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Biosolids inhibit bioavailability and plant uptake of triclosan and triclocarban

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# Biosolids inhibit bioavailability and plant uptake of triclosan and triclocarban

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## Highlights

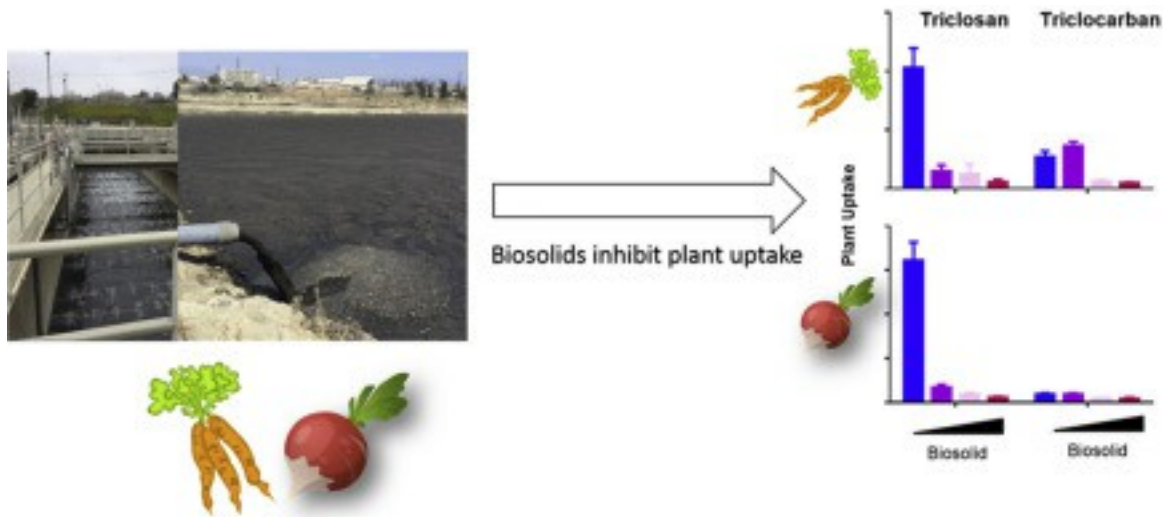
- Systematic experiments to evaluate plant uptake of triclosan and triclocarban before and after biosolid amendment.
- Comprehensive statistical analysis of results from this study and all relevant literature to discern biosolids effects.
- Use of a thin-film passive sampler to provide direct evidence on reduced bioavailability from biosolids amendment.
- Establishment of inhibition in plant uptake of triclosan and triclocarban by biosolids.

## Abstract

**Biosolids** from [wastewater treatment](#) are primarily disposed of via land applications, where numerous pharmaceuticals and personal care products (PPCPs) may contaminate food crops and pose a human [exposure risk](#). Biosolids are rich in [organic carbon](#) and addition of biosolids can increase the [sorption](#) of certain PPCPs in soil, decreasing their [bioavailability](#). This study tested the hypothesis that the relative plant uptake of PPCPs decreases with increasing biosolids amendment. Accumulation of triclosan and triclocarban was measured in roots of radish and carrot grown in soils with or without biosolids. Addition of biosolids significantly prolonged the persistence of triclosan in soil. When expressed in bioaccumulation factor (BCF), accumulation of triclosan drastically decreased in biosolids-amended soils, while the effect was limited for triclocarban. Compared to the unamended soil, amending biosolids at 2% (w/w) decreased BCF of triclosan in the edible tissues of radish and carrot by 85.4 and 89.3%,

respectively. Measurement using a thin-film passive sampler provided direct evidence showing that the availability of triclosan greatly decreased in biosolids-amended soils. Partial correlation analysis using data from this and published studies validated that biosolids decreased plant uptake primarily by increasing soil organic carbon content and subsequently sorption. Therefore, contamination of food crops by biosolids-borne contaminants does not linearly depend on biosolids use rates. This finding bears significant implications in the overall risk evaluation of biosolids-borne contaminants.

### Graphical abstract



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### Keywords

Biosolids  
Triclosan  
Triclocarban  
PPCPs  
Plant uptake  
Bioavailability

### 1. Introduction

[Biosolids](#) are the byproduct of [wastewater treatment](#) operations. Agricultural land is increasingly used as the disposal destination of biosolids, as the beneficial reuse may improve [soil health](#) and increase [crop yields](#). According to the U.S. EPA, about 8 million

tons of biosolids are produced by 16,500 [wastewater treatment plants](#) (WWTPs) in the U.S., of which 60% is eventually applied to agricultural lands ([United States Environmental Protection Agency, 2009](#), [United States Environmental Protection Agency, 1999](#)). However, studies to date have shown that numerous trace organics such as pharmaceutical and personal care products (PPCPs) are present in biosolids, with levels ranging from  $\mu\text{g kg}^{-1}$  to  $\text{mg kg}^{-1}$ . In particular, due to their high affinity for organic matter, heavily used antibacterial agents such as triclosan and triclocarban are enriched in biosolids and may reach several hundreds of  $\text{mg kg}^{-1}$  in biosolids ([Halden and Paull, 2005](#), [Walters et al., 2010](#)).

Once in agricultural fields, PPCPs in biosolids may be taken up by food crops, constituting a risk for human exposure through [dietary intake](#) ([Aryal and Reinhold, 2011](#), [Holling et al., 2012](#), [Pannu et al., 2012](#), [Prosser et al., 2014a](#), [Prosser et al., 2014b](#), [Sabourin et al., 2012](#), [Wu et al., 2012a](#), [Wu et al., 2010](#), [Wu et al., 2016](#)).

Several field studies have demonstrated the potential for plants to accumulate PPCPs. For example, [Wu et al. \(2010\)](#) found that [soybean](#) plants grown in biosolids-amended soils could accumulate triclosan, triclocarban, carbamazepine, fluoxetine, and diphenhydramine, with the maximum level of triclocarban at  $168 \pm 34 \text{ ng g}^{-1}$  (dry weight) in roots. [Sabourin et al. \(2012\)](#) also reported the occurrence of 24 PPCPs, 17 hormones, and 6 parabens at trace levels ( $0.33\text{--}6.25 \text{ ng g}^{-1}$ ) in tomatoes, [potatoes](#), carrots, and sweet corn grown in a biosolids-amended field. In a comprehensive field study, we grew eight types of vegetables with treated wastewater irrigation and detected a range of PPCPs in the edible parts at harvest ([Wu et al., 2014](#)).

While biosolids serve as the main route for soil contamination of PPCPs, amendment of biosolids may also induce drastic effects on the soil-plant continuum, which can in turn influence the plant uptake of PPCPs. Most noticeably, biosolids are rich in organic matter and may contain up to 38% [organic carbon](#) on a dry mass basis ([Kinney et al., 2006](#)). Therefore, application of biosolids may greatly increase a soil's organic carbon content (OC), leading to enhanced [sorption](#) or reduced chemical [bioavailability](#). It may be hypothesized that biosolids amendment has dual effects: while it introduces PPCPs into soil, it also simultaneously inhibits plant uptake of PPCPs due to reduced bioavailability. However, although a number of studies have considered plant accumulation of PPCPs from soil with or without biosolids ([Carter et al., 2014](#), [Macherius et al., 2012b](#), [Pannu et al., 2012](#), [Prosser et al., 2014a](#), [Wu et al., 2012a](#)), to date there lacks a clear understanding of how biosolids affect plant accumulation of PPCPs.

In this study, we used triclosan and triclocarban, two high-production-volume chemicals found in numerous household and healthcare products, as model PPCPs and tested the hypothesis that biosolids amendment had an inhibitory effect on their plant accumulation. Two root vegetables, i.e., radish (*Raphanus sativus*) and carrot (*Daucus carota* ssp. *sativus*), were considered because they present a potentially greater risk for human exposure as the edible organ is in direct contact with soil ([Prosser and Sibley, 2015](#)). To substantiate that biosolids inhibited plant uptake by decreasing chemical bioavailability, we further used a thin film-based passive sampler to detect the bioavailable concentrations in soils. Given that application of biosolids is the primary route for PPCPs to contaminate food crops, findings from this study may bear significant implications for better understanding the overall risk of biosolids-borne contaminants.

## 2. Materials and methods

### 2.1. Chemicals

Standards of triclosan (CAS# 3380-34-5) and triclocarban (CAS# 101-20-2) with >98% purity were obtained from Alfa Aesar (Ward Hill, MA) and TCI America (Portland, OR), respectively. The isotope-labeled standards of triclosan- $d_3$  and triclocarban- $d_4$  were purchased from C/D/N Isotopes (Pointe-Claire, Quebec, Canada). A stock solution was prepared by dissolving the standards in [methanol](#) and kept in amber glass vials at  $-20\text{ }^{\circ}\text{C}$  before use. All [organic solvents](#) and other chemicals were of HPLC grade and obtained from Thermo Fisher (Fair Lawn, NJ).

### 2.2. Soils and biosolids

Plant accumulation of triclosan and triclocarban was determined in plants grown in different soils and biosolids-amended soils. Three soils (abbreviated as soil A, B, and C herein) with different [physicochemical properties](#) were collected from the surface layer (0–10 cm) in fields in Riverside, CA and Irvine, CA. The aerobically treated, dewatered [biosolids](#) were obtained from a local [wastewater treatment plant](#). The soils and biosolids were sieved through a 2-mm mesh. The [organic carbon](#) content (OC) of the biosolids was determined to be 36.2% (dry weight basis). The field water holding capacity of each soil was determined using a standard method ([Gardner, 1986](#)). Biosolids-amended soils were prepared by incorporating biosolids to soil A at 2% (Soil A<sub>2</sub>), 5% (Soil A<sub>5</sub>), and 10% (Soil A<sub>10</sub>) (dry weight basis), representing approximately 8, 20, and 40 tonnes/ha with a 25 cm depth of incorporation and a soil [bulk density](#) at  $1.6\text{ g/cm}^3$ . Selected properties of soils and biosolids-amended soils used in this study are summarized in [Table 1](#).

Table 1. [Physicochemical properties](#) of soils and biosolids-amended soils.

Soil no.	Soil type	pH (H <sub>2</sub> O)	TOC <sup>a</sup> (%)	Sand (%)	Silt (%)	Clay (%)	CEC <sup>b</sup> Meq 100g <sup>-1</sup>
A	Sandy loam	6.42	0.41	64	24	12	9.6
A <sub>2</sub> <sup>c</sup>	Sandy loam	6.57	1.21	67	22	11	11.1
A <sub>5</sub> <sup>c</sup>	Sandy loam	6.51	2.45	67	22	11	14.4
A <sub>10</sub> <sup>c</sup>	Sandy loam	6.42	3.50	62	26	12	18
B	Sandy loam	7.67	0.50	73	13	14	18.5
C	Sandy Clay	7.39	1.25	57	23	20	27

a

TOC: total organic carbon.

b

CEC: cation exchange capacity.

c

A<sub>2</sub>: soil A applied with 2% of biosolids; A<sub>5</sub>: soil A applied with 5% of biosolids; A<sub>10</sub>: soil A applied with 10% of biosolids.

### 2.3. Plant cultivation and treatments

Radish and carrot were grown in soils with and without biosolids in a [greenhouse](#) (day/night: approximately 16/8 h, 25/20 °C; relative air humidity: 30–65%). Briefly, an aliquot of 100 g soil or biosolids-amended soil was spiked with 1.0 mL working solution containing triclocarban or triclosan in methanol and mixed thoroughly in a fume hood using a stainless steel blender. After the methanol was evaporated, the spiked soil was mixed thoroughly with the same type of soil (7900 g) using a motorized cement mixer. The nominal concentration of triclosan or triclocarban was 2 mg kg<sup>-1</sup>. The background concentrations of triclosan and triclocarban in biosolids were determined to be 2.5 ± 0.54 and 1.5 ± 0.39 mg kg<sup>-1</sup>, respectively. Therefore, even at the highest biosolids amendment rate (10%), the contribution of background triclosan or triclocarban to the overall chemical concentration in soil should be less than 12%. The treated soils were then transferred to individual pots of 27 cm in diameter and 30 cm in height. Seeds of radish and carrot were directly sowed in the containers. After [germination](#) and establishment, 6 radish and 10 carrot [seedlings](#) were retained in each pot while the extra plants were thinned out. To maintain soil moisture, deionized

water was added to each container twice daily. No drainage was visible during the experiment.

Subsamples of radish and carrot plants were removed after 35 and 70 d of cultivation, respectively. The leaves were separated from the roots, and the roots were carefully rinsed with deionized water, dried and divided into root skin (2 mm surface tissue) and root core. The soil in the container was mixed thoroughly using a stainless steel spatula and an aliquot of 10 g was removed. All samples (soil, root core, root skin, and leaf) were freeze-dried in a freeze dryer (Labconco, Kansas city, MO). After [freeze-drying](#), plant tissues were ground into fine powder using a stainless steel coffee grinder (Hamilton Beach, Picton, Ontario, Canada) and stored at  $-20\text{ }^{\circ}\text{C}$  before extraction and analysis.

#### 2.4. Assessment of bioavailability using film-based passive sampler

[Bioavailability](#) of triclosan and triclocarban in soil A, soil A<sub>2</sub>, soil A<sub>5</sub>, and soil A<sub>10</sub> was determined using a film-based [sampling technique](#). Thin films of different polymers have been previously used to determine the freely dissolved concentration of organic compounds in water, sediment or soil as a measurement of bioavailability ([Cornelissen et al., 2008](#), [Haftka et al., 2013](#), [Jia et al., 2012](#), [Mayer et al., 2000](#)). In this study, polymethylmethacrylate (PMMA) film (50  $\mu\text{m}$ ; Goodfellow, Coraopolis, PA) was selected through preliminary experiments. The PMMA film was cut into  $2 \times 1$  cm pieces with a [razor blade](#) and cleaned with water/methanol (95:5, v/v). The PMMA strips were further swelled in ethyl [ether](#) for 30 min and dried overnight to increase their [sorption](#) capacity before use. The pretreated PMMA strips were then embedded in 10 g (dry weight) of soil containing  $2\text{ mg kg}^{-1}$  of triclosan or triclocarban. The soil samples were incubated at room temperature ( $25\text{ }^{\circ}\text{C}$ ) in the dark and the soil moisture was maintained at 60% of the field water holding capacity throughout the incubation. All treatments were conducted in triplicates. After 35 d of incubation, the films were removed from the soil and rinsed with deionized water. Both soil and film samples were kept at  $-20\text{ }^{\circ}\text{C}$  before analysis.

#### 2.5. Sample preparation and analysis

The plant samples were extracted with methyl *tert*-butyl ether (MTBE) and [acetonitrile](#) according to a previously published method ([Wu et al., 2012b](#)). Briefly, a 0.2 g aliquot of plant tissue (dry weight) was placed in a 50-mL glass [centrifuge](#) tube, spiked with 50  $\mu\text{L}$  of  $10\text{ mg L}^{-1}$  triclosan-*d*<sub>3</sub> and  $1.0\text{ mg L}^{-1}$  triclocarban-*d*<sub>4</sub> as recovery surrogates and extracted with 20 mL [MTBE](#) for 20 min in a 50/60 Hz Fisher Scientific

FS1 10H [ultrasonic](#) water bath (Pittsburgh, PA). The sample mixtures were then centrifuged at 2000 rpm for 20 min. The supernatant was gently decanted into a 40-mL glass tube, and the residue was extracted once more with 20 mL fresh acetonitrile. The combined supernatant was concentrated to near dryness under nitrogen gas at 30 °C. The residue was recovered in 1.0 mL methanol, followed by addition of 20 mL deionized water before loading onto a 150-mg HLB cartridge (Waters, Milford, MA) for clean-up. The cartridge was preconditioned with 7 mL of methanol and then 7 mL of deionized water. The analytes were eluted under gravity using 10 mL methanol. The eluent was dried using a gentle nitrogen stream and reconstituted in 1.0 mL methanol.

Soil samples were extracted according to a previously published method ([Higgins et al., 2011](#)). In brief, an aliquot of 1.0 g of soil (dry weight) was placed in 50 mL glass centrifuge tube, spiked with 50 µL of 10 mg L<sup>-1</sup> triclosan-*d*<sub>3</sub> or 1.0 mg L<sup>-1</sup> triclocarban-*d*<sub>4</sub> as the recovery surrogate, and extracted with 20 mL methanol/acetone (1:1, v/v) for 20 min in the sonication water bath. The soil [slurry](#) was centrifuged for 20 min at 2000 rpm to recover the supernatant. The same extraction was repeated for two additional times and the extracts were combined. The combined extract was dried under nitrogen gas, followed by clean up with a HLB cartridge as described above for plant tissues.

Upon retrieval, PMMA film strips were spiked with 50 µL of 10 mg L<sup>-1</sup> triclosan-*d*<sub>3</sub> or 1.0 mg L<sup>-1</sup> triclocarban-*d*<sub>4</sub> (as recovery surrogates) and were subsequently subjected to triple extractions with 10 mL of hexane-acetone-methanol (8:1:1, v/v/v) under sonication for 20 min each time. The pooled extracts were dried under nitrogen gas and then reconstituted in 1.0 mL methanol. All samples were filtered through a 0.22-µm [polytetrafluoroethylene](#) (PTFE) filter membrane (Millipore, Carrigtwohill, Cork, Ireland) and stored at -20 °C before instrumental analysis.

Instrumental analysis for triclosan and triclocarban was performed on a Waters ACQUITY ultra-performance [liquid chromatography](#) (UPLC) combined with a Waters Micromass electrospray [ionization](#) tandem [mass spectrometer](#) (ESI-MS/MS) (Waters, Milford, MA). Chromatographic separation of analytes was achieved on an ACQUITY UPLC BEH C18 column (2.1 mm × 100 mm, 1.7 µm particle size, Waters) at 40 °C. Water (containing 5% methanol and 0.001% formic acid) and pure methanol were used as the mobile phase A and B, respectively, which was programmed as below (with respect to mobile phase A): 0–5 min: 90%–0%; 5–6 min, 0%; and 6–8 min, 90%. The flow rate was kept at 0.2 mL min<sup>-1</sup> and the injection volume was 5 µL. The mass data were acquired using the multiple reactions monitoring (MRM) in the negative ESI mode. The following transitions and collision energies were optimized for confirmation and quantification: triclosan, 287 > 35 at 8 eV; triclosan-*d*<sub>3</sub>, 290 > 35 at 10 eV; triclocarban,



313 > 160 at 20 eV; and triclocarban- $d_4$ , 317 > 160 at 10 eV. The specific instrumental settings were: capillary [voltage](#) 3.2 kV, cone voltage 30 V, collision gas (Argon, 99.99%) 0.2 mL/min, source temperature 120 °C, desolvation temperature 350 °C, desolvation gas 600 L h<sup>-1</sup>, and cone gas 50 L h<sup>-1</sup>.

## 2.6. Quality assurance and quality control

All treatments were performed in triplicates. Non-spiked soils and non-planted soils were included as treatment blanks. Triclosan- $d_3$  and triclocarban- $d_4$  were spiked into each sample before extraction to correct for potential analyte losses or gains during sample preparation, matrix effects in ionization, and variations in instrumental response. The recovery of the target analytes and their deuterated analogues varied from 30 to 70% in plant samples, from 75 to 105% in soil samples, 61–98% in the PMMA film. All data were calculated as mean and standard deviation of triplicates. A one-way ANOVA test was conducted at  $\alpha = 0.05$  to evaluate the significance of difference among the different treatments. Statistical analysis was performed using the SPSS 19.0 software (IBM SPSS Statistics, Armonk, NY).

## 3. Results and discussion

Amendment of [biosolids](#) did not significantly change the soil pH ([Table 1](#)). It was estimated that 92–95% of triclosan ( $pK_a = 7.9$ ) was in its neutral form in soil A with or without biosolids amendment, while all triclocarban ( $pK_a = 12.7$ ) was in the neutral form. The [total organic carbon](#) content (TOC) of the three unamended soils ranged from 0.41% in soil A to 1.25% in soil C. Addition of biosolids substantially increased soil TOC, and the increase was proportional to the rate of biosolids amendment, with final TOC content at 1.21% for soil A amended with 2% biosolids (soil A<sub>2</sub>), 2.45% with 5% biosolids (soil A<sub>5</sub>), and 3.50% with 10% biosolids (soil A<sub>10</sub>) ([Table 1](#)).

### 3.1. Chemical dissipation in soils

Radish was harvested at 35 d after planting, while carrot was sampled after 70 d of growth. At the time of harvest, soil samples were analyzed for remaining concentrations of triclosan and triclocarban ([Table 2](#)). When expressed on the basis of the initial total concentration (sum of spiked and residue in biosolids), triclosan dissipated quickly in the unamended soils. Among the three unamended soils, triclosan dissipation was more rapid in soils A and B than in soil C ([Table 2](#)). For example, after 35 d, 2.9–3.4% of the initial concentration was found in soils A and B, while 15.3% still remained in soil C. A similar difference was also observed after 70 d in the carrot containers ([Table 2](#)). Compared to triclosan, triclocarban was much more persistent in the unamended soils,

with 35.2–55.7% of the initial concentration still remaining in the growth media at 70 d after treatment ([Table 2](#)). Moreover, the influence of [soil types](#) was smaller than that for triclosan. The increased persistence of triclosan in soil C may be partially attributed to its higher TOC content, suggesting that organic matter likely increased [sorption](#) of triclosan and consequently inhibited its microbial degradation due to reduced [bioavailability](#).

Table 2. Concentrations (ng g<sup>-1</sup>, dry weight) and fractions of triclosan and triclocarban remaining in soils at time of plant harvest (n = 3).

Soil <sup>a</sup>	Triclosan				Triclocarban			
	Radish (35 d)	% <sup>b</sup>	Carrot (70 d)	%	Radish (35 d)	%	Carrot (70 d)	%
A	67.6 ± 12.0	3.4 ± 0.6	51.5 ± 4.1	2.6 ± 0.2	1003.2 ± 100.9	50.2 ± 5.0	1024.3 ± 233.7	51.2 ± 11.7
A <sub>2</sub>	549.6 ± 47.3	26.8 ± 2.3	433.9 ± 62.0	21.2 ± 3.0	583.3 ± 36.6	28.7 ± 1.8	544.0 ± 9.8	26.8 ± 0.5
A <sub>5</sub>	944.0 ± 76.2	44.4 ± 3.6	802.3 ± 72.2	37.8 ± 3.4	766.3 ± 75.3	36.9 ± 3.6	737.6 ± 18.3	35.5 ± 0.9
A <sub>10</sub>	1637.9 ± 136.3	72.8 ± 6.1	1148.1 ± 91.1	51.0 ± 4.1	1394.9 ± 113.3	64.9 ± 5.3	1054.1 ± 136.2	49.0 ± 6.3
B	57.4 ± 20.6	2.9 ± 1.0	25.9 ± 3.3	1.3 ± 0.2	819.2 ± 129.6	41.0 ± 6.5	703.4 ± 73.6	35.2 ± 3.7
C	305.4 ± 21.1	15.3 ± 1.1	116.0 ± 22.0	5.8 ± 1.1	1320.0 ± 100.4	66.5 ± 5.0	1114.4 ± 63.2	55.7 ± 3.2

a

A<sub>2</sub>: soil A amended with 2% of biosolids; A<sub>5</sub>: soil A amended with 5% of biosolids; A<sub>10</sub>: soil A amended with 10% of biosolids.

b

Calculated as the initial concentration, which is the sum of the spiked concentration and the native chemical concentration in biosolids.

With the amendment of biosolids, the residual level of triclosan at the end of cultivation was significantly higher than that in the unamended soil, and the higher the biosolids amendment rate, the higher the residual concentration of triclosan ([Table 2](#)). For example, after 35 d of cultivation, residual triclosan in the 2% amended soil (549.6 ng g<sup>-1</sup>) was 8.1 times that in the unamended soil (67.6 ng g<sup>-1</sup>). The remaining level of triclosan further increased by 14.0 and 24.2 times in the soils amended with biosolids at 5 and 10%, respectively. A pronounced effect of biosolids on the persistence of triclosan was also observed in the carrot containers after 70 d of cultivation ([Table 2](#)). It must be noted that biosolids used in this study contained some triclosan and that the “native” triclosan may have contributed to the increased residue in biosolids amended soils. However, the native triclosan was estimated to be less than 12% of the initially spiked triclosan concentration at the highest amendment rate, and was proportionally smaller in the soils amended at the lower rates. Therefore, the influence of biosolids on

the persistence of triclosan may be mainly attributed to other factors, such as sorption and bioavailability.

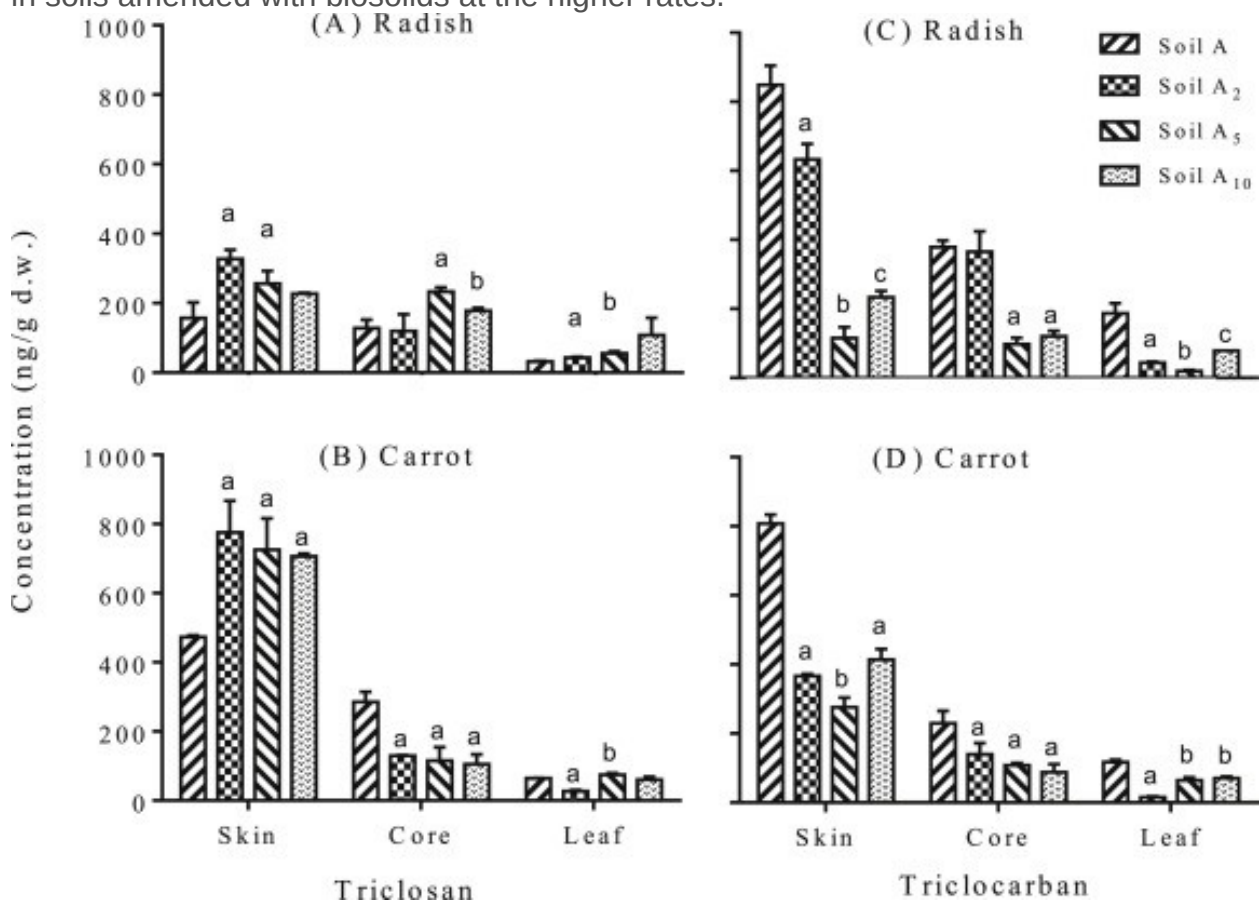
Compared to triclosan, amendment of biosolids generally did not elicit a similar effect on the dissipation of triclocarban ([Table 2](#)). The difference between triclosan and triclocarban may be due to the fact that triclocarban was already relatively persistent as compared to triclosan in the unamended soil and the effect of biosolids on its persistence was not discernable over the short duration of the [greenhouse](#) experiments. It is also likely that triclocarban was degraded via different mechanisms, and that addition of biosolids stimulated the [biodegradation](#) of triclocarban, offsetting any effect due to increased sorption or reduced bioavailability from biosolids. However, the substantially increased persistence of triclosan in biosolids-amended soils validated that biosolids are capable of imparting significant effects on the fate and availability of some PPCPs in soil, which may subsequently influence their uptake by plants.

### 3.2. Accumulation of triclosan and triclocarban in edible tissues

The edible parts of radish and carrot were harvested at 35 d and 70 d, respectively. To better understand plant uptake and biosolids effect, the skin, core, and leaf were separated and analyzed individually. The concentrations of triclosan and triclocarban in different plant tissues grown in unamended soils are shown in [Fig. S1](#). In general, triclosan and triclocarban were detected in all plant tissues. Accumulation of triclosan and triclocarban varied among the different soil types ([Fig. S1](#)). The lowest accumulation was observed in plants grown in soil C, while the highest uptake occurred mostly in soil A. In most of the treatments, concentrations of triclosan or triclocarban in the root skin, core and leaf followed the order of soil A > soil B > soil C for both radish and carrot, except for triclosan in radish, which followed the order of soil B > soil C > soil A in radish skin and core, and soil C > soil A > soil B in radish leaf. The difference among the different soils may be attributed to variations in the bioavailability of triclosan and triclocarban as influenced by soil properties such as pH, TOC, and indigenous [microbial communities](#). As soil pH increased from 6.42 in soil A to 7.67 in soil C, the neutral fraction of triclosan decreased from 97% to 63%. It is known that plant uptake of [anions](#) is often inhibited as compared to uncharged molecules, because the negative charged cell membrane may repel the transport of negatively charged anions across cell membranes ([Trapp, 2000](#)). Moreover, due to the higher TOC in soil C, increased sorption or reduced bioavailability may have also contributed to the lower plant uptake. In addition, the higher concentration of triclocarban than that of triclosan may be due to recalcitrance of triclocarban to plant metabolism. Compared to

triclocarban, triclosan was previously found to be rapidly transformed in plants ([Macherius et al., 2012a](#), [Wu et al., 2016](#)).

The concentrations of triclosan and triclocarban in different plant tissues grown in biosolids-amended soils are shown in [Fig. 1](#). Both triclosan and triclocarban were found in all parts of the plants. For the same treatment, the levels of triclosan or triclocarban in the different samples generally followed skin > core > leaf. For example, in the 2% amended soil, the average levels of triclosan were  $775.3 \pm 91.9$ ,  $129.9 \pm 1.7$ , and  $27.0 \pm 3.3$  ng g<sup>-1</sup> in the carrot skin, core, and leaf, respectively. Likewise, the corresponding values for triclocarban were  $365.9 \pm 6.0$ ,  $139.8 \pm 32.6$ , and  $15.2 \pm 2.6$  ng g<sup>-1</sup>. Similar trends were also observed in radish, as well as for both plants in soils amended with biosolids at the higher rates.



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Fig. 1. Concentrations of triclosan and triclocarban in carrot and radish grown in soil A amended with 0% (Soil A), 2% (Soil A<sub>2</sub>), 5% (Soil A<sub>5</sub>), and 10% (Soil A<sub>10</sub>) of [biosolids](#). (A) triclosan in radish; (B) triclosan in carrot; (C) triclocarban in radish; (D) triclocarban in carrot. Different letters (a, b, c) show significance of difference at level  $\alpha = 0.05$

(Student *t*-test) compared to soil A. Error bars represent the standard deviation of triplicates.

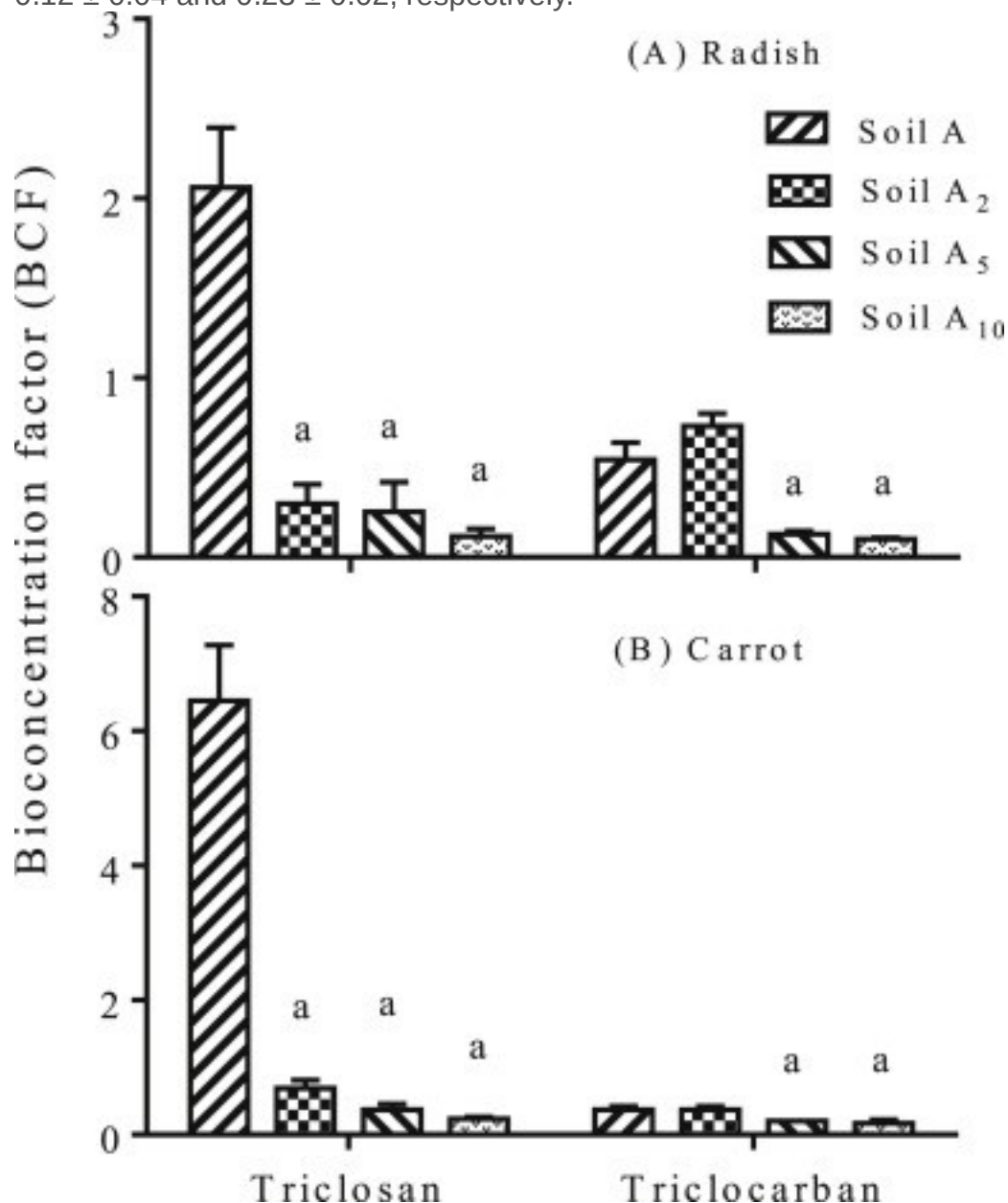
As the biosolids amendment rate increased, concentrations of triclosan or triclocarban in the various tissues did not exhibit a clear trend. For example, the concentration of triclosan was  $156.8 \pm 45.6 \text{ ng g}^{-1}$  (d.w.) in the skin of radish grown in the unamended soil A, which increased to  $327.0 \pm 27.1 \text{ ng g}^{-1}$  in the 2% amended soil, but decreased to  $256.3 \pm 36.3 \text{ ng g}^{-1}$  in the 5% amended soil and further to  $227.4 \pm 2.4 \text{ ng g}^{-1}$  in the 10% amended soil ([Fig. 1A](#)). A similar pattern was observed in the carrot skin, but not in the core or leaf samples. For triclocarban, the concentration in the skin, core or leaves in the biosolids-amended treatments decreased compared to the unamended treatment, although there were exceptions.

It was evident that the distribution of either triclosan or triclocarban in the root vegetables did not change linearly in response to biosolids amendment. This may be due to the complexity of interactions of multiple processes and factors among soil, biosolids, plant and [soil microorganisms](#). As a source of triclosan or triclocarban, application of biosolids may have increased the net amount of chemicals in the system. However, biosolids amendment resulted in a significant increase in soil TOC ([Table 1](#)), leading to increased sorption or reduced bioavailability. Moreover, addition of biosolids introduced exogenous microorganisms into the soil, which may have contributed to enhanced chemical transformations, also leading to reduced bioavailability of chemicals for plant uptake.

### 3.3. Influence of biosolids on plant accumulation of triclosan and triclocarban

To better evaluate the influence of soil properties and biosolids on plant uptake of triclosan and triclocarban, [bioconcentration factors](#) (BCFs) were calculated and used for comparative analysis ([Fig. 2](#) and [Fig. S2](#)). BCF was calculated by dividing the concentration in plant tissues over that in soil at the time of sampling. Among the unamended soils, BCFs of triclosan and triclocarban in soil C were much smaller than those in soil A or B, and the difference may be partly attributed to the relatively higher TOC content in soil C ([Fig. S2](#)). For both radish and carrot, BCFs of triclosan significantly decreased in the biosolids-amended soils, while decreases were limited for triclocarban. For example, the mean BCF of triclosan in the radish skin was  $2.4 \pm 0.8$  in the unamended soil, which decreased to only 0.3–0.6 in the biosolids-amended soils, reflecting a 4–8-fold reduction ([Fig. S2](#)). Similar decreases were also observed for the core of radish and carrot. For example, the mean BCF for triclosan in the radish core was  $1.9 \pm 0.4$ , which decreased to 0.2–0.3 in the biosolids-amended soils, suggesting a

6.3–9.5-fold reduction. When values in both skin and core were averaged, BCFs in radish and carrot were  $2.1 \pm 0.1$  and  $6.5 \pm 0.5$ , respectively, in the unamended soil, which decreased significantly ( $p < 0.05$ ) to  $0.30 \pm 0.1$  and  $0.69 \pm 0.06$  in the 2% amended soil, and then to  $0.25 \pm 0.17$  and  $0.37 \pm 0.05$  in the 5% amended soil. In the 10% amended soil, the average BCFs in the edible part of radish and carrot were only  $0.12 \pm 0.04$  and  $0.23 \pm 0.02$ , respectively.



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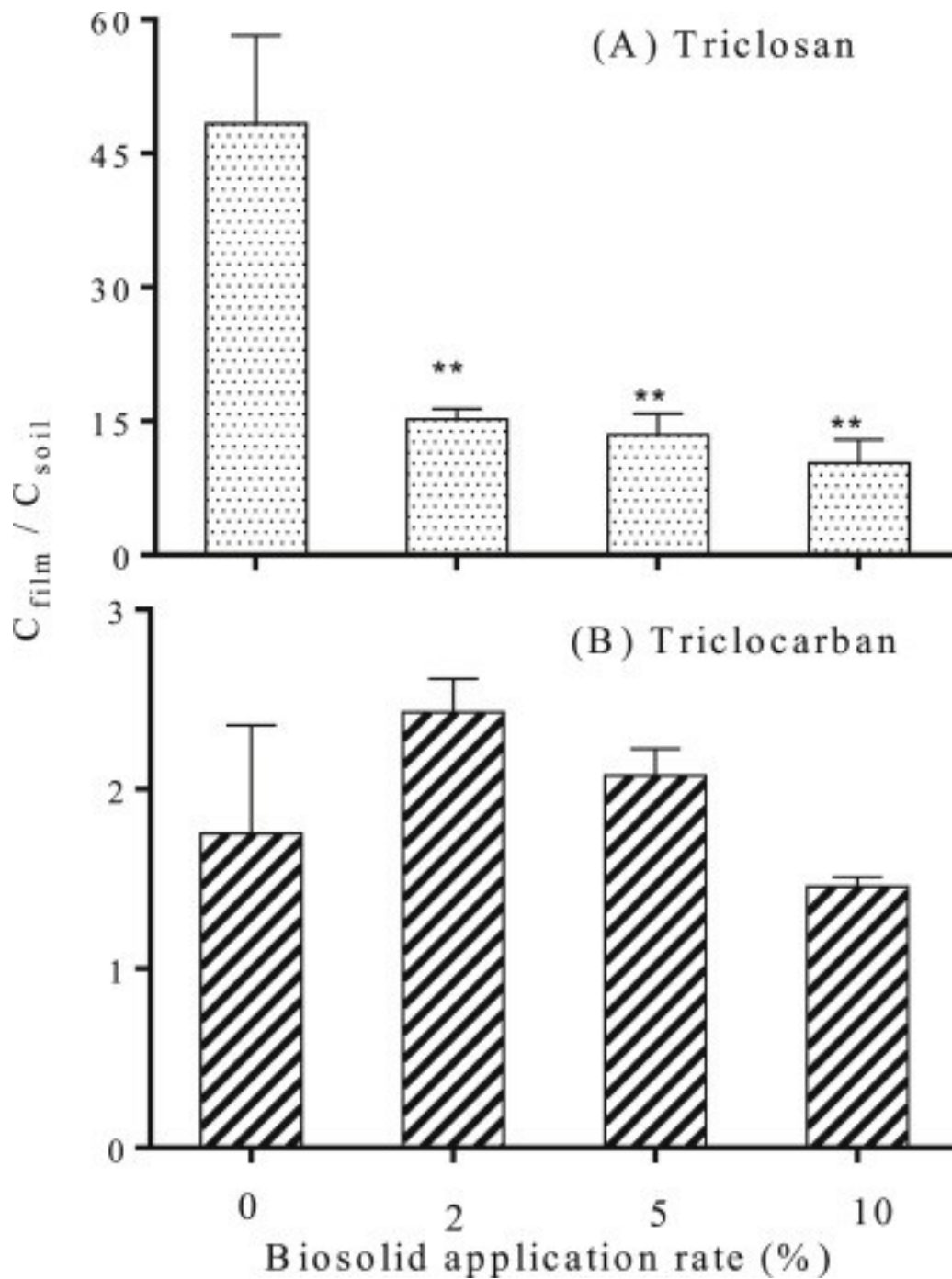
Fig. 2. Root [bioconcentration factors](#) (BCF) of triclosan and triclocarban in the root of radish and carrot grown in biosolids-amended soils. (A) BCFs of radish. (B) BCFs of

carrot. Different letters (a, b, c) show significance of difference at level  $\alpha = 0.05$  (Student *t*-test) compared to soil A. Error bars represent the standard deviation of triplicates.

A similar effect was not observed on triclocarban. The BCFs in the soil amended with 2% biosolids were similar to those in the unamended soils for both carrot and radish ([Fig. 2](#)). After averaging the core and skin data, BCFs of triclocarban in carrot in the soils with 5 or 10% biosolids were lower than the unamended soil or 2% amended soil, but the difference was not statistically significant for carrot.

The significant inhibitory effect of biosolids on plant uptake of triclosan suggested that in biosolids-amended soils, triclosan became less bioavailable. This occurred even though addition of biosolids greatly elevated the residual concentration of triclosan in soil at the time of plant sampling due to slower degradation ([Table 2](#)). In a previous study, addition of biosolids was found to rapidly increase sorption of triclosan ([Fu et al., 2016](#)). For instance, in the 2% amended soil,  $K_d$  of triclosan was 3.9-fold that in the unamended soil. Therefore, biosolids likely decreased the chemical concentration in soil solution due to enhanced sorption, leading to the reduced plant uptake.

Evaluation using a thin-film passive sampler offered a direct evidence on the effect of biosolids on bioavailability of triclosan and triclocarban. In this study, a strip of thin PMMA film was imbedded in the treated soils, and the level of triclosan or triclocarban in the film was determined after 35 d. The concentrations of triclosan and triclocarban in the film in different soil treatments are shown in [Fig. S3](#). Chemical availability was calculated as the ratio of the concentration in the PMMA film to that in the soil. As shown in [Fig. 3](#), the availability of triclosan in the unamended soil ( $48.3 \pm 9.9$ ) decreased drastically after amendment of biosolids at 2% ( $15.2 \pm 1.2$ ), and additional decreases were further observed in the soils with 5 or 10% amendment. In contrast, no significant difference was observed between the unamended and amended soils for triclocarban ([Fig. 3B](#)). Thin-film samplers operate on the basis of equilibrium partition, and the level in the film has been related to the freely dissolved concentration of a chemical in the sediment or soil solution, and further to bioaccumulation ([Haftka et al., 2013](#), [Jia et al., 2012](#)). Therefore, measurement using the thin-film sampler further corroborated that biosolids significantly decreased the availability of triclosan in soil, but not that of triclocarban. This modification to chemical availability likely resulted in the observed differences in the plant uptake of triclosan and triclocarban.



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Fig. 3.  $C_{\text{film}}/C_{\text{soil}}$  of triclosan and triclocarban in (A) triclosan and (B) triclocarban in different soils. \* indicates statistical significance at  $\alpha = 0.05$  (\*) or 0.01 (\*\*) level. Error bars represent the standard deviation of triplicates.

### 3.4. Evaluation of factors affecting plant uptake

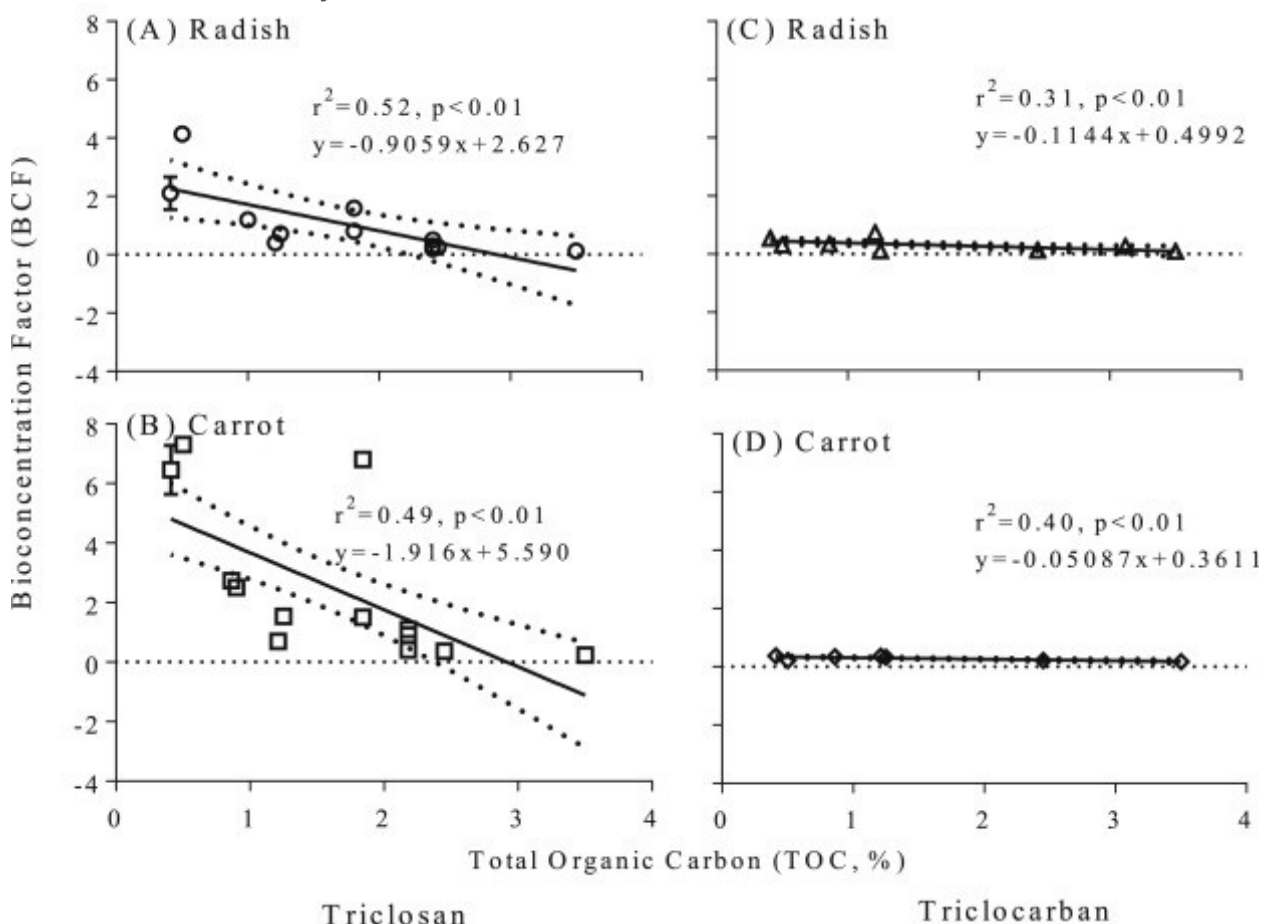


Sorption ( $K_d$ ) and persistence (measured as half-time  $T_{1/2}$ ) are considered the two primary variables controlling the availability and hence offsite transport potential of contaminants in soil ([Roberts et al., 2007](#), [Semple et al., 2013](#)). The availability of a chemical decreases with increased sorption, while it increases as the chemical becomes more persistent. To extrapolate observations from this study and further understand the effects of biosolids on plant uptake of PPCPs, several correlation analyses were performed using data from this study alone or data from both this and published studies.

A simple correlation analysis was first performed between  $K_d$  and plant uptake of triclosan or triclocarban using data from this study. The  $K_d$  values for the different soils were taken from a recent soil-incubation study ([Fu et al., 2016](#)). A significant and negative relationship was found between plant uptake and  $K_d$  for triclosan ( $r^2 = 0.40$ – $0.65$ ,  $p < 0.05$ ) or triclocarban ( $r^2 = 0.21$ – $0.74$ ,  $p < 0.05$ ), suggesting sorption played a dominant role in the inhibition of biosolids on plant uptake of these compounds. From the derived regression, as  $K_d$  increased by 50%, plant uptake of triclosan would decrease by 25.3–25.9%, while that of triclocarban would decrease by only 0.9–1.6%. In a recent study, sorption ( $K_d$ ) was found to increase  $T_{1/2}$  of both triclosan and triclocarban, and the effect was more pronounced for triclosan ([Fu et al., 2016](#)). Because of the co-dependence of  $K_d$  and  $T_{1/2}$ , a partial correlation analysis was conducted to find a unique variance between  $T_{1/2}$  and plant uptake while eliminating the variance from  $K_d$  ([Wang, 2013](#)). The relationships were found to be poor between  $T_{1/2}$  and plant uptake of triclosan ( $r^2 = 0.007$ – $0.20$ ,  $p > 0.05$ ) or triclocarban ( $r^2 = 0.007$ – $0.52$ ,  $p > 0.05$ ), implying that persistence in soil alone did not impart a discernable effect on plant uptake of these compounds. These analyses highlighted that enhanced sorption from biosolids was likely the predominant cause for the decreased plant uptake, especially for triclosan.

A subsequent correlation analysis was undertaken to examine dependence of plant uptake on soil TOC. To strengthen the power of analysis, BCFs of triclosan or triclocarban in radish and carrot root (with skin and core combined), as well as TOC values in the corresponding soil or biosolids-amended soils, from this and relevant references ([Carter et al., 2014](#), [Macherius et al., 2012b](#), [Pannu et al., 2012](#), [Prosser et al., 2014a](#), [Wu et al., 2012b](#)) were used as the data input ([Fig. 4](#)). Detailed information about the fit parameters is given in [Table S1](#). It is evident that BCFs of both triclosan and triclocarban were significantly ( $p < 0.01$ ) negatively related to soil TOC. From the slopes of the linear relationships, the effect of soil TOC on plant uptake was far greater for triclosan than triclocarban. For triclocarban, with a 1% increase in soil

TOC, the BCF would decrease by only 0.11 for radish and by 0.051 for carrot, reflecting a limited effect. In contrast, as soil TOC increased by 1%, BCF of triclosan may be expected to decrease by 0.91 for radish and 1.92 for carrot, suggesting a profound influence. These analyses imply that because amendment of biosolids leads to increases in soil TOC and stronger sorption, plant uptake decreases due to reduced chemical bioavailability.



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Fig. 4. Correlation of soil [total organic carbon](#) (TOC, %) content and root [bioconcentration factors](#) (BCFs) of triclosan in radish (A) (n = 12) and carrot (B) (n = 13), and triclocarban in radish (C) (n = 8) and carrot (D) (n = 7). Data points are from this study and published studies in the literature. Error bars represent the standard deviation of triplicates.

#### 4. Conclusions

In this study, we quantified plant uptake of two model biosolids-borne PPCPs by two common root vegetables (i.e. radish and carrot) grown in soils with or

without [biosolids](#) amendment. Among the different plant parts analyzed, the edible root tissue was found to readily accumulate triclosan and triclocarban. Bioaccumulation of triclosan in soil was substantially inhibited after the amendment of biosolids, and the inhibition was in close agreement with the chemical availability reflected in measurement with a thin-film passive sampler. Correlation analyses using data from this and earlier studies uncovered that the change in [sorption](#), not persistence, was primarily responsible for the effects of biosolids. While biosolids amendment serves as an important route for soil contamination of PPCPs, biosolids also simultaneously inhibit plant uptake of certain PPCPs due to increased sorption or reduced [bioavailability](#). Therefore, plant uptake of PPCPs cannot be simply predicted from the biosolids use rate.

Results from this and other studies also show that while biosolids displayed a generally inhibitory effect on plant uptake, the magnitude of effect may vary among different compounds. A pronounced effect may be expected for compounds for which sorption is easily influenced by changes in soil TOC. More research is needed to determine the generality of biosolids-induced inhibition of plant uptake among the different PPCPs and other biosolids-borne contaminants. The knowledge of non-linear response of plant uptake to biosolids application should be further incorporated in predictive models to better evaluate human dietary exposure and potential effects on other [non-target organisms](#).

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## Appendix A. Supplementary data

The following is the supplementary data related to this article:

[Download Word document \(212KB\)](#)[Help with docx files](#)

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