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Decreases in the bioconcentration of triclosan in wheat plants according to increasing amounts of biosolids added to soil

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ABSTRACT

The uptake of triclosan (TCS) and its metabolite methyl-triclosan (MTCS) by plants is poorly discussed in the current literature. The aim of this research was to analyze the extent of absorption of these compounds by the tissues of wheat plants by quantifying their bioconcentration factors (BCFs). Plants were grown for 30 days under controlled greenhouse conditions in two types of Chilean soil (Taqueral, TQ and Cuesta Vieja, CV) obtained from the metropolitan region of Santiago, Chile, that were amended with different amounts of biosolids containing indigenous and spiked TCS (10 mg kg^{-1}). Once the plants were harvested, both the root and the aerial parts of the plants were treated separately in each biomass sample. From the results, the extent of absorption of the compounds by the root was determined by measuring the BCF; the determined BCFs for the lowest and highest biosolid doses were 0.64 and 0.34 for TQ soil and 1.06 and 0.30 for CV soil, respectively. These decreases were significant (p *value* < 0.05). In the case of plants grown in soils treated with biosolids spiked with an extra amount of TCS, a higher amount of TCS was available (labile fraction) and higher BCF values were obtained. The contributions of organic matter from biosolid doses and pH of the matrix as well as the additional loads of TCS from the biosolids were evaluated through a multi-factorial design. A mathematical expression was derived using this model, which was applied to predict BCFs using data reported in the literature. Predicted values showed great variability mainly due to variations in the plant species and harvest times, indicating that these factors should also be included for the development of a more complete model.

1. Introduction

Various pharmaceutical and personal care products and their metabolites are continuously discharged into the environment through wastewater. Depending on their molecular structures and physicochemical properties, they can be degraded through the biological processes that occur at treatment plants or can be incorporated into the environment, causing toxic effects on living organisms. Triclosan (TCS) is one of these compounds, which due to its structural similarity to xenoestrogens, is classified as an endocrine disruptor (Cabana et al., 2007; Banihashemi and Droste, 2014; Provencher et al., 2014). Once methylated, TCS is transformed to methyl-triclosan (MTCS), which increases its lipophilicity and tends to bioaccumulate in lipid tissues; it is also less susceptible to photodegradation and is thus more stable in the presence of natural light (Chen et al., 2009).

TCS, which is released by personal care products, is one of the emerging contaminants that is present in higher concentrations in biosolids (Sabourin et al., 2012; Prosser et al., 2014; Sánchez-Brunete et al., 2010; Liu et al., 2009; Jachero et al., 2013). According to its K_{ow} value (4.8), TCS is a hydrophobic compound (Cha and Cupples, 2009), which tends to accumulate in the sediment or particulate matter of aqueous eco-systems (Aryal and Reinhold, 2011; Durán et al., 2012).

Biosolids are employed to amend agricultural soil, which benefits crops due to the contribution of nutrients, improvements in texture and other modifications of the physical properties of soil as well as through the incorporation of organic matter. In soils amended with biosolids, TCS has a half-life of 73–301 days (DT_{50}) and tends to degrade under aerobic conditions (Langdon et al., 2011). However, biosolids are also a source of trace amounts of organic and inorganic pollutants, which are discharged through domestic or industrial emissions; most of these pollutants are potentially toxic and generate problems linked to environmental pollution (Mikes et al., 2009) because the compounds present in the sludge can be mobilized to contaminate groundwater or be captured by living organisms. Consequently, this uptake affects the bioavailability of contaminants (Lavado et al., 2005). TCS accumulation in organisms has been reported in the literature including accumulation in plants (Zarate et al., 2012; Stevens et al., 2009; Macherius et al., 2012), humans (Allmyr et al., 2006) and animals (Coogan et al., 2007). In all these cases, the negative effects of TCS and its bioconcentration in each of the organisms were mentioned.

The plant uptake of organic compounds from the soil depends on their hydrophobicity, water solubility, vapor pressure, partition coefficient K_{ow}, concentration, persistence in the environment, environmental

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conditions and biological factors related to the ability of organisms to metabolize the organic compounds (Meylan et al., 1999; Staci et al., 1995). The presence of such compounds in some biological receptors tends to cause toxicity in the organism. This effect is generally related to the freely dissolved fraction (labile fraction) of the contaminant, which is available to be absorbed by biota and can be evaluated through measurements of bioconcentration factors (BCFs), (Wu et al., 2013; Wen et al., 2012; Mackay, 1982). BCFs are indicators that are widely used in risk analysis, toxicology studies and in protocols that test for chemicals that present potential hazards to the environment (Veith et al., 1979). Taking into account the fact that bioconcentration depends on the hydrophobicity of compounds (Wu et al., 2010), K_{ow} has been used as a fundamental parameter to estimate the bioconcentration of organic compounds that are introduced from the soil into root tissues (Paterson et al., 1994). Moreover, bioconcentration is pH-dependent and is affected by the pK_a of compounds (Wu et al., 2010). While most methods estimate BCFs based on physicochemical properties of the compounds, few are based on the studies of BCFs that consider matrix features. Thus, the aim of this study was to develop a model using a multilevel experimental design taking into account the influence of factors such as soil pH, dose of biosolid, and the presence of the analytes (TCS and MTCS); (either in indigenous form or added to the matrix). BCFs were evaluated for two emerging organic pollutants that were present in the biosolid samples, such as TCS and its metabolite MTCS. These results were compared with the uptake of the compounds by wheat plants that were evaluated through bioassays in soil with different doses of biosolids and in consideration of the two following scenarios: biosolids containing indigenous triclosan and biosolids spiked with an additional load of TCS.

2. Materials and methods

2.1. Reagents

Nano-pure water from a Barnstead water system (Dubuque, IA, USA) was used throughout this study. The TCS and MTCS (both 99.5% purity) analytes were purchased from Dr. Ehrenstorfer (Augsburg, Germany). A standard stock solution of the analytes was prepared in methanol (GC–MS/pesticides grade analysis; Fisher Scientific, Fair Lawn, NJ, USA). Irgasan® (containing a ≥97.0% TCS), purchased from Sigma–Aldrich (Milwaukee, WI, USA), was used to enrich the biosolid. Hexachlorobenzene (HCB, 99.5% purity), used as an internal standard, was purchased from Dr. Ehrenstorfer. $^{13}C_{12}$ Labeled triclosan, purchased from Wellington Laboratories (Ontario, Canada), was used as a surrogate standard. Nitrogen 5.0 and helium 5.0 were purchased from Linde (Santiago, Chile) and were used to evaporate the final extract and as the chromatographic carrier gas, respectively. Ethyl acetate, acetone, acetonitrile (HPLC-grade, 99.8% purity) and sodium chloride (99.5% purity) were purchased from Merck (Darmstadt, Germany). *N*-Methyl-*N*-(tert-butyldimethylsilyl) trifluoroacetamide (MTBSTFA) was purchased from Sigma–Aldrich (Milwaukee, WI, USA) and was used as a derivatizing agent.

2.2. Soil and biosolid characterization

Two soil samples were obtained from the metropolitan region in Chile: Taqueral (TQ) and Cuesta Vieja (CV) located at 6309.5 Km Lat. and 331.4 Km Long. UTM and 6292.9 km from Lat. and Long. 317.9 Km UTM, respectively.

The soil samples were collected at the surface level (0–10 cm). Compound samples from each sample site were air dried, passed through a 2 mm sieve and stored in plastic containers until use. The supernatants of soil-water suspensions with 1:2.5 (w/v) ratios were used to determine the pH of each sample. The organic carbon (OC) content of the soil and biosolid samples were determined with wet oxidation/digestion methods using dichromate, combined with a colorimetric method for the quantification of chromic oxide. Cation exchange capacities (CECs) were determined using a sodium acetate procedure at pH 7, and the sample textures were determined using the Boyoucus method (Zagal and Sadzawka, 2007).

The soil samples and biosolid properties were determined prior to the development of the bioassays; Table 1 shows the chemical features that were determined. The particle sizes showed that both soils were equivalent and had the same sandy-loam texture. The pH values of both soils were different; CV was slightly acidic and TQ was slightly alkaline. The soil samples showed differences in their organic matter content, with TQ having the highest content.

The biosolids resulting from anaerobic digestion were collected from a wastewater treatment plant in the Santiago Metropolitan Region. The biosolids were air-dried and passed through a 2-mm sieve. The biosolids were then mixed thoroughly into the soil samples at rates equivalent to 0, 30, 60, 90 and 200 Mg ha^{-1} and were incubated at 25 °C for 15 days under field capacity moisture conditions.

The biosolids were spiked with additional amounts of TCS dissolved in acetone (10 mg L^{-1}) using the following procedure: 500 g of the biosolid sample was placed in a separate 500 mL round flask, and an additional amount of TCS (10 mg kg^{-1}) was added using the commercial formulation, Irgasan®. The sample was evaporated in a rotary evaporator at 200 rpm for 24 h at room temperature in the dark to prevent photo-degradation of the compounds. The biosolids were then transferred to a dish and left to dry in the dark.

2.3. Greenhouse experiment

Wheat plants (*Triticum aestivum*) were grown in soil samples treated with natural biosolids and biosolids spiked with a commercial product-based TCS (Irgasan). Plastic pots were used for the plant assays. The pots were filled with the different soil samples and soil-biosolid samples with biosolids added to soils at 0, 30, 60, 90 and 200 Mg ha^{-1}. This study was performed in triplicate.

Each pot containing the equivalent of 500 g soil (dry weight) were irrigated to field capacity and allowed to stand for 15 days before sowing with wheat. A 10 g sample of wheat seed was planted in each pot. After the germination period (about one week), the automatic greenhouse lighting was set to produce a 14/10 h (day/night) cycle with a temperature of 25 ± 5 °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 35 °C for 30 days.

2.4. Determination of TCS and MTCS in plant tissues

The roots and aerial parts of the plants were analyzed separately. Prior to analysis, each sample was weighed on a dry basis and the analytes were extracted using the matrix solid phase dispersion (MSPD) technique (Sánchez-Brunete et al., 2010). A 100 μL aliquot of a 2000 μg L^{-1} solution of $^{13}C_{12}$ TCS was added to each sample prior to the extraction as a method to control the quality of the recovery values.

The acetonitrile extract containing the concentrated analyte was then evaporated to dryness under a stream of N_2, and the residue was

Table 1
Characterization parameters of soil and biosoil samples.

Parameters	Taqueral	Cuesta Vieja	Biosolid
pH	8.2	6.5	6.9
OM (%)	5.9	3.3	–
Sand (%)	76	71	
Clay (%)	6	9	
Silt (%)	18	20	
OC (%)	3.4	1.9	28.1
Apparent density (g/cm^3)	1.25	1.12	
CEC (cmolkg^{-1})	22.3	18.5	71.6

re-dissolved in 1 mL of ethyl acetate. MTBSTFA (50 μL) was added to this extract (500 μL) and derivatization was performed for 45 min at 80 °C. Before injection, 10 μL of a 5 mg L^{-1} HCB solution was added as an internal standard and the analytes were characterized by GC–MS.

2.5. Instruments and software

A Thermo Scientific Focus (Milan, Italy) gas chromatograph coupled to a mass-selective Thermo Fisher Scientific model ISQ (Austin, TX, USA) instrument was used to make final determinations. A Restek RTX-5MS (Bellefonte, PA, USA) (30 m × 0.25 mm id; 0.25 μm film thickness) coated with 5% phenyl – 95% methylpolysiloxane was used as the fused silica capillary column. Two microliters of the sample extract was injected into the GC, which was operated in splitless mode. The injector temperature was 250 °C. The initial column temperature was 100 °C (1 min) and was increased to 300 °C at a rate of 10 °C min^{-1}. Helium was used as a carrier gas at a constant flow of 1.0 mL min^{-1}. The solvent delay was 7 min. A dwell time of 0.1 s was employed for each *m/z*. The MS transfer line was maintained at 250 °C and quantifications were based on calibrations with the standard analyte using mass spectrometric parameters in selective ion monitoring (SIM) mode.

2.6. Modeling and data analyses

The main factors (biosolid doses, soil pH and the additional load of TCS) were evaluated with a multilevel factorial design ($2^2 \times 4^1$) using Statgrafics XV.I Centurion software. The design consisted of 16 runs, was executed in a single block, and the log BCF TCS and log BCF MTCS were used as response variables. A one-way analysis of variance (ANOVA) was performed, treating the types of treatments as independent variables. A two-way ANOVA was performed to compare the concentration of TCS in aerial parts and MTCS in roots, considering the system (spiked/non spiked) and dose of bisolid as independent variables. Basic assumptions of normal distribution and homogeneity of variances were tested before comparison. The differences between means were considered significant when the *p values* were <0.05. The results presented are the average of three replicates for each dose of biosolid and soil type. Tukey's tests (with 95% confidence level) were applied to determine differences between treatments.

3. Results and discussion

3.1. Effects of applying biosolids

This biosolid was incorporated into both soils (TQ and CV soils) at different rates: 30, 60, 90 and 200 Mg ha^{-1}. Wheat plants were grown in both soil samples using the following three systems: soil without biosolids or control soil (S), soil amended with biosolids containing only indigenous TCS (S + B), and soil amended with biosolids spiked with an extra amount of TCS (S + B*). The additional load of TCS (10 mg L^{-1}) was chosen based on indigenous TCS concentrations measured in biosolids. The concentrations of indigenous TCS and MTCS in the biosolid were 12.5 mg kg^{-1} and 0.17 mg kg^{-1} respectively. Meanwhile, biosolid doses were selected according to the levels reported by the Chilean legislation, where amendment doses of 30 and 90 Mg ha^{-1} were reported. In this study, an intermediate dose of 60 Mg ha^{-1} was also tested and a higher dose of 200 Mg ha^{-1} was considered to mimic the effect of subsequent amendments.

The effects of utilizing biosolids in plant development have been previously observed in other studies (Cheng et al., 2007; Oleszczuk and Hollert, 2011). The addition of biosolid to soil was shown to reduce the plant biomass, which depends on both the dose of biosolid and the conditions in which the TCS was found in the biosolid (*i.e.*, indigenous or spiked). In the present study, significant differences ($p < 0.05$) were observed in ANOVA between samples with control soil (S) and some treatments in the same soil. The biomass of the plants, upon treatment amended with biosolid, was lower compared with plants grown only in the control soil (S). At the same time, soils amended with the biosolid containing an additional dose of TCS (S + B*) showed lower plant biomass compared with plants grown in soils amended with biosolid containing only indigenous TCS (S + B). Fig. 1 shows that the biomass followed the following trend: (S + B*) < (S + B) < S. Despite the fact that biosolid amendment improves water retention and increases the nutrient content of the soil, biosolid also introduces excessive amounts of heavy metals, organic pollutants and soluble salts, particularly with high soil amendment rates. In addition, this study confirmed that the available fraction (spiked) of a pollutant, TCS in this case, had a more critical effect on the plant biomass grown compared with the indigenous fraction of the same contaminant (Fig. 1).

It is known that the amount of organic matter is related to soil fertility, which leads to greater crop development. Consequently, higher biomass crops in TQ soil would be expected due to its higher content of organic matter (Table 1) compared with plants grown in CV soil. When comparing both soils, only few treatments show significant differences (Fig. 1). However plants from CV control soil denotes a statistically significant difference and exhibit higher biomass development than TQ soil. This effect can be attributed to the higher pH of TQ soil (pH 8.2) than CV soil (pH 6.5); because crops have more difficulty adapting to basic pH. It was previously observed that the pH should be in the range of 5.6–7.5 for the optimum growth of wheat plants (CIREN, 1989).

Plant growth is associated with both the dose of biosolid application and crop resistance to certain levels of salt; this is because biosolid tends to increase the electrical conductivity of soil by reducing the ability of plants to absorb water (Bohn et al., 2001). Moreover, it has been reported in the literature that some emerging organic pollutants inhibit the transport of essential elements, minimizing plant growth (Wu et al., 2012; Carter et al., 2014). In the bioassays carried out in the S + B system, the average growth inhibition relative to the control S was 15% and 32% for doses of 30 and 200 Mg ha^{-1}, respectively. In plants grown in the S + B* system, this inhibition increased to 24% for the lowest dose and 42% for the highest dose (Fig. 2).

3.2. Concentrations of TCS and MTCS in wheat plants

Considering that TCS is initially contained in biosolids, either in indigenous or spiked form, the concentration of this contaminant increases in soil with increasing doses of biosolids. This is reflected in a statistically significant increase of the compound in the roots of plants grown in the TQ soil (Fig. 3A and B). This trend is less evident in the CV soil where significant differences only occur between some treatments in both the S + B and S + B* systems (Fig. 3A and B). Notably, the presence of the target compounds was a result of the incorporation of biosolids into the soil samples; these compounds were not detected

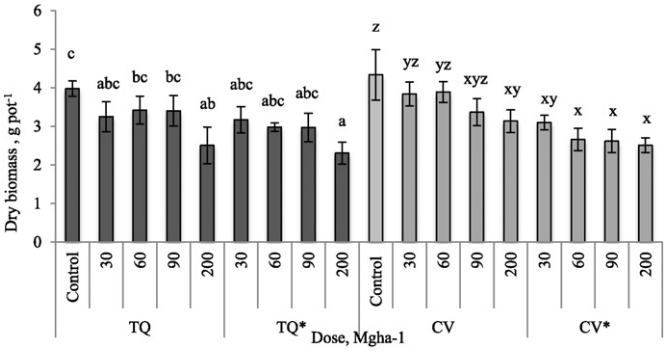

Fig. 1. Effects of increasing the soil amendments of biosolid on plant biomass. Dry weights of plants were determined after the culture period. Different letters indicate significant differences by determined by Tukey's test (*p value* < 0.05). *Refers to conditions that used biosolid samples spiked with an additional load of TCS.

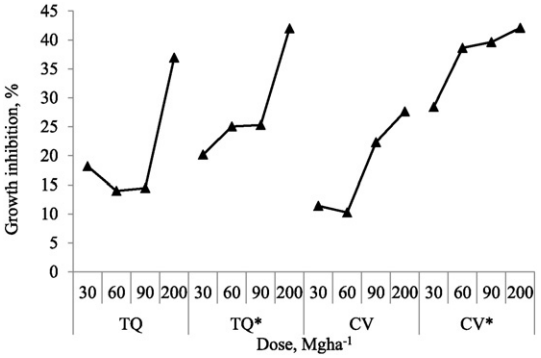

Fig. 2. Percentage of plant growth inhibition for each dose of biosolid relative to the control. *Refers to conditions that used biosolid spiked with an additional load of TCS.

in the original soil samples, and irrigation records with wastewaters were not detected.

For MTCS (Fig. 3C and D), the application of biosolids did not generate a predictable pattern and statistical analyses reported no significant differences regarding biosolid doses in S + B* (Fig. 3D). This may be because MTCS concentrations are nearly 100 times less than TCS in biosolids and comparing the contents of MTCS between the S + B (Fig. 3C) and S + B* systems (Fig. 3D), in this latter system, higher levels of MTCS are observed because part of the TCS is metabolized in the plant. As can be observed in Fig. 3C and 3D a higher concentration of TCS was found in plant root grown in the S + B* than in S + B system due to the additional content of TCS. Under this condition, the spiked compound has less time to interact or bind soil particles and is consequently more available to be taken up by plant roots. In contrast, when the TCS contained in biosolids is indigenous, the increased residence time of the compound reinforces binding of the analyte-matrix and results in reduced analyte availability (Chung and Alexander, 1998).

Since differences in MTCS concentrations were limited (Fig. 3C and 3D), the concentration of MTCS in roots as dependent variable was compared using a two-ways ANOVA considering two factors: system (spiked/non spiked) and dose of bisolid (details in supplementary information S1). In both soils the factors have a statistically significant effect (p *value* < 0.05) on MTCS concentration. Significant differences in TCS concentrations were observed in the roots of plants grown in both soils, particularly in systems spiked with TCS; these differences can be attributed to differences in soil pH, which affect the extent of ionization of TCS (Trapp, 2000; Briggs, 1981). In CV soil, the TCS fraction existing in the ionic form is approximately 4% compared with 67% in TQ soil (calculated from the Henderson-Hasselbalch equation); thus, the mobility of TCS tends to be much higher in the latter (Fig. 3A) and results in easier incorporation into the soil solution and absorption by the root. In any case, the last step of absorption into the root requires the rapid protonation of the anionic form of TCS in the root-soil solution interface, as anions are not directly absorbed by root plants. This is because cell membranes have negative electrical potentials and consequently repel negatively charged anions (Wu et al., 2013).

In the aerial tissues of the plants (stem and leaves), TCS concentrations were significantly different and exhibited variable behavior depending on the treatments (Fig. 4A and 4B). Since differences were limited between 4A and 4B (details in supplementary information S1), the concentration of TCS in aerial parts was compared using a two-ways ANOVA. In the aerial tissues of the plants (stem and leaves), TCS concentrations showed significantly differences depending on the treatments (Fig. 4A and 4B). MTCS was not detected in the aerial parts of the plants.

The translocation factor of TCS, which accounts for the ratio between the concentrations of the compound in the aerial parts of the plant and in the root (Wu et al., 2010; San Miguel et al., 2013), was found to be lower than one in every biosolid dose. This was because TCS exhibited significantly higher accumulation in the root in the time scale implicit in the experiment. The mobility of TCS toward the aerial parts of the plant occurs passively, depending on the concentration gradient (Macherius et al., 2012).

The absence of MTCS in the aerial parts of the plant would be conditioned by its lipophilicity because this property is involved in the

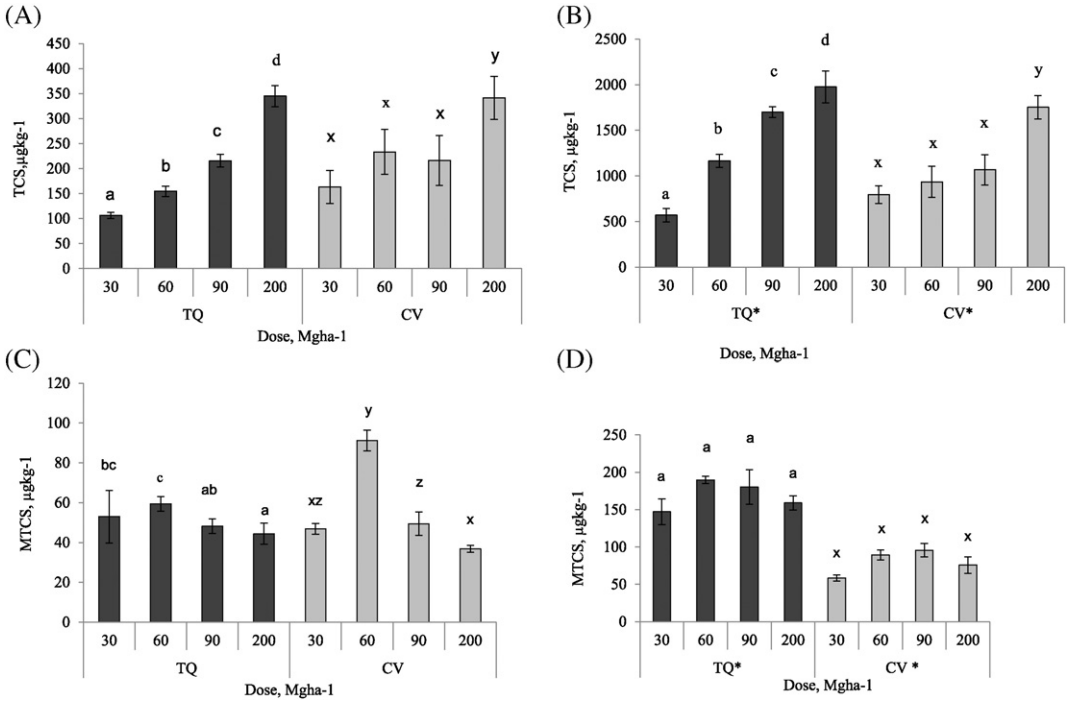

Fig. 3. Concentration of TCS and MTCS in root plants grown in soil amended with biosolid. (A) and (B) correspond to concentrations of TCS in root plants grown in soil amended with biosolid and with spiked biosolid samples, respectively. (C) and (D) correspond to concentrations of MTCS in root plants grown in soil amended with biosolid and with spiked biosolid samples, respectively. Different letters indicate significant differences between treatments that were determined using Tukey's test (p *value* < 0.05).

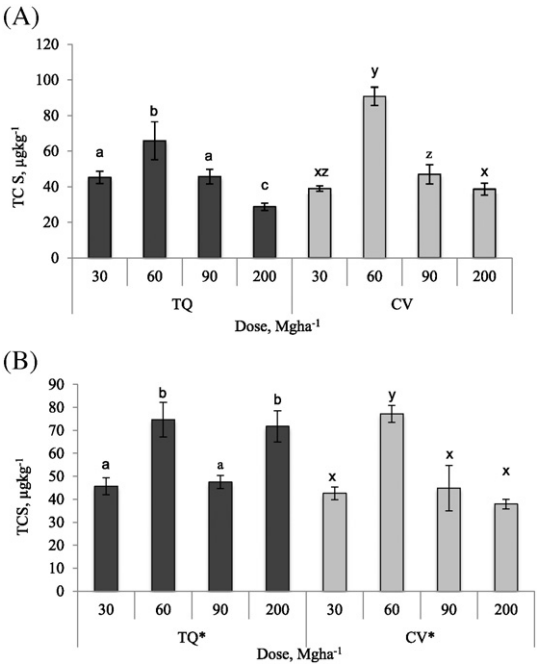

Fig. 4. Concentration of TCS in the aerial parts of the plants grown in (A) soil amended with biosolid and (B) in soil amended with spiked biosolid. Different letters indicate significant differences between treatments that were determined using Tukey's test (p *value* < 0.05).

transport of compounds toward aerial parts of the plant (Briggs et al., 1982). Lipophilic organic compounds have a greater tendency to be partitioned into the lipids of the plant roots compared with hydrophilic compounds (Collins et al., 2006). In this context, organic compounds with log K_{ow} > 4 were expected to have a high degree of retention by the roots and a low capacity of translocation (Carter et al., 2014), as the observed for MTCS.

Metabolization of TCS in plant roots can also reduce translocation, similar to the observations reported by Briggs et al. (1982) for simazine in the roots of plants.

3.3. Bioconcentrations of TCS and MTCS

Taking into account that the texture, apparent density and CEC of the two soils were similar, these parameters were not considered for the evaluation of the BCFs. In contrast, results showed that the dose of biosolids, soil pH and additional concentration (spike) of TCS incorporated in the biosolids influenced the uptake of TCS by the roots. In this context, these three factors were evaluated using a multilevel factorial design. Table 1 shows that the BCF decreased with increasing doses of biosolids. Significantly higher values of BCF were not produced by the absorption of MTCS, but rather obtained by the transformation of TCS absorbed during plant growth (Lyndall et al., 2010). These observations were notable considering that hydrophobic compounds are poorly absorbed by plants (Carter et al., 2014).

Table 2 shows the 16 experiments used in this experimental design and the log BCF values that were obtained using the bioassays for TCS and MTCS. Regression of these values on the matrix of experiments was performed in coded terms. The Pareto charts (Fig. 5) show the standardized effects of the studied factors on BCF.

The dose of biosolids had a significant negative effect (p *value* < 0.05) on the BCF of both analytes, whereas MTCS had higher values (Fig. 5B); these results are consistent with those found in the bioassays in wheat plants refer of the present study. In these assays, bioavailability decreased by sorption of the compounds in the biosolids, which in turn increased with the dose of the compound and the value of K_{ow} (higher for MTCS). Meanwhile, incorporating an additional charge of TCS in the biosolids generated a positive effect on the response of log BCF.

Table 2
Design matrix and responses (log BCF) for each experiment.

Experiment	Dose Mgha^{-1}	pH	Biosol. spike	Log BCF TCS	Log BCF MTCS
1	30	6.5	NS	0.041	1.274
2	60	6.5	NS	−0.155	1.269
3	90	6.5	NS	−0.398	0.832
4	200	6.5	NS	−0.523	0.380
5	30	8.2	NS	−0.222	1.372
6	60	8.2	NS	−0.301	1.127
7	90	8.2	NS	−0.398	0.863
8	200	8.2	NS	−0.523	0.505
9	30	6.5	S	0.398	1.369
10	60	6.5	S	0.146	1.257
11	90	6.5	S	0.041	1.120
12	200	6.5	S	−0.155	0.690
13	30	8.2	S	0.301	1.815
14	60	8.2	S	0.296	1.630
15	90	8.2	S	0.230	1.437
16	200	8.2	S	0.041	1.056

NS (no spike refers to the system S + B).
S (spike refers to the system S + B*).

The pH of the soil samples had no significant effect on the BCF for TCS. However, the interaction of this factor with the dose of biosolids (AB) or spike (BC) positively influenced the BCF. This implies that an increase in the BCF of TCS was produced with increases in both pH and biosolid doses. It can be observed that the effect of BC interactions over the BCF was significant for both compounds (Fig. 5A and 5B). The spiked TCS had less time to interact with the organic matter of the matrix compared with indigenous TCS. This made it more susceptible to be absorbed by the biota or metabolized to MTCS in the case of TCS, which is favored for higher pH conditions. Different behaviors of indigenous and spiked TCS have been reported in degradation studies, and it was shown that indigenous TCS degraded at a rate 1.6 times slower than

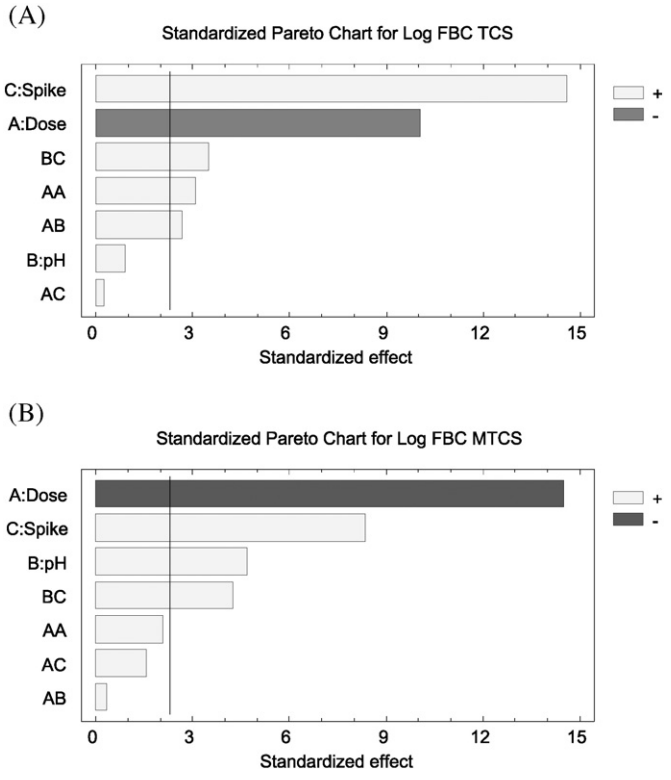

Fig. 5. Pareto chart for the factors evaluated on log BCF (A) TCS and (B) MTCS The negative effects are shown as black color and the positive effects in white color, in this Chart influence variable and interations are ordered according to their relative importance. Vertical line in the chart defines 95% confidence level.

Table 3
BCFs of TCS in roots of different plants reported and predicted by the model (Eq. (1)).

Root	Ref.	Reported		Dose biosolid Mg ha^{-1}	soil pH	Harvested days	Predicted.	
		BCF	BCF[a]				BCF	BCF[a]
Radish	Prosser et al. (2014)	0.5	0.3	12	7.9	34	0.84	2.92
Carrot	Prosser et al. (2014)	7.5	2.4	22	7.9	45	0.74	2.58
Soybean	Prosser et al. (2014)	11.1	7.6	29	7.9	54	0.68	2.37
Festuca	Macherius et al. (2012)	–	0.36	6	5.5	60	–	3.39
Barley	Macherius et al. (2012)	–	1.43	6	5.5	60	–	3.39
Carrot	Macherius et al. (2012)	–	0.18	6	5.5	60	–	3.39
Radish	Pannu et al. (2012)	0.1	0.93	228	6	40	0.3	0.6
Soybean	Wu et al. (2010)	–	2.25	12	5.1	60	–	3.08
Pumpkin	Aryal and Reinhold (2011)	–	972	7.3	7	165	–	3.18
Zucchini	Aryal and Reinhold (2011)	–	1822	7.3	7	165	–	3.18
Switch grass	Aryal and Reinhold (2011)	–	874	7.3	7	165	–	3.18

[a] Refers to the system S + B*.

the spiked compound (Langdon et al., 2013). The mathematical models describing the BCFs for TCS and MTCS as a function of the significant experimental factors are presented in Eqs. (1) and (2), respectively.

$$\log BCF\ TCS = -0.200 - 0.211 * Dose + 0.236 * Spike + 0.121 * Dose^2 + 0.049 * Dose * pH + 0.053 * pH * Spike \quad (1)$$

$$\log BCF\ MTCS = 1.030 - 0.397 * Dose + 0.100 * pH + 0.172 * Spike + 0.086 * pH * Spike \quad (2)$$

The equations for both analytes generated a good fit with the experimental data ($R^2 > 0.9$). Moreover, the consistency of the model was checked using a leave-one-out cross validation set of experiments for the matrix and also resulted in a good correlation ($R^2 > 0.9$) (details in supplementary information S2).

To verify if the plant species significantly affected the bioavailability of TCS, this model was used to estimate the BCF values already reported in the literature in which bioassays were performed under different experimental conditions. The conditions and values for the BCFs of TCS estimated from Eq. (1) are presented in Table 3.

When the model proposed in this study was applied to other reported bioassays involving TCS, different orders of approximation were obtained for the BCFs of TCS (Table 2). Independent of the biological variability inherent to *in vivo* assays, it is clear that the plant species is a fundamental variable that should be considered in the model. In addition to the type of plant, harvest times should also be considered (Pannu et al., 2012; Stevens et al., 2009). Despite this, BCFs close to the reported values for radish, carrot, barley, soybean (Table 3), were found in some cases by applying the present model (Prosser et al., 2014; Wu et al., 2010; Pannu et al., 2012).

Moreover, other models have been reported to predict BCFs of different compounds in vegetation (barley root) taking into account variables other than the ones considered in this study including the following: K_{oc} absorption coefficient, soil organic carbon content (Topp et al., 1986), molecular connectivity index (MCI) (Dowdy and McKone, 1997) and the octanol/water (Travis and Arms, 1988) partition coefficient. These models take into account the basic physical and chemical parameters of compounds and, only in some cases, consider characteristics of the medium in which the vegetation is grown.

4. Conclusions

This study demonstrates that TCS and MTCS accumulate in the tissues of wheat plants grown in soils amended with biosolids containing the target compounds. The abilities of the compounds to translocate to the aerial part of the plants depended on the physical and chemical characteristics of the compounds. Other factors such as the dose of biosolids, soil pH and the additional concentration of TCS added to biosolids were decisive in the bioaccumulation of the compounds and also influenced the growth inhibition of biomass.

It was observed that the bioconcentration of TCS decreased with increasing doses of amendment. At the same time, bioavailability of TCS varied depending on the nature of the analyte present in the biosolid, which was proven to be less bioavailable when it was in an indigenous form. The effects of adding an additional amount of TCS to the biosolid were noted in the quantification of MTCS in the plant at higher concentrations than TCS due to the biodegradation of this compound.

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