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Influence of triclosan and triclocarban antimicrobial agents on the microbial activity in three physicochemically differing soils of south Australia

Abid Ali[1*], Muhammad Arshad[1], Zahir A. Zahir[1] and Amer Jamil[2]
[1]Institute of Soil and Environmental Sciences, University of Agriculture, Faisalabad
[2]Biochemistry Department, University of Agriculture, Faisalabad

Abstract

Antimicrobial agents are being used in numerous consumer and health care products on account of which their annual global consumption has reached in millions of kilograms. They are flushed down the drain and become the part of wastewater and sewage sludge and end up in the ultimate sink of agricultural soils. Once they are in the soil, they may disturb the soil's ecology as a result of which microbial activity useful for soil fertility and biodegradation of xenobiotics may severely be impacted. The present study was designed to assess the influence of two antimicrobial agents triclosan (TCS) and triclocarban (TCC), commonly used in consumer and health care products, on the microbial activity in the three agricultural soils from South Australia having different characteristics. The study was laid out following the two factors factorial design by applying ^{14}C-glucose at 5 µg g^{-1} with either TCS at 0, 30, 90 and 270 µg g^{-1} or TCC at 0, 50, 150 and 450 µg g^{-1} in three agricultural soils, Freeling (Typic Rhodoxeralf–sodic), Booleroo (Typic Rhodoxeralf) and Avon (Calcixerralic Xerochrepts). The $^{14}CO_2$, which was released as a result of microbial respiration, was trapped in 3 mL 1M NaOH and was quantified on Wallac WinSpectral α/β 1414 Liquid Scintillation Counter. The results revealed a significant difference in amounts of ^{14}C-glucose mineralized in the three soils. A significant concentration dependant suppressive effect of TCS on the biomineralization of ^{14}C-glucose appeared in all the tested soils as opposed to TCC where no such concentration dependent effect could be recorded. The reduction in ^{14}C-glucose biomineralization in the Freeling, Booleroo and Avon soils was recorded up to 53.6, 38.5 and 37.4 % by TCS at 270 µg g^{-1} and 13.0, 5.8 and 1.6 % by TCC at 450 µg g^{-1} respectively. However, a significant negative correlation of CEC and pH was recorded with TCS and TCC effects. These results may imply that presence of such antimicrobial agents in the soil environment may also be considered while designing the bioremediation strategy for any xenobiotic pollutants.

Keywords: Biodegradation, xenobiotics, antimicrobial agents, biomineralization, bioremediation

Introduction

Many beneficial processes such as transformation of inorganic molecules, symbiotic associations with plants, organic matter decomposition, soil formation and aggregation, prevention of plant diseases and degradation of toxic xenobiotics owe to microbial activity in soil ecosystem (University of Minnesota, 2000; Vidyalakshmi *et al.*, 2009; Stout, 2010). Glucose can be used as a model substrate for assessing the microbial activity in a soil (Sheehan, 1997) because glucose is a highly water-soluble, easily degradable and readily bioavailable compound characterized with least adsorption in the soil (Johns and Edwards, 1998; Nguyen and Guckert, 2001). Moreover, glucose biodegradation owes to the enzyme glucose oxidase which is ubiquitous among soil bacteria (Paul, 2007).

Since microbial activity is a physiological state where cellular functions allow the organism to grow and reproduce, therefore, it may considerably be influenced by antimicrobial agents who have the intrinsic capacity to inhibit the growth of microorganisms. Besides antimicrobial agents, microbial activity may markedly be impacted by soil characteristics which primarily determine the soil environment. Triclosan (TCS) and triclocarban (TCC) are two potent antimicrobial agents which have broadspectrum activity against a wide range of bacteria and fungi. They are extensively being used in many contemporary consumer and professional health care products (Jones *et al.*, 2000). Most of the products containing TCS and TCC are mainly used in the kitchens and washrooms, wherefrom a large fraction of both of these chemicals enter the sewage water. The reports indicate that influent reaching the wastewater treatment plants (WWTPs) may contain TCS and TCC up to 6.1 and 6.7 µg L^{-1}, respectively, (Halden and Paull, 2005; Heidler, *et al.*, 2006). Moreover, at least 20 million hectares in 50 countries, including Pakistan, are being irrigated with untreated or partially treated sewage water due to shortage

*Email: abidkhokhar@yahoo.com

of canal water (Ensink *et al*., 2004; Mahmood, 2006; Jiménez and Asano, 2008). The irrigation by such wastewater may transfer heavy quantities of TCS and TCC to agricultural soils. Moreover, in WWTPs, 50% of TCS and 76% of TCC remain undegraded on account of their high recalcitrance, low water solubility and high adsorption capacity to influent sediments. Hence the sewage sludge may contain TCS and TCC up to 30 and 51 mg kg^{-1}, respectively, on dry weight basis (Halden and Paull, 2005; Heidler, *et al.*, 2006). In many advanced and advancing countries of the world, the dry sewage sludge (biosolids) is being applied as plant nutrients' source and soil conditioner. This practice may also cause the transfer of TCS and TCC to agricultural soils. Once, these antimicrobial agents enter the agricultural soils, they may severely disturb the soil ecology, and in turn soil health, by suppressing microbial activity (Hammesfahr *et al.*, 2008; Liu *et al.*, 2009).

Little information is available on the role of TCS and TCC on microbial activity in soils of different physicochemical characteristics. Therefore, the present study was carried out to assess microbial activity as impacted by TCS and TCC antimicrobial agents and different physicochemical characteristics of soils of South Australia.

Materials and Methods

Reagents and equipments

Triclosan (97% pure) and triclocarban (99% pure) were obtained from Aldrich (Sydney, Australia). Scintillation liquid, 'OptiPhase HiSafe 3'was purchased from PerkinElmer Life and Analytical Sciences B.V., Groningen, the Netherlands. Triclosan and triclocarban solutions were prepared in acetone.

Collection and analysis of soils

The soils were air dried, sieved through 2 mm sieve and preserved in plastic buckets. A sub-sample of each soil was used for the determination of various physicochemical characteristics using the methods described by US Salinity Laboratory Staff (1954), Walkely and Black (1934), Piper (1966) and others. From the collected soils, three soils viz. Freeling (Typic Rhodoxeralf–sodic), Booleroo (Typic Rhodoxeralf) and Avon (Calcixerralic Xerochrepts) were selected (Figure 1) on the basis of their marked contrasting characteristics. Their physicochemical properties are given in the Table 1.

Multiplication and stabilization of microbial population

One kilogram of each of the three soils (Freeling, Booleroo and Avon) was taken in a plastic container separately. The moisture level in each container was adjusted to 48% of the maximum water holding capacity (MWHC) using sterile deionised water. The containers were moved to the incubator at 28±1 ^{o}C for 3 weeks to multiply and stabilize microbial population. The moisture evaporated meanwhile was regularly checked and maintained to the initial level of moisture content. Occasionally, the soils were stirred with a spoon for the proper aeration.

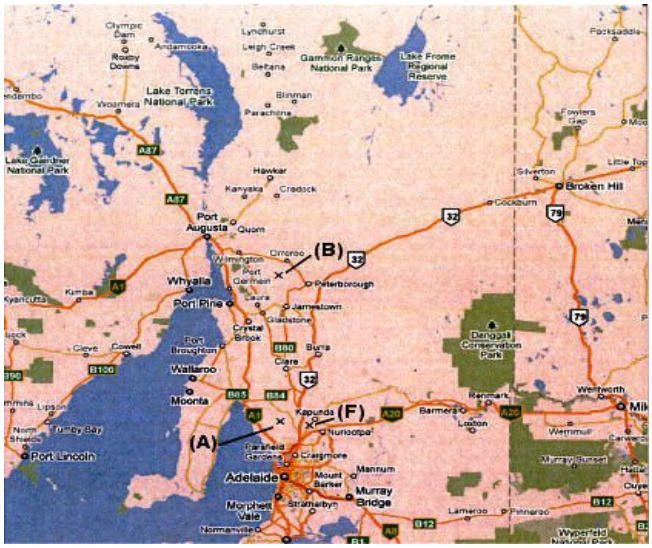

Figure 1: Sampling sites in Freeling (F), Booleroo (B) and Avon (A), South Australia, Australia. The soils were named after their sites of collection

Treatments of ^{14}C-glucose, TCS and TCC

From each container, 10 g soil on dry weight basis was taken in small plastic vials of 45 mL. The vials were divided into two halves in a manner that each set comprised equal number of vials of the Freeling, Booleroo and Avon soils. One set of vials was sterilized by autoclaving them at 120 ^{o}C under 300 kPa chamber pressure for 30 minutes for three consecutive days (called sterile soils). The other set of vials was kept as such without sterilization (called nonsterile soils). Both the sets were amended with triclosan at 0, 30, 90 and 270 µg g^{-1} and triclocarban at 0, 50, 150 and 450 µg g^{-1} soil. The vials were left in the fume hood for ~1 hour to allow the carrier acetone of the antimicrobial agents to evaporate. Subsequently, the vials were taken to the fume hood of the radiation laboratory. Here, they were spiked with ^{14}C-glucose at 5 µg g^{-1} soil. All the treatments were applied in triplicate. The soils were thoroughly mixed to ensure uniform distribution of the added chemicals. Sterile deionized water was added to adjust their water content to 48% of MWHC. The vials were immediately

transferred to large sealable vessels of 250 mL as one vial in each vessel. Beside each vial in sealable vessels, one vial of 20 mL containing 3 mL of 1*M* NaOH was placed to trap $^{14}CO_2$ and the vessels were immediately sealed. The sealable vessels were shifted to the incubator where temperature had already been set as 28 °C. The sampling was carried out 1, 2, 4, 7, 14, 21 and 28 days after the treatment of the soils.

Table 1: Basic soil properties of the three agricultural soils

Soils (USDA classification)	Sand (%)	Silt (%)	Clay (%)	Textural Class	pH	Organic Carbon (%)	MWHC (%)	Ca	Mg	K	Na	CEC
								(cmol kg^{-1})				
Freeling (Typic Rhodoxeralf–sodic)	72	12	16	Sandy loam	5.8	0.9	35	0.939	3.670	1.152	0.081	5.9
Booleroo (Typic Rhodoxeralf)	55	21	24	Sandy clay loam	7.3	1.0	41	4.769	7.966	0.169	0.129	13.9
Avon (Typic Calcixerallic Xerochrepts)	76	10	14	Sandy loam	9.0	1.9	40	11.050	1.590	1.000	0.045	13.7

MWHC: maximum water holding capacity

Radiorespirometric Analysis

Total microbial activity (TMA) was assessed by using the method of 'Indirect Estimation of Soil Microflora.' The background theory of this method is that when carbon-containing substrates are oxidized in soil, carbon dioxide is evolved which is generally taken as an index of the total activity of soil microflora. The total $^{14}CO_2$ produced by microbial activity as a result of glucose mineralization was analyzed following the protocols set by Bergman, *et al.* (2000) and Organization for Economic Co-operation and Development (2000). For measuring the quantity of $^{14}CO_2$ trapped, 10 mL scintillation liquid 'OptiPhase HiSafe 3, was taken into a scintillation vial. From a NaOH trap (which had trapped $^{14}CO_2$), 1 mL volume was also poured into the scintillation vial. The contents were mixed gently and left for ~5 hours to get stable. Subsequently, the samples were loaded on the Wallac WinSpectral α/β 1414 Liquid Scintillation Counter. The release of $^{14}CO_2$ was taken as an indicator of the capacity of the soils to biomineralize the radiolabeled $^{14}CO_2$ compound.

Statistical analysis

All the experiments were laid out with three replications of each treatment. An analysis of variance (ANOVA) was performed to determine the significance of the effect of two antimicrobial agents on glucose mineralization and interactions of these treatments with soil type. To prepare ANOVA-Two Way, the following statistical model was followed:

$Y_{ijk} = \mu + \alpha_j + \beta k + (\alpha\beta)_{jk} + \varepsilon_{ijk}$

Where

Y_{ijk} = ith (i = 1, 2, ……, r) observation from the experimental unit with jth (j = 1, 2, …., a) level of factor A and kth (k = 1, 2, ……, b) level of factor B.

μ = overall mean

α_j = the effect of jth level of factor A

βk = the effect of kth level of factor B

$(\alpha\beta)_{jk}$ = the interaction effect

ε_{ijk} = the error component, assumed N (0, σ^2)

Fisher's Least Significance Difference (LSD) test was applied to compare the cumulative mineralization values during the study period from different antimicrobial agents or soils.

Results

Biomineralization of ^{14}C-glucose in the three soils

In all the nonsterile soils, substantial amounts of ^{14}C-glucose were mineralized. The mineralization rate of ^{14}C-

glucose was markedly high at the start of the incubation which gradually declined over the time. At the end of the study, the total mineralized amounts of ^{14}C-glucose were noted as 63.7% in the Freeling, 78.0% in the Booleroo and 73.1% in the Avon soils. The analysis of variance showed significant differences in mineralized amounts of ^{14}C-glucose in the three soils. The greatest amount of ^{14}C-glucose was mineralized in the Booleroo soil followed by Avon and Freeling soils (Table 3). ^{14}C-glucose degradation kinetics was calculated using the first-order decay model. It was found that the kinetics of ^{14}C-glucose biodegradation varied substantially in the three soils. A marked difference existed in the rate constants (k) and half-lives ($t_{1/2}$) of ^{14}C-glucose in the three tested soils. For example, rate degradation of ^{14}C-glucose took place with rate constants 0.039878, 0.059109 and 0.051199 which resulted in half-lives of 17.4, 11.7 and 13.5 days in the Freeling, Booleroo and Avon soils, respectively. Thus degradation of ^{14}C-glucose followed the order of Booleroo > Avon > Freeling soils (Table 3).

biodegradation and cation exchange capacity of the soils. However, pH, clay and organic carbon content of the soils did not seem to have any major role in the mineralization of the spiked ^{14}C-glucose (Table 4).

Influence of TCS on biomineralization of ^{14}C-glucose

It was observed that the suppression of biomineralization of ^{14}C-glucose in all the soils occurred in the similar pattern, i.e. the effect of exogenously applied TCS was relatively weak in the start of the experiment which became very distinct in the later days. At the end of the study, the amounts of ^{14}C-glucose mineralized were recorded as 29.6, 47.9 and 45.7% by the dose of TCS at 270 μg g^{-1} compared to 63.7, 78.0 and 73.1% in their respective controls (Table 5). The analysis of variance showed a significant difference in the mineralized amounts of ^{14}C-glucose under different doses of TCS. The ^{14}C-glucose mineralization was significantly suppressed with every increasing level of TCS antimicrobial agent. Similarly, the

Table 2: General properties of triclosan and triclocarban using the US EPA Estimation Programs Interface Suite (EPI Suite v3.10) and PBT Profiler

Property	Triclosan	Triclocarban
CAS number	3380-34-5	101-20-2
Molecular formula	$C_{12}H_7Cl_3O_2$	$C_{13}H_9Cl_3N_2O$
Boiling point (°C)	373.62	434.57
Melting point (°C)	136.79	182.04
Vapour pressure (mm Hg at 25 (°C)	4.65 x 10^{-6}	3.61 x 10^{-9}
Water solubility (mg L^{-1} at (°C)	4.621	0.6479
Log K_{ow}	4.7	4.9
Log K_{oc}	4.265	3.732

Table 3: Percent cumulative biomineralization, rate constant (k) and half-life ($t_{1/2}$) of ^{14}C-glucose in Freeling, Booleroo and Avon soils

	SOILS		
	Freeling	Booleroo	Avon
% Cumulative Biomineralization	63.7 **c**	78.0 **a**	73.1 **b**
Rate constant (k)	0.039878	0.059109	0.051199
Half-life ($t_{1/2}$)	17.4	11.7	13.5

LSD (α=0.05) 2.98

Table 4: Correlation of ^{14}C-glucose degradation with different soil properties. R^2 is the square of correlation coefficient (r). Probability (two-tailed) was estimated at 0.05

Soil Property							
CEC		pH		Clay		Organic Matter	
R^2	p-value	R^2	p-value	R^2	p-value	R^2	p-value
0.8617	0.0003	0.3649	0.0849	0.3792	0.0774	0.0673	0.5004

Correlation analysis between ^{14}C-glucose mineralization and soil physicochemical properties revealed a positive significant correlation between ^{14}C-glucose amounts of ^{14}C-glucose mineralized were also significantly different in different soils. The highest amount of ^{14}C-glucose was mineralized in the Booleroo soil (61.8%)

followed by Avon (60.3%) and the Freeling soil (47.5%) (Table 7).

Freeling, from 11.7 to 24.9 days in Booleroo and from 13.5 to 26.6 days in the Avon soil (Table 6).

Table 5: The statistical computation of the effect of triclosan, soil and their interaction on the biomineralization of unsupplemented ^{14}C-glucose

	Freeling	**Booleroo**	**Avon**	Marginal Means
TCS 0	63.7 c	78.0 a	73.1 b	**71.6** A
TCS 30	54.9 d	65.0 c	65.3 c	**61.7** B
TCS 90	41.9 f	56.1 d	57.3 d	**51.8** C
TCS 270	29.6 g	47.9 e	45.7 e	**41.1** D
Marginal Means	**47.5** C	**61.8** A	**60.3** B	

Each value in the regular font is a mean of three replicates whereas, each value in the bold font is a marginal mean. The means sharing similar letters are not significantly different at probability level 0.05 by Fischer LSD Test. The capital letters with marginal means show the main effect of the factors whereas, the small letters with replication means show the interaction between the various levels of the two factors. The critical values for comparison are 1.46 for main effect of triclosan, 1.27 for main effect of soil and 2.54 for interaction.

Table 6: Rate constant (k), half-life ($t_{1/2}$) and percent reduction of ^{14}C-glucose as influenced by different levels of triclosan antimicrobial agent

(µg/g)	**Rate Constant (k)**			**Half-life ($t_{1/2}$)**			**% Reduction**		
	Freeling	**Booleroo**	**Avon**	**Freeling**	**Booleroo**	**Avon**	**Freeling**	**Booleroo**	**Avon**
TCS 0	0.039878	0.059109	0.051199	17.4	11.7	13.5	-	-	-
TCS 30	0.030932	0.042338	0.041128	22.4	16.4	16.8	13.9	16.7	10.7
TCS 90	0.021703	0.034154	0.034444	31.9	20.3	20.1	34.3	28.0	21.6
TCS 270	0.014895	0.027874	0.026024	46.5	24.9	26.6	53.6	38.5	37.4

Table 7: Correlation between TCS effect on ^{14}C-glucose mineralization and different soil properties. R^2 is the square of correlation coefficient (r). Probability (two-tailed) was estimated at 0.05

	Soil Property							
	CEC		**pH**		**Clay**		**Organic Matter**	
	R^2	*p*-value	R^2	*p*-value	R^2	*p*-value	R^2	*p*-value
TCS 0	0.8617	0.0003	0.3649	0.0849	0.3792	0.0774	0.0673	0.5004
TCS 30	0.9383	0.0000	0.6948	0.0052	0.0876	0.4399	0.3334	0.1033
TCS 90	0.9621	0.0000	0.7538	0.0024	0.0667	0.5014	0.3859	0.0743
TCS 270	0.9698	0.0000	0.6006	0.0142	0.1801	0.2551	0.2282	0.1933

The data were fitted to the first-order decay model and ^{14}C-glucose degradation kinetics was calculated. It was found that the kinetics of ^{14}C-glucose biodegradation substantially differed with the various levels of TCS. For example, the rate constants (k) varied from 0.039878 to 0.014895 in Freeling, from 0.059109 to 0.027874 in Booleroo and 0.051199 to 0.0260224 in Avon soil in the presence of TCS in the range of 0-270 µg g^{-1}. It extended the half-lives of ^{14}C-glucose from 17.4 to 46.5 days in

The R^2 and *p*-values showed a strong negative relationship between some physicochemical properties (CEC and pH) and the effect of TCS on the mineralization of ^{14}C-glucose (p-value < 0.05) (Table 7). It was revealed that in soils with higher CEC and pH values, the effect of TCS became relatively weak, as a result of which, the greater biodegradation of ^{14}C-glucose took place in those soils. However, such relationship could not be found between some other physicochemical properties (clay and

organic carbon content) and TCS effect (p-value > 0.05) (Table 7).

Influence of triclocarban on biomineralization of ^{14}C-glucose

The application of various levels of triclocarban (TCC) also decreased the biomineralization of ^{14}C-glucose in all the soils, however unlike TCS, the effect of TCC was not concentration-dependent. In Freeling and Booleroo soils, the effect of TCC was significant over the controls (no TCC), whereas in Avon soil, the effect of TCC was nonsignificant even over the control (Table 8). The application of TCC at 450 µg g^{-1} caused a reduction of 12.1% in Freeling, 5.2% in Booleroo and only 1.5% in Avon soil over their controls (no TCC) (Table 8).

The kinetics of ^{14}C-glucose biodegradation did not vary considerably in the presence of different levels of TCC antimicrobial agent because biodegradation of ^{14}C-glucose took place at almost the same rate. Similarly, the half-lives were also of almost the same period of length. For example, the half-lives varied from 9.5 to 14.3 days in the Freeling, from 11.7 to 14.0 days in the Booleroo and from 13.5 to 14.3 days in the Avon soils with TCC ranging from 0 to 450 µg g^{-1} (Table 9).

The R^2 and p-values showed a strong negative relationship between the soil physicochemical properties of CEC and pH and TCC effect on ^{14}C-glucose mineralization (p-value < 0.05) (Table 10). It was revealed that in soils of higher CEC and pH values the effect of TCC became weaker as a result of which greater degradation of ^{14}C-glucose was recorded in those soils. However, clay and organic carbon content of the soils did not have such correlation with effect of TCC on mineralization of ^{14}C-glucose (p-value > 0.05) (Table 10).

Discussion

Very small amounts of ^{14}C-glucose were mineralized in all the sterile (autoclaved) soils, most likely, due to chemical (abiotic) processes. In contrast, substantial amounts of ^{14}C-glucose were degraded in the nonsterile soils. This implies that biological activity of soil was primarily responsible for the biomineralization of ^{14}C-glucose in the soils. The results are in conformity with the findings of other researchers who found that microorganisms are primarily responsible for the degradation of glucose in the soil (Sheehan, 1997). The biodegradation started immediately after the addition of ^{14}C-glucose to the soils which indicates that the soils were well conditioned with microbial communities that could degrade ^{14}C-glucose. Actually, glucose is catalyzed with glucose oxidase enzyme, which is a ubiquitous bacterial enzyme, i.e. this enzyme is found in almost all the bacteria. The degradation of ^{14}C-glucose started at a fairly rapid rate which gradually slowed down in the subsequent days. The reason might be that a large amount of ^{14}C-glucose was available to the microorganisms in the beginning of the experiment which decreased over the time. This might have resulted in gradual decline in biodegradation rate of ^{14}C-glucose.

The comparison of soils showed the highest degradation in the Booleroo soil followed by the Avon and the Freeling soils (Table-2). This might be ascribed to the slightly alkaline pH (pH = 7.3) of the Booleroo soil because a slightly alkaline pH has been found favorable for the growth and activity of bacteria (Burns, 1976; Somasudaram *et al.,* 1987), and the bacteria are generally the major contributor to biodegradation activity in the soil. Furthermore, the pH and CEC are closely linked with each other. Normally, the soils of higher pH also have high values of CEC.

A strong concentration-dependent suppressive effect of TCS on the biomineralization of ^{14}C-glucose was observed in all the soils which implies that TCS was fairly effective against the soil microflora involved in the biomineralization of ^{14}C-glucose. The literature indicates, TCS is a broadspectrum antimicrobial agent which is predominantly static in action when present in low concentration and has a cidal effect on bacteria and fungi when present in high concentration (Schweizer, 2001). For example, TCS has been found effective against Nitrifying bacteria (Federle *et al.*, 2002; Stasinakis *et al.*, 2007), *Escherichia coli* (Sivaraman *et al.*, 2004; Stickler and Jones, 2008), *Staphylococcus aureus* (Stickler and Jones, 2008), *Proteus mirabilis* (Stickler and Jones, 2008) and *Vibrio fischeri* (Stasinakis *et al.*, 2007; Farre *et al.*, 2008). Some others have reported such antimicrobial effect of TCS without specifying/identifying the soil microorganisms (McMurry *et al.,* 1999; Suller and Russell, 2000; Denyer and Maillard, 2002; Reiss *et al.*, 2009; Waller and Kookana, 2009; Butler *et al.*, 2011).

The comparison of soils showed that TCS caused reduction in the order of Freeling > Booleroo > Avon soils (Tables-6). This variation in effectiveness of TCS in different soils could be attributed to the physicochemical properties of the tested soils. It was noted that in soils having higher pH, CEC and organic matter values, the intensity of TCS effect on biomineralization of ^{14}C-glucose decreased (Table 5). Since TCS is a weak acid (pk_a = 7.9), it is likely that greater amounts of TCS got dissociated in the soils of higher pH values (Loftsson, 2005) and that might be a reason of its mild effects in the Booleroo and Avon soils (pH 7.3 and 9.0, respectively) (Table 1).

Table 8: The statistical computation of the effect of triclocarban, soil and their interaction on the biomineralization of unsupplemented ^{14}C-glucose

	Freeling	**Booleroo**	**Avon**	Marginal Means
TCC 0	63.7 d	78.0 a	73.1 bc	**71.6** A
TCC 50	57.7 e	74.8 b	72.2 c	**68.2** B
TCC 150	56.8 e	73.6 bc	72.0 c	**67.5** B
TCC 450	55.5 e	73.5 bc	71.9 c	**66.9** B
Marginal Means	**58.4** C	**75.0** A	**72.3** B	

Each value in the regular font is a mean of three replicates whereas, each value in the bold font is a marginal mean. The means sharing similar letters are not significantly different at probability level 0.05 by Fischer LSD Test. The capital letters with marginal means show the main effect of the factors whereas, the small letters with replication means show the interaction between the various levels of the two factors. The critical values for comparison are 0.72 for main effect of triclocarban, 1.29 for main effect of soil and 2.59 for interaction.

Table 9: Rate constant (k), half-life ($t_{1/2}$) and percent reduction of ^{14}C-glucose as influenced by different levels of triclocarban antimicrobial agent

	Rate Constant (k) in ^{14}C-Glucose			**Half-life ($t_{1/2}$)**			**% Reduction**		
µg/g	**Freeling**	**Booleroo**	**Avon**	**Freeling**	**Booleroo**	**Avon**	**Freeling**	**Booleroo**	**Avon**
TCC 0	0.039878	0.059109	0.051199	17.4	11.7	13.5	-	-	-
TCC 50	0.032453	0.051899	0.049467	21.4	13.4	14.0	9.5	4.1	1.2
TCC 150	0.031126	0.049933	0.049041	22.3	13.9	14.1	10.8	5.7	1.4
TCC 450	0.029930	0.049636	0.048574	23.2	14.0	14.3	13.0	5.8	1.6

Table 10: Correlation between TCC effect on ^{14}C-glucose mineralization and different soil properties. R^2 is the square of correlation coefficient (r). Probability (two-tailed) was estimated at 0.05

	Soil Property							
	CEC		**pH**		**Clay**		**OM**	
	R^2	*p*-value	R^2	*p*-value	R^2	*p*-value	R^2	*p*-value
TCC 0	0.8617	0.0003	0.3649	0.0849	0.3792	0.0774	0.0673	0.5004
TCC 50	0.9595	0.0000	0.5677	0.0191	0.2042	0.2221	0.2015	0.2256
TCC 150	0.9689	0.0000	0.6236	0.0114	0.1588	0.2878	0.2502	0.1705
TCC 450	0.9814	0.0000	0.9907	0.0109	0.9814	0.2898	0.9814	0.1675

Moreover, clay and organic matter have been reported to have positive correlation with adsorption for TCS in soils. This premise was supported by the findings of Waller and Kookana (2009) who reported greater respiration inhibition in a clay soil than a sandy soil spiked with TCS at 10 mg kg^{-1}. They attributed this difference of TCS intensity with the organic matter content because clay soil had a higher content of organic carbon than the sandy soil. They presumed that in clay soil (having higher organic matter content), there was reduced bioavailability of TCS due to its greater adsorption which resulted in reduction in its expected toxicity. There are some reports which indicate that TCS can also adsorb to the clay colloids in soil. That is why, in soils of higher clay content, the reduced intensity of TCS might be due to the decreased bioavailability of TCS caused by its diffusion into the smallest pores, which are inaccessible to microbes (Hamscher *et al.*, 2002; Semple *et al.*, 2003). Therefore, in our case, it is possible that greater amount of TCS was adsorbed in soils of Booleroo and Avon soils (which had higher content of clay and organic matter contents) due to which the intensity of TCS on biomineralization of ^{14}C-glucose decreased in these soils.

In contrast with TCS, the addition of triclocarban (TCC) did not reduce the biomineralization of ^{14}C-glucose substantially in the tested soils (Tales 9). Moreover, the action of TCC as antimicrobial agent was not concentration-dependent, as all the three levels (50, 150 and

450 µg g^{-1}) had almost the same impact on biomineralization of spiked ^{14}C-glucose in all the soils. This implies that TCC is not a very effective antimicrobial agent or it gets degraded quickly after its entry into soil environment. Moreover, it could be explained in the perspective of lower intrinsic antimicrobial potential of TCC than that of TCS (Suller and Russell, 1999). Secondly, TCC is less soluble in water (0.6479 mg L^{-1}) than TCS (4.621 mg L^{-1}). Thirdly, TCC has greater K_{oc} value (54,800) than TCS (47,500). Our results are similar to those of Yang *et al.* (2007) who compared the growth-inhibitory and binary ion effects of TCS and TCC on the freshwater green alga, *Pseudokrichneriella subcapitata* over 72-hr exposure. They found smaller toxicity values (the median inhibitory concentration value, in micromoles) for TCS (0.0018) than TCC (0.054).

Conclusion

A significant concentration dependant suppressive effect of TCS on the biomineralization of ^{14}C-glucose appeared in all the tested soils as opposed to TCC where no such concentration dependent effect could be recorded. The reduction in ^{14}C-glucose biomineralization in the Freeling, Booleroo and Avon soils was recorded up to 53.6, 38.5 and 37.4% by TCS at 270 µg g^{-1} and 13.0, 5.8 and 1.6% by TCC at 450 µg g^{-1}, respectively. However, a significant negative correlation of CEC and pH was recorded with TCS and TCC effects. It implies that the presence of antimicrobial agents has a significant effect on the microbial activity in soil due to which the soil ecology, and in turn, soil health and quality may remarkably be impacted. An agricultural soil with low microbial activity may not be fertile and productive. In addition to this, it may have low capacity of xenobiotics cleanup. Thus, such soil will not only supply plants with poor nutrition but also pose serious health risks to humans on account of accumulation of toxic xenobiotics in it. Therefore, there is a dire need to take measures which would not let the antimicrobial agents reach the agricultural soils. However, if the entrance of antimicrobial agents could not be stopped, the steps should be taken for their cleanup so that their steady addition could not result in their accumulation to the level where it would significantly suppress microbial activity in the soil.

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