to the other formulations may be related to moisture content and the concentration of microconstituents such as nitrogen and phosphorus. For example, the moisture content of LMB was 14%, 35%, and 4% greater than DMB, CMB, and AMB, respectively. The injection of LMB to agricultural fields, in the form of liquid manure, offers a high moisture environment that creates the physical characteristics of a slurry, including increased water content, inorganic nitrogen, and readily oxidizable carbon, all of which are favorable for rapid plant growth [48,49]. The LMB formulation used in the present study possessed these favorable characteristics, with high concentrations of nitrate (NO_3^-: 9.76 mg/kg dry) and ammonia (NH_4^+: 19 300 mg/kg dry), as well as high concentrations of carbon (total C: 28.8% dry) and phosphorus (total P: 4.51% dry). Phosphorus-rich environments encourage robust plant growth, which may have been the case for LMB. Among the 4 biosolids, LMB (9.32% dry) had the smallest and CMB (44.80% dry) the greatest percentage of moisture content. A comparatively low input of plant-available nutrients in CMB and all soil controls may partially explain the lower stimulation of plant growth in this treatment relative to the other treatments. Notably, corn experienced growth at a similar rate as the soil controls in CMB, and lacked ear development in soil controls across all experiments.

In view of the fact that soybean is capable of fixing nitrogen by forming symbiotic relationships with rhizobia bacteria (*Bradyrhizobium* sp.), it was not surprising that all 4 formulations of biosolids had little positive effect on soybean

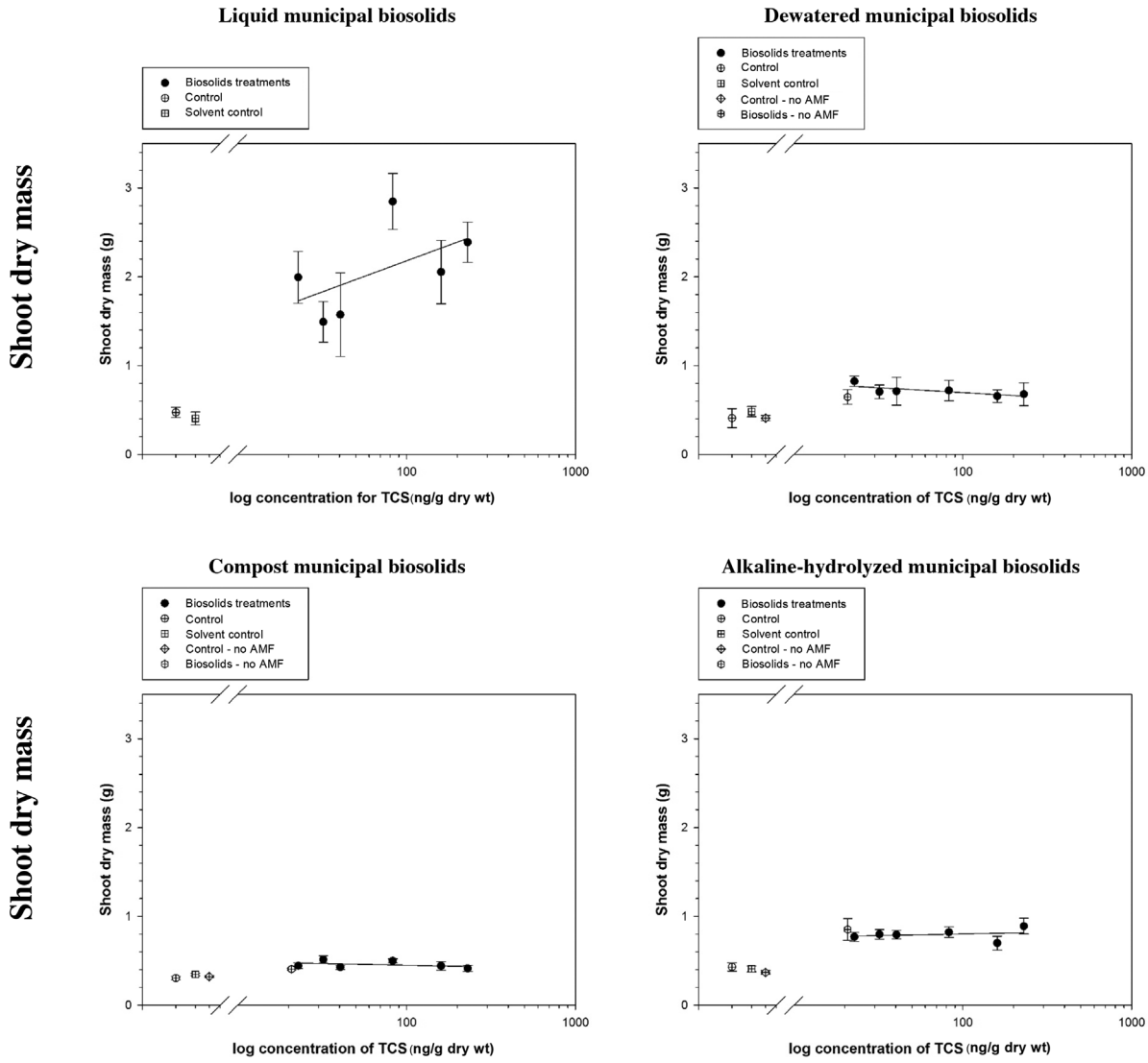


Figure 6. Mean shoot dry mass for spring wheat grown in soil without biosolids and triclosan (control and solvent control), soil without arbuscular mycorrhizal fungi (AMF; CTNA), biosolids without AMF (BSNA), and biosolids amended soils with increasing triclosan (BS1 to BS6). Liquid municipal biosolids: $y = 1.162 + (0.00686x)$; $r^2 = 0.427$; $p = 0.427$. Dewatered municipal biosolids: $y = 0.585 + (0.000654x)$; $r^2 = 0.127$; $p = 0.313$. Compost municipal biosolids: $y = 0.393 + (0.000303x)$; $r^2 = 0.112$; $p = 0.345$. Alkaline-hydrolyzed municipal biosolids: $y = 0.601 + (0.00137x)$; $r^2 = 0.275$; $p = 0.120$.

growth [50]. Root nodules of soybean plants appeared to be fewer in number in LMB, DMB, and AMB treatments relative to CMB treatments but this was not quantified. Previous studies have shown that in nutrient-deficient conditions, like those of the CMB treatments, nitrogen fixation by rhizobial bacteria and nutrient acquisition by AMF increase [51]. In nutrient-rich conditions, such as those in the LMB, DMB, and AMB treatments, AMF and rhizobial activity is reduced because of the readily increased availability of nutrients to plants [51].

There was also an unusually high fruit yield observed in soybeans grown in DMB compared with LMB, CMB, and AMB. Fruit yield in the control group, too, was greater compared with the other biosolids formulations. The reasons for this increase are unknown but did not appear to be related to date of planting, duration of flowering, or composition and compaction of soil. The LMB soybean experiment was conducted from late summer (August) to early autumn (October), which is considered off-season for this crop species. Tremblay et al. [52] found that planting soybean from mid to late May resulted in the highest grain yields in Québec, Canada. Thus, it is generally understood that planting soybean late after the optimum dates will produce less grain yield [53]. In addition, Mattioni et al. [54] proposed that the availability of oxygen in the rhizosphere, soil composition, soil compaction, and climate could greatly affect the physiology and potential for soybean growth. This, however, was not the case for the present study because the experimental conditions were constant across all soybean experiments. Therefore, although the literature offers findings that are consistent with the fruit yields seen in the LMB, CMB, and AMB experiments, it remains unknown why the fruit yield was higher in the DMB experiment.

Results from the no-AMF soil control and no-AMF biosolids control treatments in spring wheat showed that a lack of AMF inoculation did not impede plant emergence and growth. On the contrary, in CMB treatments the root wet mass in the no-AMF biosolids control treatment was higher than its AMF-inoculated equivalent; in the same experiment, the no-AMF soil control treatment was higher than its AMF-inoculated equivalent. Spring wheat normally colonizes with AMF; therefore, in a nutrient-deficient condition such as the CMB treatment, roots in the no-AMF soil and no-AMF biosolids controls may have had to spread further out in the pot soil to acquire the

nutrients necessary for optimal growth, resulting in larger root wet mass [55].

CONCLUSIONS

The application of municipal biosolids to agricultural soils can offer nutrients and organic matter needed for plant emergence and growth. The present study adhered to current best management practice for the application rate of biosolids in the province of Ontario, and demonstrated that triclosan with background concentrations of numerous emerging substances of concerns did not adversely impact growth of corn, soybean, and spring wheat cultivated in soil amended with 4 formulations of biosolids. Amending soil with biosolids either benefited or, at worst, had no effect on crop growth. From the standpoint of triclosan and background concentrations of emerging substances of concerns, our results support the land application of municipal biosolids as an economical and sustainable approach to nutrient recycling, restoration of soil fertility, and boost in crop yield [3,6,56]. Stakeholders can utilize the information generated from the present study to improve sustainable biosolids management practices in their geographical areas as a means to strengthen the credibility of municipally land-applied biosolids to local authorities and the general public.

Supplemental Data—The Supplemental Data are available on the Wiley Online Library at DOI: 10.1002/etc.3712.

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