

Determination of the bioavailable fraction of triclosan in biosolid-treated soils using a predictive method and wheat plant bioassays

Yanina Corrotea¹ · Pablo Richter¹ · Sally Brown² · Betsabet Sepúlveda¹ · Loreto Ascar¹ · Inés Ahumada¹

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Abstract

Purpose Triclosan (TCS, 5-chloro-2-(2,4-dichlorophenoxy)phenol) an antimicrobial compound used in a range of household products, is an emerging hydrophobic organic contaminant, that may be incorporated into soil through the application of biosolids. The present study assessed the bioavailable fraction of TCS in a soil-biosolid system using wheat (*Triticum aestivum*) plant assays and a predictive extraction method using a solution of hydroxypropyl- β -cyclodextrin (HPCD) to determine if it was a reliable surrogate for this bioassay.

Materials and methods Three soils were obtained from the central region of Chile (Cuesta Vieja, Polpaico, and Taqueral). Biosolid was obtained from a regional wastewater treatment plant. The soils were amended with biosolids at different rates (30, 60, 90, and 200 Mg ha⁻¹). The TCS concentration was determined in biosolids, soil, and plant samples via gas chromatography coupled with mass spectrometry (GC-MS).

Results and discussion The total TCS concentration in the biosolids was 5.45 mg kg⁻¹. The results of the TCS extraction from the wheat plants (roots and shoots) indicated that TCS was primarily found in the roots. TCS uptake by the plant varied based on soil properties. The predictive capability of

the HPCD extraction was assessed using a simple linear correlation test for TCS concentration in wheat plants.

Conclusions The study yielded a linear relationship, which demonstrated the validity of the chemical method as a biosimulation technique.

Keywords Bioavailability · Biosolids soil · Cyclodextrin · Triclosan

1 Introduction

Land application of biosolids has been shown to contribute to improved soil physical and chemical conditions, including porosity, aggregate stability, water retention, aeration, and nutrient content, all of which help to prevent erosion and favor root growth and plant and soil nutrition (Khaleel et al. 1981; Wallace et al. 2009; Brown et al. 2011). However, when biosolids are applied to soils, organic contaminants may be retained in the soil, leached through the liquid phase of soil, taken up by plants and/or bio-accumulated in soil organisms (Epstein 2003).

Broad-spectrum antimicrobial agents such as triclosan (TCS) are among the emerging organic contaminants detected in wastewater treatment plants. Triclosan (5-chloro-2-(2,4-dichlorophenoxy)phenol) is an antimicrobial agent widely used in many personal care products such as soaps, detergents, toothpastes, deodorants, disinfectants, cosmetics, and pharmaceuticals. It is also used as an additive in plastics, polymers, and textiles (De Vere and Purchase 2007; Orhan et al. 2007). Therefore, discharge of effluents from wastewater treatment plants and the application of biosolids to soils are the main routes through which TCS enters the environment after its use (Ying and Kookana 2007). Triclosan exhibits a broad antimicrobial spectrum against gram⁺ and gram⁻ bacteria. In

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✉ Inés Ahumada
iahumada@ciq.uchile.cl

¹ Facultad de Cs. Químicas y Farmacéuticas, Universidad de Chile, Casilla 233, Santiago, Chile

² University of Washington, Box 352100, Seattle, WA 98195, USA

addition, TCS is effective against fungi and yeast (Stewart et al. 1999; McBain et al. 2003; Frantz et al. 2008; Clarke and Smith 2011; Gasperi et al. 2014). Its antimicrobial effect occurs through the inhibition of the carrier protein, which is responsible for the biosynthesis of the fatty acids necessary for cell wall formation and bacterial reproduction (Coogan et al. 2008). The presence of triclosan in the environment can lead to the development of resistant microorganism strains (Stevens et al. 2009).

Triclosan is a hydrophobic, chlorinated phenoxy phenol with a pKa of 7.9 and an estimated octanol-water partition coefficient ($\log K_{ow}$) of 4.8 (Wu et al. 2009). Given its hydrophobic nature, triclosan is not completely eliminated from either water or the solid generated at wastewater treatment plants. In addition, the conversion of triclosan into methyltriclosan (MTCS) has been observed, and MTCS has been measured in a variety of environmental samples, including influents and effluents from wastewater treatment plants (Cheng et al. 2011; Jachero et al. 2013).

Triclosan has been considered as endocrine disruptor which implies that TCS may alter the growth, development, reproduction, and behavior of living organisms, interfering with their normal hormone activity by mimicking hormones (Bedoux et al. 2012). It is found that the antimicrobial agents triclosan (TCS), triclocarban (TCC), and their associated transformation products are of increasing concern as environmental pollutants due to their potential adverse effects on humans and wildlife, including bioaccumulation and endocrine-disrupting activity (Matsumura et al. 2005).

To assess the potential environmental hazard produced by TCS, one must determine its concentration, behavior, fate in biosolid-amended soils, and in wastewater effluents. The land application of biosolids is the main route through which TCS enters the soil. The plants grown in soil amended with biosolids at agronomic application rates for multiples years should never experience TCS toxicity, but TCS may accumulate in their tissues (Pannu et al. 2012).

Triclosan uptake has been assessed in bean plants (*Phaseolus vulgaris*) in which the TCS was primarily distributed in the roots but not in the shoots (Karnjanapiboonwong et al. 2011). Triclosan has been observed in various plants, including food crops such as lettuce and radish, as well as bahiagrass grown in soils fertilized with biosolids (Pannu et al. 2012). Other researchers have examined triclosan phytoaccumulation from biosolid-amended soils in pumpkins, zucchini, and grass, these results showed more potential for phytoaccumulation than other crops (Aryal and Reinhold 2011).

An assessment of contaminant bioavailability in soils is necessary to understanding potential associated hazards. Hydroxypropyl- β -cyclodextrin (HPCD) has recently been used in a non-exhaustive extraction technique to determine the bioavailability of hydrophobic organic contaminants in

soil (Ried et al. 2000b; 2004). The HPCD offers the advantage that cyclodextrins can form inclusion complexes with several compounds because they have a hydrophilic outer surface with an apolar cavity that provides a hydrophobic matrix. This cavity enables the formation of inclusion complexes with a wide variety of hydrophobic molecules. Thus, HPCD is an appropriate extractant for simulating the bioavailable fraction of some soil contaminants (Wong and Bidleman 2010). Other researchers have found that HPCD extraction is a good method for predicting PAH bioavailability (Cuyppers et al. 2002; Doik et al. 2006).

The present study intends to assess the bioavailable fraction of TCS in a soil-biosolid system using wheat (*Triticum aestivum*) assays and to determine if HPCD extraction is a reliable surrogate for this bioassay.

2 Materials and methods

2.1 Reagents

Triclosan (97 %) was obtained from Dr. Ehrenstorfer Gmb H (Augsburg, Germany). Carbon 13-labeled TCS ($^{13}\text{C}_{12}$ -TCS, 50 mg L⁻¹) was purchased from Wellington Laboratories (Ontario, Canada), was used as a surrogate standard. Hexachlorobenzene (HCB, 99.5 % purity) was used as internal standard and was purchased from Dr. Ehrenstorfer Gmb H (Augsburg, Germany). The N-methyl-N-(tert-butyl)dimethylsilyl trifluoroacetamide (MTBSTFA) derivatizing agent was obtained from Sigma Aldrich (Milwaukee, WI, USA). The HPLC grade solvents ethyl acetate, methanol, acetone, hexane, and dichloromethane were purchased from Merck (Darmstadt, Germany). Hydroxypropyl- β -cyclodextrin was obtained from Sigma Aldrich. The Oasis HLB cartridges for the solid-phase extraction were obtained from the Waters Corporation (Milford, MA, USA). Nitrogen 5.0 and helium 5.0 were purchased from Linde (Santiago, Chile) and were used in the final extract evaporation and as the chromatographic carrier gas, respectively.

2.2 Soil and biosolid samples

Three soil samples were obtained from the Santiago Metropolitan Region, Chile: Polpaico, Taqueral, and Cuesta Vieja. The soil samples were collected from the surface level (0–20 cm). Compound samples from each sample site were air-dried and passed through a 2-mm sieve. The biosolid of anaerobic digestion was collected at a wastewater treatment plant in the Santiago Metropolitan Region. The biosolid was air-dried and passed through a 2-mm sieve.

The pH, electrical conductivity (EC), organic carbon content, and cation exchange capacities of the soil and biosolid

samples were determined. Texture analysis was performed on the soil samples by Bouyoucus methods (Sadzawka et al. 2006).

2.3 Spiking the biosolids with TCS

The biosolids were spiked with an additional TCS concentration (10 mg kg^{-1}) using the following procedure: 500 g of biosolids was placed in a separate 500 mL round flask, and an additional TCS concentration of 10 mg kg^{-1} in acetone was added using the commercial formulation IrgasanTM. The sample was covered in acetone, evaporated in a rotary evaporator at 200 rpm for 24 h at room temperature in darkness to prevent photo-degradation of the compound. The biosolid was then transferred to a dish and left to dry in darkness for 2 weeks. The addition of TCS to the biosolids resulted in an approximate total concentration of 15 mg kg^{-1} . The TCS-spiked biosolid was later added to pots with 500 g the soil at rate of 30, 60, 90, or 200 Mg ha^{-1} . Soils and biosolids were mixed until homogeneous before the soil was seeded.

2.4 Determination of the total TCS concentration in the soils and biosolid

The samples of soils, biosolids, soil-biosolids, and soils with biosolids spiked with TCS, were spiked with a surrogate standard $^{13}\text{C}_{12}$ -TCS (200 ng g^{-1}) and extracted three times with ethyl acetate ($3 \times 4 \text{ mL}$) in a sonication bath (15 min each step). The samples were then centrifuged for 15 min at 2500 rpm (Ying and Kookana 2007). The extracts were concentrated to dryness under a gentle nitrogen stream and then dissolved in 5 mL of methanol. To each extract sample, 5 mL of Milli-Q water were added.

2.5 Extract purification

Purification was carried out with Oasis HLB solid-phase extraction cartridges.

The cartridges were successively conditioned with 3 mL of each of the following solvents: methanol, acetone, dichloromethane (DCM), and hexane (Shaogang and Chris 2007). The samples were loaded into the cartridges and washed three times with 2 mL hexane and twice with 3 mL Milli-Q water. The compounds were eluted three times in 3 mL 50:50 (v/v) methanol/acetone mixture. The eluate was evaporated with nitrogen and reconstituted in 1 mL ethyl acetate for GC-MS analysis.

2.6 Determination of the bioavailable triclosan fraction

Plastic pots (12.5 cm diameter and 11 cm high) were used for the plant assays. The pots were fitted at the bottom with plastic grids to support quartz which was added to prevent sample

loss. The pots were filled with the different soils and soil-biosolid samples with biosolids added to soils at 0, 30, 60, 90, and 200 Mg ha^{-1} . Three replicates of each treatment were included.

Rates of biosolids addition were made based on Chilean regulations that allow biosolid application of 30 and 90 Mg ha^{-1} in agricultural soils and in degraded soils, respectively. The 200 Mg ha^{-1} rate was used for to represent repeated biosolid applications.

Pots containing the equivalent to 500 g dry weight of soil were irrigated to field capacity and allowed to stand for 15 days before sowing with wheat. Ten grams of wheat seeds were planted in each pot. After germination period (about 1 week), the automatic greenhouse lighting was set to produce a 14/10 h (day/night) cycle with a temperature of $25 \pm 5 \text{ }^\circ\text{C}$.

The moisture content was controlled with daily watering with distilled water at 60–70 % of the soil field capacity. After the growth period (30 days), the wheat plants, were removed from the each pot and washed with distilled water. The roots and shoots of each pot were separated and oven-dried at $30 \text{ }^\circ\text{C}$.

Plant samples (root and shoot) were ground and homogenized. For the extraction, 0.5 g root or shoot was weighed and placed in a conical glass tube. The TCS was then extracted, purified, and analyzed as previously described using $^{13}\text{C}_{12}$ -TCS. Concentration of TCS in the roots that were measured during the study, include both the TCS taken up into roots and the TCS sorbed to the outer surface of the roots.

2.7 Estimating the bioavailable TCS fraction

The bioavailable fraction of TCS was estimated through an extraction with HPCD by weighing 1 g of each soil (the three soils with varying biosolid rates and the control soils), transferring the weighed sample to a 15-mL tube and adding 10 mL of 50 mM HPCD. The mixture was stirred for 1 h and then centrifuged for 30 min at 2500 rpm. The supernatant was filtered through a fiberglass filter pad ($1 \text{ }\mu\text{m}$ pore size). Then, 10 mL of methanol was added to the solution in addition to the $^{13}\text{C}_{12}$ -TCS standard surrogate. The methanol/HPCD mixture was sonicated for 1 h and then left to stand for 24 h to ensure total compound release from the HPCD. The HPCD mixture was extracted three times with 10 mL of a 50:50 (v/v) dichloromethane/ethyl acetate mixture. The combined extract was evaporated to dryness in a nitrogen stream and re-dissolved into ethyl acetate (1 mL). The extraction process was modified from Wong and Bidleman (2010).

2.8 GC-MS

Both the unlabeled TCS and the stable-label $^{13}\text{C}_{12}$ -TCS were derivatized with $20 \text{ }\mu\text{L}$ MTBSTFA at $80 \text{ }^\circ\text{C}$ for 45 min. The derivatized extracts were injected ($2 \text{ }\mu\text{L}$) into a gas

chromatograph (Thermo Fisher, Focus model) with a mass spectrometry detector, an ISQ, and a SSL injector. The ionization mode was electron impact (EI) and used 99.999 % pure helium as a carrier gas at a flow rate of 1 mL min⁻¹. A Restek RTX-5MS capillary column 30 m long, 0.32 mm ID, film thickness 0.25 µm df and a maximum temperature of 350 °C were used. The initial oven temperature was 100 °C, which was maintained for 1 min then heated to 300 °C at a rate of increase 10 °C/min. The chromatographic run required a total of 21 min with a solvent delay of 7 min, a transfer line at 250 °C, an ionization source at 200 °C, a carrier gas flow rate of 1 mL/min, and an injector temperature of 240 °C in the splitless mode. The ions used to quantify and confirm the TCS were m/z 345 and 347. The ions used to quantify and confirm the ¹³C₁₂-TCS were m/z 357 and 359. The same extraction methodology, purification, and GC-MS analysis were used to determine TCS concentration in soil, plants (shoot and root), and biosolids.

The accuracy of the method was calculated by the recovery of the standard surrogate, which was always similar to found by Ying and Kookana (2007) for biosolids, (73–74 %).

2.9 Statistical analysis

Linear correlation tests were applied to assess the relationship between the plant TCS concentration and the estimated bioavailable TCS fraction obtained from cyclodextrin extraction. All statistical tests were carried out using Statgraphic 5.0 software. The level of significance for all comparisons was 95 % ($P < 0.05$). ANOVA was used to evaluate the differences between treatments.

3 Results and discussion

3.1 Physical and chemical sample characterization

Table 1 presents the properties of the soil and biosolid samples. The Cuesta Vieja soil was more acidic than the other

soils, while the Taqueral soil was the most basic with the lowest organic carbon (OC) content. The Polpaico soil had the highest values of cation exchange capacity (CEC) and electrical conductivity (EC). In respect of texture, Cuesta Vieja and Taqueral soils are classified as sandy loam and Polpaico as sandy clay loam; Polpaico showed the highest values of clay content. As expected, the biosolids contained more organic carbon and had a higher CEC. The uptake and distribution of organic contaminants into plants depends on the physical and chemical properties of the contaminants, plant factors, and on the soil characteristics (Bedoux et al. 2012). Triclosan concentration in biosolids was 5.45 ± 0.13 mg kg⁻¹, but it is not detected in any of the soils before the addition of biosolids.

3.2 TCS in wheat plants

Figure 1 depicts concentration of TCS in tissue of plant grown in the soils treated with different rates of biosolids, unspiked, and with TCS-spiked. In general, plant concentrations of TCS were higher in the roots than in the shoots. In addition, the TCS concentration in plant tissue varied as a function of soil type. The relative uptake for TCS into plant tissue was as follows: Cuesta Vieja > Polpaico > Taqueral. In the Cuesta Vieja and Polpaico soils, the TCS content in the roots increased as the rate of biosolids addition increased. However, this increase peaked when at the 90 Mg ha⁻¹ biosolid amendment rate. The TCS concentration in wheat roots grown in the 200 Mg ha⁻¹ biosolid treatment decreased in comparison to the 90 Mg ha⁻¹. This decrease may be related to the higher organic matter in this treatment, which could retain TCS into organic matter, thus decreasing its availability. Pannu et al. (2012) suggested that high biosolid application rates increase the OC content of soil, reducing TCS bioavailability. In contrast to root samples, the concentration of TCS in plant shoots was similar across all treatments except for the samples treated with 200 Mg ha⁻¹ biosolids in which a slight increase in TCS concentration was observed. Researchers have found that root concentration of TCS was generally higher than concentration

Table 1 Some physical and chemical properties of the soils and biosolids

	Cuesta Vieja	Polpaico	Taqueral	Biosolid
pH	6.1	7.2	8.2	6.8
OrganicC (%)	1.9	1.8	1.4	27.8
CEC (cmol · kg ⁻¹)	18.6	39.2	22.3	71.6
EC (µScm ⁻¹)	291	346	59	–
Sand (%)	70	55	76	–
Clay (%)	10	27	6	–
Silt (%)	20	17	20	–
Texture TCS (mg kg ⁻¹)	Sandy loam	Sandy clay loam	Sandy loam	–
	nd	nd	nd	5.45

nd non-detected

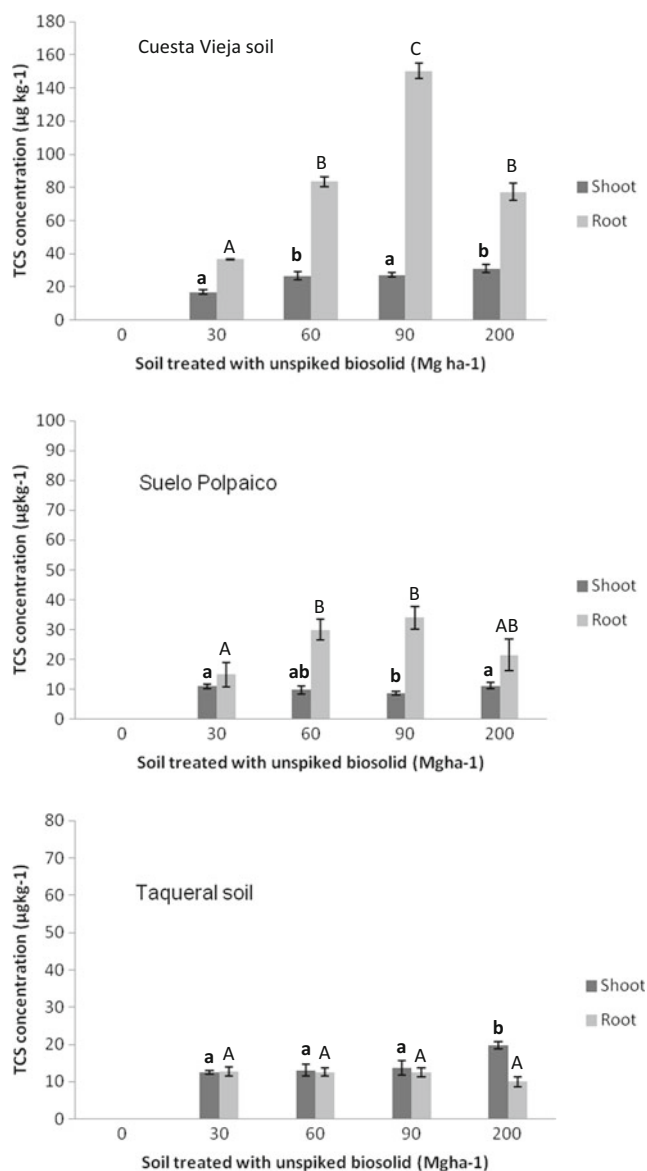


Fig. 1 TCS concentration in the shoots and roots of wheat plants grown in the three soils treated with different rates of unspiked biosolid. Values with the *same letter* are not significantly different at $P \geq 0.05$ (Fisher's least significant difference (LSD) test) within roots (*capital letter*) or shoot (*lowercase*) and biosolid rates, respectively. Means \pm standard deviation ($n=3$) are shown

in stem and leaves (Ried et al. 2000b; Karnjanapiboonwong et al. 2011; Aryal and Reinhold 2011).

Unlike the plants grown in other soils, the TCS concentration in roots and shoots of plants grown in Taqueral soil were similar with respect to TCS accumulation, with greater concentrations observed when the soil was treated with the highest biosolid rate. The pH of the Taqueral was 8.2 and pKa of TCS is 7.9. In this condition, a significant portion of the TCS was deprotonated, facilitating a flow from the roots to the other plant parts. Organic compounds that are more water-soluble are more readily translocated into the plant parts (Stevens et al. 2009), which could account for the low TCS

concentration in the root of the plants grown in the Taqueral soil with respect to the other soils. In general, molecular dissociation leads to reduced bioaccumulation by roots (Wu et al. 2010; Simonich and Hites 1995; Trapp 2000). The plants grown in the Cuesta Vieja soil had higher TCS concentrations in roots than any of the other soils. This may be related to the more acidic pH (6.1) of this soil. The pKa of TCS is 7.9 meaning that it is neutral at a pH of 6.1, which favors its transport to the roots. In addition, the roots have a high potential for accumulating lipophilic contaminants (Aryal and Reinhold 2011).

When the soils were treated with the spiked biosolids, the TCS concentration in the roots was found to increase with increasing biosolid application rate (Fig. 2), a result that differs from the findings in soils treated with the unspiked biosolids in which the maximum TCS accumulation was observed in the roots of plants grown in soils treated with 90 Mg ha⁻¹ biosolids. The difference in the TCS concentration in the roots of the wheat plants grown in soils treated with the unspiked biosolids and those with the spiked biosolids can be explained by the higher TCS concentration in the spiked biosolids. The difference in behavior between the biosolid-TCS and the TCS added as a spike may be related to different bonding strength of TCS and biosolid matrix. The TCS may have formed stronger bonds with the biosolid organic matter resulting in lower mobility. Due to shorter incubation time, the spiked TCS may have been less strongly bond to the organic matter in the biosolids. A similar effect was observed by Wu et al. (2010).

3.3 Bioconcentration factor

The bioconcentration factor (BCF) describes the contaminant transport from the soil to the plant and is calculated based on the equation that relates the concentration of the contaminant found in the plant tissue to that of the contaminant found in the soil (Karnjanapiboonwong et al. 2011):

$$\text{BCF} = \frac{\text{Concentration in plant tissue (mg kg}^{-1}\text{)}}{\text{Concentration in soil (mg kg}^{-1}\text{)}}$$

Figure 4 shows that the TCS bioconcentration factor decreased with increasing biosolid rates. The greatest bioconcentration factor in the roots and shoots was obtained when the soils were treated with 30 Mg ha⁻¹ biosolids. This was observed for both the unspiked and spiked biosolids. The TCS bioconcentration factor was lower in the soils treated with the spiked biosolids, in comparison with the unspiked biosolids. This could indicate, that plants have a limited TCS uptake capacity, either could be due to a defense mechanism that prevents translocation of the contaminant to the other plant parts, or due to degradation of TCS within the plant

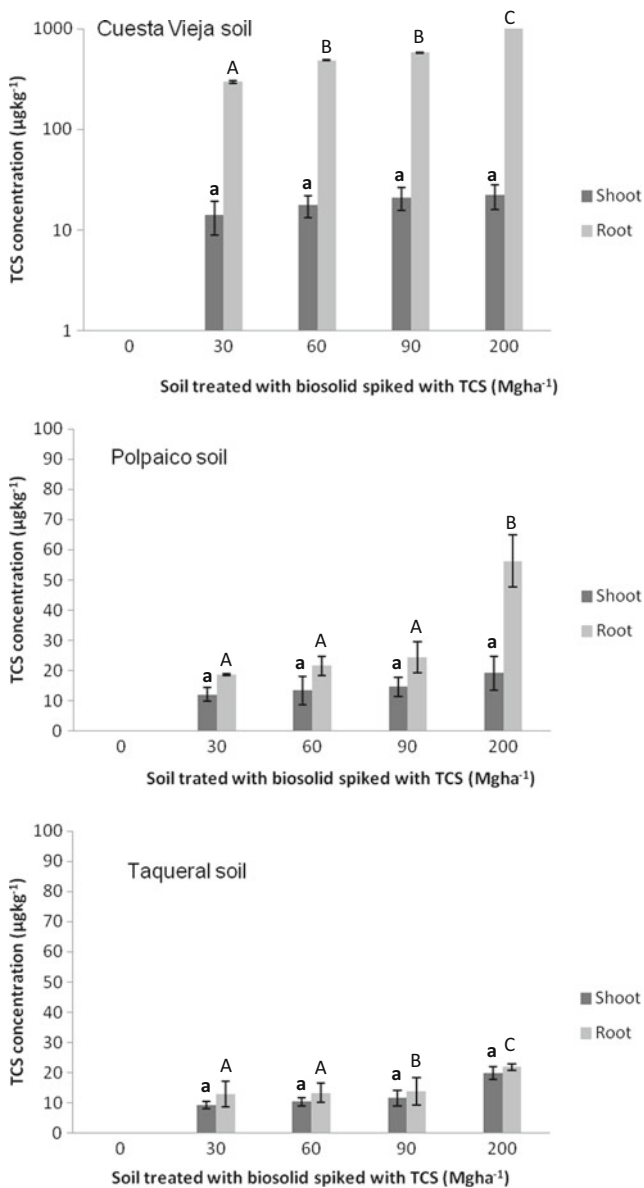


Fig. 2 Concentration of TCS in the shoots and roots of plants grown in the three soils treated with different rates of TCS-spiked biosolids. Values with the same letter are not significantly different at $P \geq 0.05$ (Fisher's least significant difference (LSD) test) within roots (*capital letter*) or shoot (*lowercase*) and biosolid rates, respectively. Means \pm standard deviation ($n = 3$) are shown

via some biological mechanism (Coleman et al. 1997). Plants endogenously methylate xenobiotics, thus, they would likely convert TCS to methyl-triclosan (Stevens et al. 2009). The pathways of chemical uptake by plants depend on the chemical properties, environmental conditions, and plant species characteristics (Pannu et al. 2012).

In general, the low bioconcentration factor for TCS observed could be attributed to the TCS retention in the soil-biosolid system, which would lower the phytoavailability of the compound. This hypothesis is supported by the high octanol-water ($\log K_{OW} = 4.8$) and

organic carbon-water ($\log K_{OC} = 3.8-4.0$) partition coefficients of TCS, which indicate that TCS would bind preferentially to the hydrophobic organic matter in the biosolids. This suggests that higher rates of biosolids addition would increase the binding of TCS by increasing the organic matter concentration of the soil/biosolid matrix. Therefore, as the rate of biosolids added to soil increases, the TCS bioavailability decreases due to the addition of organic matter capable of retaining TCS. Lipophilic compounds with $\log Kow > 4$ generally exhibit limited transport across the endodermis membrane from the soil solution (Pannu et al. 2012; Wu et al. 2013). Log Kow of TCS indicates that triclosan has a higher tendency to sorb to the soil resulting in less of the chemical available for plant uptake (Karnjanapiboonwong et al. 2011).

Triclosan degradation is another important factor to consider. TCS may be biotransformed to methyl-triclosan (MTCS). Degradation compounds are more persistent and lipophilic than the parent compound (Jachero et al. 2013; Aranami and Readman 2007). However, the present study did not assess these TCS degradation products.

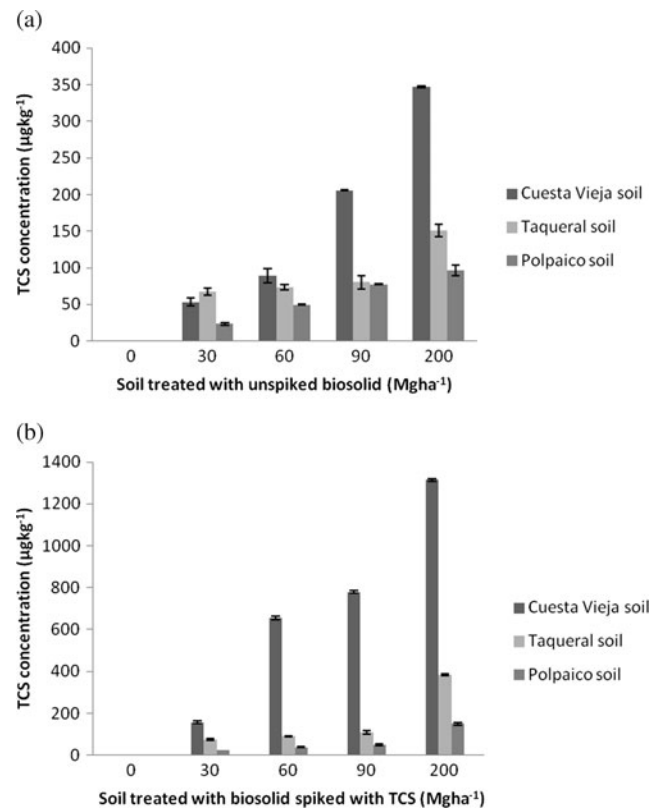


Fig. 3 Concentration of TCS in the soils treated with unspiked and spiked biosolids, as determined via HPCD extraction. Values are shown as means \pm standard deviation ($n = 3$). **a** Soil-biosolid mixtures and **b** soil-spiked biosolid mixtures

3.4 Estimation of the bioavailable TCS fraction

The HPCD-extractable TCS concentration, increased as biosolid application rates increased across all soil types. The Cuesta Vieja soil exhibited a greater TCS concentration than the other soils. As expected, the soils treated with the TCS-spiked biosolid rates had higher TCS concentrations (Fig. 3). The HPCD extraction indicated TCS availability than was observed in the plants. However, the relative trends across the different soils and rates of biosolids and TCS addition were similar between the HPCD extraction and plant. These results suggest that there is a potential value for the HPCD extract as a surrogate for predicting bioavailability of TCS in soil system.

3.5 Bioconcentration factor

The TCS bioconcentration factor was calculated using the bioavailable fraction as defined predicted by the HPCD extraction. Figure 4 provides this factor for the three soils treated with different rates of unspiked and spiked biosolids. The TCS bioconcentration in the three soils decreased as amendment addition rates increased. The bioconcentration factor was

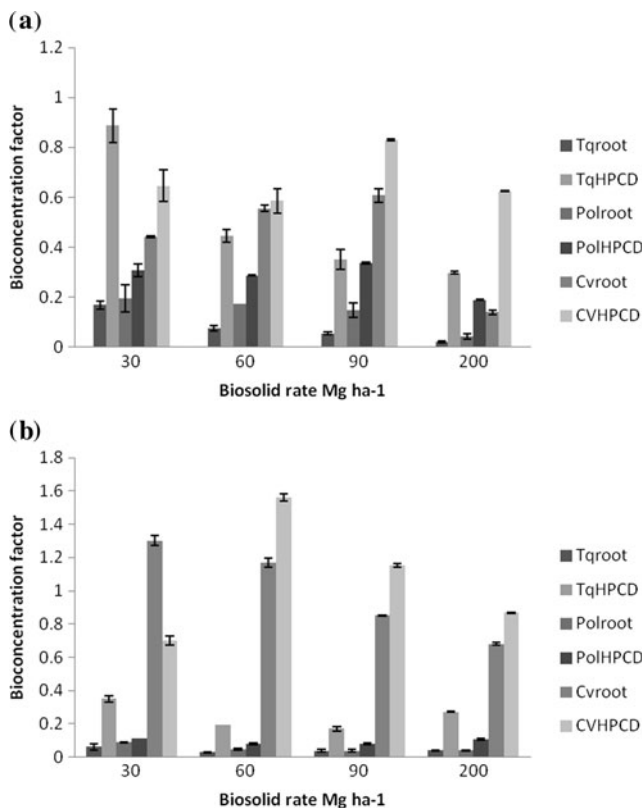


Fig. 4 TCS bioconcentration factor in plant roots and TCS bioconcentration factor as determined by HPCD extraction with respect to the biosolid rates in the different soils. **a** Soil-biosolid mixture and **b** soil-spiked biosolid mixture. Data are shown as means \pm standard deviation ($n=3$)

lower in the three soils when the biosolid rate was 200 Mg ha⁻¹ than at the minimum biosolid rate (30 Mg ha⁻¹). In addition, the TCS concentration increased markedly at higher biosolid rates, even though the bioavailability decreased.

Figure 5 provides the results obtained when the TCS bioconcentration factors calculated from the HPCD extraction were correlated with the TCS bioconcentration factors from the wheat plant roots. Correlation coefficients of 0.98 and 0.99 were obtained in the soils treated with unspiked and TCS-spiked biosolids respectively, indicating a strong relationship between the variables. The R-squared (R^2) statistic indicated that the model as fitted explained 96.97 and 99.84 % of the variability in root. The P value was below 0.05 at the 95 % confidence level. The values found indicated that the model was able to simulate the bioavailability of TCS.

The overestimation of results with HPCD extraction, indicate that the method needs further study to find a better fit, for example, with respect to study the best HPCD concentration and extraction time.

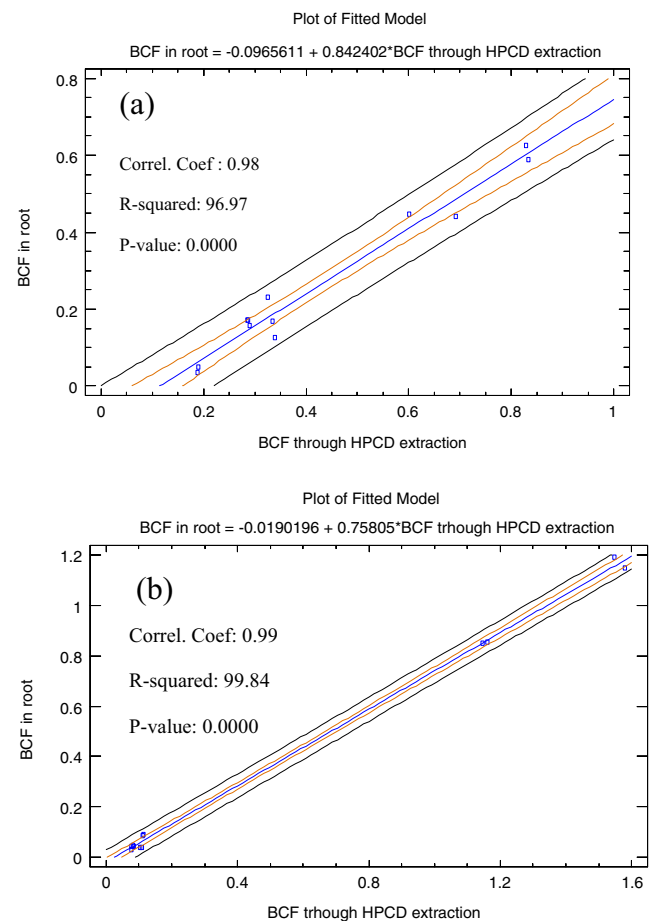


Fig. 5 Comparison between the TCS bioconcentration factor in wheat plants (roots) and the TCS bioconcentration factor obtained via HPCD extraction with unspiked (**a**) and spiked biosolids (**b**)

4 Conclusions

In general, plant concentrations of TCS were higher in the roots than in the shoots. Soils treated with the unspiked biosolid exhibited maximum TCS concentrations in their roots when treated with a biosolid rate of 90 Mg ha⁻¹. On the other hand, when was used spiked biosolids, a progressive TCS buildup was observed in the roots up to the highest biosolid rate.

The TCS was successfully extracted from the soil-biosolid system using HPCD. Unlike the results obtained with the plants, the soils treated with both the unspiked and spiked biosolids exhibited the same tendencies in TCS concentration, with a greater concentration observed in soils treated with 200 Mg ha⁻¹ biosolids. The same was observed in the bioconcentration factor in plant. The correlation coefficients for the TCS bioconcentration factors obtained from the HPCD extraction with those obtained from the wheat roots were 0.98 and 0.99, respectively, for the unspiked, and spiked biosolids. Both samples yielded *P* values below 0.05, demonstrating the validity of the HPCD extraction method as a surrogate for in vivo plant studies. This study assessed a chemical extraction, for determining the bioavailable portion of total soil TCS. The determination of the bioavailable fraction of triclosan in soils treated with biosolids using a predictive method and wheat plant bioassays is acceptable, however, the overestimation of the values obtained with HPCD extraction, indicate that the method needs further study, to find a best fit, for example, study the best HPCD concentration, and extraction time.

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