The Fate and Environmental Impact of Antibacterial Agents Triclosan and Triclocarban in Soil Receiving Long Term Biosolids Application

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Introduction

Farmland application of biosolids is the most sensible and cost-effective option for many municipalities and it provides tremendous savings in fertilizer costs to the farmers. However, environmentalists continue to raise concerns about the safety of this practice because biosolids may contain traces of organic compounds, such as pharmaceuticals and personal care products (PPCPs). Triclosan and triclocarban, both are widely used antibacterial agents in a variety of personal care products, are of particular interest because of concerns about their accumulation in soil receiving biosolids application may result in increased antibiotic resistance population of microorganisms in the environment. Up to date, there is limited information on the occurrence of those two compounds in biosolids, their long term fate in biosolids-applied soils, and their environmental impact on soil microorganisms.

Objectives

- To investigate occurrence of triclosan and triclocarban in biosolids collected from 16 U.S. wastewater treatment plants.
- To determine levels of triclosan in soils receiving biosolids annually for 33 years.
- To test the populations of antibiotic resistance microorganisms in soils receiving long-term biosolids application.
- To study the transformation rates of triclosan and triclocarban in soils.

Materials and Methods

- Biosolids were collected from 16 wastewater treatment plants in five U.S. states
- Soil samples from 0-6 in, 6 in – 12 in, and 24 in – 48 in were collected from control plots and plots receiving 16.8, 33.6, and 67.2 Mg dry biosolids per hectare per year for 33 years (1973 to 2005).
- About 3 g of air-dried and homogenized soil or biosolids sample was sonicated for 15 min in acetone and centrifuged.
- The supernatant was evaporated to complete dryness using turbo evaporator at 40°C, re-dissolved in certain volume of acetonitrile, filtered with Millipore filter (0.45 µm, PTFE, 13 mm), and finally transferred to a LC vial for analysis of triclosan and triclocarban on LC/MS.
- LC/MS analysis of the two target compounds used electrospray ionization in negative ion mode by monitoring ions with m/z 287 ($[M-H]^-$) for triclosan, and m/z 313 ($[M-H]^-$) for triclocarban.
- Bacterial antibiotic resistance in surface soil of the field receiving the highest biosolids application rate were characterized and compared to that in the control soil.
- For the bacterial antibiotic resistance study, 1 g of soil sample was diluted in saline solution stepwise from 10^{-1} to 10^{-8}. One mL each of the 10^{-5}-, 10^{-6}-, 10^{-7}-, 10^{-8}-diluted sample and saline solution (control) were pipetted on to separate plates, immediately covered with 15 mL warm Tryptic soy broth growth medium, and gently swirled to evenly distribute the inoculums. After the medium was solidified the plates were sealed and incubated at 30°C. The plates were observed daily and colonies were counted.

Materials and Methods (continued)

- Each sample was simultaneously cultured in four separate plates with growth medium containing 50 mg L^{-1} triclosan, 50 mg L^{-1} tetracycline, 50 mg L^{-1} triclosan + 50 mg L^{-1} tetracycline, as well as control. Certain amount of cycloheximide was added to each medium in order to prevent fungal growth. There were 2 replicates per treatment.
- The transformation rates of triclosan and triclocarban in two soils (McLaurin sandy loam and Marietta fine sandy loam) were investigated by incubating the soils amended with the two target compounds at 1mg kg^{-1} with and without addition of 1 g biosolids. Both sterilized and non-sterilized soil were used. The field moisture capacity of each soil was maintained throughout the incubation. One g of subsample was collected from each treatment at day 1, 2, 3, 7, 15, 30, and 50 and analyzed for triclosan and triclocarban.

Results and Discussion

- There is a wide spread occurrence of triclocarban and triclosan in biosolids. They were detected in biosolids at levels up to 80 and 61 mg kg^{-1} dry weight, respectively (Figure 1). Composted biosolids had lower concentrations of both compounds than fresh biosolids.
- Both triclocarban and triclosan were detected in soils annually applied with biosolids of WWTP #15 (Figure 1) for 33 years (Figure 2).
- The concentration of triclocarban in the biosolids #15 was about 3 times as much as that of triclosan (Figure 1), while its level in the surface soil receiving the long term biosolids application was about 20 times higher than that of triclosan (Figure 2), indicating a slower degradation rate for triclocarban.
- The levels of triclocarban and triclosan in the surface soils increased with increasing biosolids application rate. Concentrations of triclocarban and triclosan in the 0-6'' surface soil receiving 67.2 Mg dry biosolids per hectare per year for 33 years were 1251 and 52 µg kg^{-1} dry weight (ppb), respectively (Figure 3).
- The levels of triclocarban and triclosan decreased significantly with soil depth. At a depth of 24-48" for plots receiving the highest amount of biosolids, only traces of triclocarban and triclosan (23 and 19 µg kg^{-1} dry weight, respectively) were observed (Figure 2).

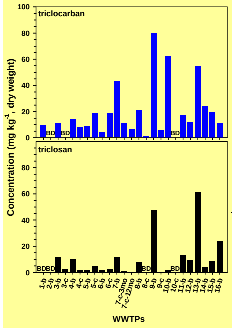


Table 1. Characteristics of the WWTPs investigated				
WWTP	**Location**	**Population**	**Community**	**Treatment**
1	GA	33,000	Mostly residential	Aerobic
2	SC	125,000	Mostly industry	Anaerobic
3	SC	30,000	Mostly residential	Aerobic
4	SC	60,000	Mostly residential	Aerobic
5	SC	50,000	Mostly residential	Aerobic
6	SC	25,000	Mostly residential	Aerobic
7	GA	40,000	Residential and industry	Anaerobic
8	GA	70,000	Residential	Aerobic
9	GA	30,000	Mostly residential	Anaerobic
10	GA	29,000	Mostly industry	Aerobic
11	GA	600,000	Residential and industry	Anaerobic
12	GA	175,000	Residential and industry	Anaerobic
13	CO	300,000	Residential and industry	Aerobic
14	IL	500,000	Residential and industry	Aerobic
15	IL	200,000	Residential and industry	Aerobic
16	CA	175,000	Residential	Aerobic

Figure 1. *Concentration of triclocarban and triclosan in biosolids (Table 1). Letters b and c indicate fresh biosolids and composted biosolids, respectively. c-3mo and c-12mo indicate composting for 3 months and 6 months, respectively. BD indicates below the detection limit (5 µg kg^{-1} dry weight)*

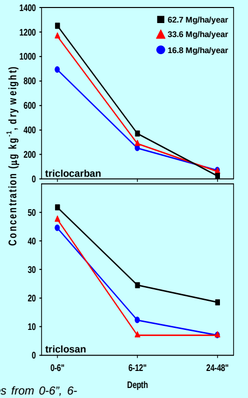
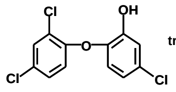
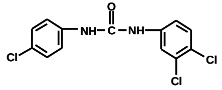


Figure 2. Concentration of triclocarban and triclosan in soil samples from 0-6", 6-12", and 24-48" depth of plots receiving biosolids of WWTP #15 at the annual rate of 16.8, 33.6, and 67.2 Mg dry biosolids per hectare.



triclosan



triclocarban

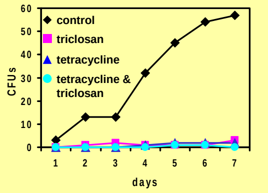


Figure 3. Bacterial plat counts for surface soil sample collected from the plot receiving the highest biosolids application rate. The dilution factor for the plating was 10^5. The growth medium in the plats were amended separately with triclosan, tetracycline, and a combination of the two antibiotics

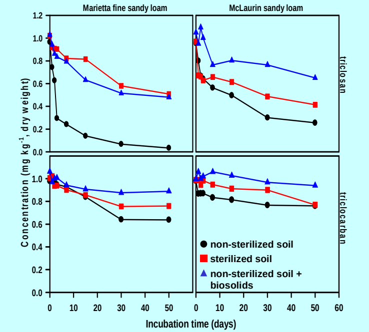


Figure 4. Transformation of triclosan and triclocarban with time in two soils under aerobic condition and field moisture capacity at 30°C.

- Triclosan and triclocarban do not accumulate extensively in surface soil. At an annual application rate of 67.2 Mg dry biosolids per hectare, about 44 kg triclocarban and 19 kg triclosan were added per hectare to the soil during the 33 years. At the time of soil collection from this plot, the total amount of triclocarban and triclosan in the top 6" soil was approximately 2.8 and 0.12 kg per hectare, respectively, about 6.4% and 0.6% of the total triclocarban and triclosan input during the 33-year biosolids application.
- The presence of low levels of both compounds below soil surface is mostly due to soil redistribution via disturbance by root/earthworm activity and plowing rather than leaching.
- There was insignificant soil bacterial antibiotic resistance to triclosan and tetracycline in the long-term biosolids-applied soils (Figure 3). However, additional experiments using growth culture containing lower levels antibiotics are needed to confirm this observation.
- Triclosan transformation in the two soils tested was significantly faster than triclocarban transformation (Figure 4).
- The transformation of both compounds followed 1st order kinetics. Triclosan had half lives of 2.3 and 16 days in non-sterilized Marietta and McLaurin soils, respectively, while the half lives for triclocarban in these soils were 72 and 244 days, respectively.
- Sterilization of the soil significantly retarded the transformation of both compounds, emphasizing the importance of microbial transformation in addition to the abiotic transformation processes.
- Laboratory incubation studies showed that addition of biosolids to soils significantly reduced the transformation rates of both compounds, suggesting that soil microorganisms might prefer biosolids rather than triclosan or triclocarban as carbon source. Sorption of both compounds to biosolids may also reduce the degradation rates.
- However, our field investigation suggested insignificant accumulation of triclosan and triclocarban in biosolids-applied soils. Faster transformation of the two compounds may occur in field conditions than in laboratory settings.