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ANTIMICROBIAL COMPOUNDS (TRICLOSAN AND TRICLOCARBAN) IN SEWAGE SLUDGES, AND THEIR PRESENCE IN RUNOFF FOLLOWING LAND APPLICATION

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Abstract

The reuse of treated municipal sewage (‘biosolids’) on land is an effective method to divert waste away from landfill and to use an alternative, low cost method of fertilisation. While
legislation has mainly focused on the control of nutrient and metal application rates to land, other potentially harmful emerging contaminants (ECs) may be present in biosolids. Up to 80% of municipal sewage sludge is reused in agriculture in Ireland, which is currently the highest rate of reuse in Europe. However, unlike other countries, no study has been conducted on the presence of ECs across a range of wastewater treatment plants (WWTPs) in this country. This study evaluated the concentrations of two ECs in sewage sludge, the antimicrobials triclosan (TCS) and triclocarban (TCC), and their presence in surface runoff following land application in controlled rainfall simulation studies. In 16 WWTPs, concentrations of TCS and TCC were 0.61 and 0.08 µg g⁻¹, which is at the lower end of concentrations measured in other countries. The concentrations in runoff post land application were also mainly below the limits of detection (90 ng L⁻¹ for TCS, 6 ng L⁻¹ for TCC), indicating that runoff is not a significant pathway of entry into the environment.

Keywords: Triclosan; triclocarban; biosolids; wastewater treatment plants; surface runoff.

Introduction

The reuse of treated municipal sewage sludge (“biosolids”) in agriculture provides the necessary nutrients and micronutrients essential for plant and crop growth (Latare et al., 2014; Liu et al., 2015). Biosolids may be used as a soil conditioner, improving its physical (e.g. water holding capacity; Cele and Maboeta, 2016) and chemical properties (e.g. soil test phosphorus; Shu et al., 2016). Their use also addresses European Union (EU) policy on sustainability and recycling of resources (COM, 2014a).
There are several issues associated with the reuse of municipal sewage sludge in agriculture (Peyton et al., 2016). While many of these are issues of perception (Robinson et al., 2012), there is considerable concern, which is scientifically based, regarding a number of substances that may be present in biosolids. There are concerns regarding pharmaceutical and personal care products (PPCPs), antimicrobial compounds, and other endocrine-disrupting compounds and synthetic compounds in biosolids (Clarke and Cummins, 2014) and the associated risk of contamination of soil, and surface and groundwater (Hanief et al., 2015; Fu et al., 2016). Toxic metals in sludge may accumulate in the soil and crops and enter the food chain following continuous applications to land (Stietiya and Wang, 2011; Latare et al., 2014; García-Santiago et al., 2016). Organic and inorganic contaminants may be lost along surface runoff and leaching pathways following land application (Gottschall et al., 2012; Peyton et al., 2016). Furthermore, there is a risk of emission and transport of bioaerosols containing manure pathogens following land application of biosolids (Brooks et al., 2005; Jahne et al., 2015). These concerns are confounded by the fact that although EU legislation controls the application of biosolids to land by setting limit values for nutrients and metals (EEC, 1986), no safety guidelines currently exist for PPCPs or many emerging contaminants (ECs).

Wastewater treatment plants (WWTPs) cannot fully remove PPCPs or other organic or synthetic compounds from wastewater, the removal of which is affected by treatment technique and operating conditions (Narumiya et al., 2013). Removal pathways include sorption onto sludge (Ternes et al., 2004) and biodegradation/biotransformation (Verlicchi et al., 2012). Despite this, several compounds have been measured in digested sewage sludge (Walters et al., 2010; Verlicchi and Zambello, 2015). Therefore, when biosolids are spread on land, there is a risk of indirect exposure to humans through several pathways, including the food chain (consumption of crops, meat, dairy products and drinking water), surface runoff,
and leaching to land drainage systems or groundwater used for abstraction by water treatment plants. Clarke et al. (2016) developed a quantitative risk ranking model for human exposure to 16 organic contaminants following biosolids application to land. They found that while nonylphenols had the highest risk, the antimicrobials, triclosan (TCS) and triclocarban (TCC), were considered more of an evolving risk, as these contaminants are emerging and have only recently been restricted within the US (US-FDA, 2015) and EU (COM, 2014b). In addition, both compounds are commonly the most abundant contaminants in biosolids (McClellan and Halden, 2010) and both are listed in the top contaminants of concern worldwide (von der Ohe, 2012; Verlicchi and Zambello, 2015). Triclosan, a broadspectrum bacteriostat and fungicide, and TCC, a fungicide and bacteriostat, are known toxins for humans and have been linked to inhibition of muscle function (Cherednichenko et al., 2012), resistance to antibiotics used in human medicine (Yazdankhah et al., 2006), and ecotoxicity in the environment such as the inhibition and killing of algae, crustaceans and fish (Chalew and Halden, 2009). On account of this, these compounds are the main focus of the current study.

In the EU there are considerable differences in national policy regarding the reuse of biosolids in agriculture. In some countries, such as Belgium (Brussels and Flanders), Switzerland and Romania, the reuse of biosolids in agriculture is prohibited (Milieu et al., 2013a,b,c), whereas in other countries, such as Ireland, up to 80% of municipal wastewater sludge is reused in agriculture (EPA, 2014; Eurostat, 2016). However, despite this, as the country with the greatest reuse of biosolids on land, no study has examined the concentrations of TCS or TCC in biosolids from WWTPs in Ireland. Such national studies of TCS and TCC have been conducted in the USA, Canada, India and South Korea (Table 1), but currently no extensive study across a range of WWTPs exists in the EU.
Once applied to land, TCS and TCC in biosolids may either accumulate in plants (Mathews et al., 2014); accumulate, biodegrade or biotransform in soil (Wu et al., 2009), or be released in surface runoff during rainfall-runoff or leaching events (Sabourin et al., 2009). The potential for loss via surface runoff or leaching depends on their availability in soil, which is a function of their persistence or half-life (Fu et al., 2016). It has been speculated that the persistence of TCS or TCC in the soil may be enhanced by the organic content of the soil (Fu et al., 2016), soil temperature (which is positively correlated to half-life), the physicochemical properties of the compounds (Wu et al., 2009), and the presence of co-contaminants (Walters et al., 2010), making them potentially more available for loss in surface runoff during rainfall events. Many studies have investigated losses of TCS and TCC in surface runoff from agricultural lands (Table 2), but few, if any, studies have investigated the surface losses from lands which have received sludge applications from the same WWTP having undergone different treatments. Such an experiment may allow the potential for surface water contamination from different sludge treatment methods to be evaluated.

Therefore, the aim of this study was to (1) characterise, for the first time, the TCS and TCC in biosolids from a range of WWTPs in Ireland, and (2) measure the surface runoff of TCS and TCC under successive rainfall simulations at 1, 2 and 15 days after application of two types of biosolids, originating from the same WWTP.

2. Methodology

2.1 WWTP identification and sample collection
In January and February 2015 (Winter in Ireland), biosolids were collected from 16 WWTPs, which had population equivalents (PEs, i.e. the amount of oxygen demanding substances in wastewater equivalent to the demand of the wastewater produced by a single person) ranging from 2.3 million to 6,500. Details of the PE and influent wastewater characteristics of each WWTP are given in Healy et al. (2016a). Most WWTPs received quantities of landfill leachate in low quantities (less than 2% of the influent biochemical oxygen demand (BOD) load), whilst others received industrial, commercial and domestic septic tank sludge comprising up to 30% of the influent BOD load. Anaerobic digestion of sewage sludge was carried out in five WWTPs, thermal drying in eight WWTPs, and lime stabilisation in four WWTPs (one WWTP carried out both anaerobic digestion and thermal drying). Discrete samples (n=8) of biosolids were collected in clean LDPE containers from each WWTP, and were pulverised in an agate ball mill (Fritsch™ Pulverisette 6 Panetary Mono Mill) with a rotational speed of 500 rpm for 5 min (repeated three times). The metal content of the biosolids are reported in Healy et al. (2016a).

2.2 Field study site description and runoff simulations

Treated municipal sewage sludge from the WWTP in which anaerobic digestion and thermal drying was carried out, was used in this study. Raw, untreated sludge from the same WWTP was modified by the authors with calcium oxide following the method outlined by Fehily Timoney and Company (1999). Therefore, the anaerobically digested (AD), thermally dried (TD) and lime stabilised (LS) biosolids used in this study originated from the same WWTP. The biosolids were applied to replicated (n=3), hydraulically isolated, field-scale micro-plots, each measuring 0.4 m-wide by 0.9 m-long. The slope of each micro-plot ranged from 2.9 to 3.7 % and each micro-plot was instrumented with a runoff collection channel, which allowed
all surface runoff to be collected over the duration of a rainfall event (Peyton et al., 2016).

The site was planted with ryegrass for over twenty years and the soil pH ranged from 5.9 to 6. The soil in all micro-plots was classified as loam and the soil organic matter ranged from 8.1 to 9.0%. Full classification of the plots is detailed in Peyton et al. (2016).

Anaerobically digested, TD and LS biosolids were applied by hand to the surface of each micro-plot at the maximum legal application rate in Ireland (Statutory Instrument 610 of 2010), which is currently 40 kg phosphorus (P) ha$^{-1}$. As the P content and the dry matter (DM) of the biosolids varied, the application rates were equivalent to 2.6 tonnes DM ha$^{-1}$ for TD biosolids (97 g per plot), 6.7 tonnes DM ha$^{-1}$ for AD biosolids (242 g per plot), and 29.5 tonnes DM ha$^{-1}$ for LS biosolids (1063 g per plot). The biosolids used in this experiment were natural and were not spiked with either triclosan or triclocarban. As the experiment was designed to compare the surface runoff from plots amended with three types of biosolids, no study control (grass only) plots were used in this experiment.

A rainfall simulator was used to apply rainfall to each micro-plot at intensity of approximately 11 mm h$^{-1}$ (corresponding to a 3 year return period in Ireland; Met Éireann, 2016) in time intervals of 1, 2 and 15 d after the time of biosolids application. Each rainfall simulation lasted 30 min from the time of first occurrence of surface runoff. Runoff water samples were collected in solvent washed amber glass Pyrex® bottles with PTFE lined lids and upon returning the laboratory, 4 mol L$^{-1}$ of sulphuric acid (H$_2$SO$_4$) was added to adjust the water to pH 3 to prevent biodegradation by microorganisms. Samples were then stored and frozen at -20°C until analysis.

2.3 Analysis of triclosan and triclocarban in biosolids and surface runoff
The method of analysis for TCS and TCC in the biosolids and surface runoff was conducted in accordance with USEPA Method 1694 (USEPA, 2007) for the determination of pharmaceuticals and personal care products in water, soil, sediment, and biosolids by liquid chromatography tandem-mass spectrometry (HPLC-MS/MS) analyses (Mc Clellan and Halden, 2010; Walters et al., 2010; Klosterhaus et al., 2013).

LC-MS-MS analyses was performed using a Waters Xevo™ TQ MS triple-quadrupole mass spectrometer (Waters Corp., Milford, MA, USA) coupled with a Waters Acquity UPLC™ system (Waters Corp., Milford, MA, USA) using reverse phase chromatography. Experimentation was based upon the conditions specified in the USEPA Method 1694, and summarised in Table S1 of the Supplementary Material. Details on the methodology employed may also be found in the Supplementary Material.

3. Results and Discussion

3.1 Triclosan and triclocarban content of biosolids from WWTPs

The TCS and TCC concentrations in the biosolids samples are shown in the Figure 1. The concentrations of compounds in sewage sludge are dependent on the influent wastewater characteristics, and up to 50% of the mass of TCS entering a WWTP may remain in the sludge (Heidler and Halden, 2007). Studies have examined the effectiveness of various sludge treatment methods (e.g. Carballa et al., 2007), but there is no indication that one form of treatment is more efficient in TCS or TCC removal.
One of the WWTPs examined (1 and 2 in Figure 1) had a history of high concentrations of TCS, with concentrations of 25 µg g⁻¹ previously being reported (EPA, 2009), but the concentrations in this study were below this. Of the previous studies that have carried out testing of TCS and TCC across a number of WWTPs in a given region, the concentrations of both parameters measured in this study ranked the lowest (Table 1). This may be due to the fact that TCS has been restricted in the EU since 2014 (COM, 2014b), whereas its use in ‘consumer antiseptic wash products’ will only be restricted in the USA from September 2017 (US-FDA, 2016). The use of TCC has been phased out by pharmaceutical companies in the last number of years (Westervelt, 2014). The biosolids samples used in this study were collected in January and February of 2015, so there may be seasonal variation in the concentrations of TCS and TCC as have been measured in other studies (Martin et al., 2012).

3.2 Triclosan and triclocarban content of surface runoff

The surface runoff concentrations of TCS and TCC were below the LOD in all cases, with the exception of TD biosolids at 15 days (0.01 µg L⁻¹) and LS biosolids (0.02 µg L⁻¹) one day after application (Table 3). These TCS and TCC concentrations in the surface runoff were lower than values observed in similar studies, and below the concentrations at which biota are considered likely to be potentially impacted (120 ng L⁻¹ for TCS, Wilson et al. (2003); 101 ng L⁻¹ for TCC, McClellan and Halden (2010)). The low concentrations in surface runoff may have been a function of the low TCS and TCC concentrations in the biosolids applied to land relative to similar studies (Table 2), but more likely were either due to their degradation or transformation to other compounds, or due to the soil characteristics at the study site. Less than 0.5% of the mass of TCS and TCC applied to each plot was lost in each rainfall event (Table 3). Similar results (expressed as a % of mass released versus mass of compound
applied) were obtained in a runoff study by Sabourin et al. (2009), who speculated that they remained sequestered in the soil or were leached to groundwater. The relationship between persistence of organic or synthetic compounds and the composition and physico-chemical properties of soil is well established in the literature (Verlicchi and Zambello, 2015). As reported in other studies (Wu et al., 2009), the high soil organic matter content in the micro-plots of the current study (8.1 to 9.0%) may have adsorbed some of the TCS and TCC. Unlike other studies which had durations ranging from 46 days (Wu et al., 2009) to 60 days (Fu et al., 2016), it was impossible to determine if the addition of the biosolids altered the soil’s physicochemical properties, as soil analysis was only conducted before the experiment commenced and at the end of the experiment (15 days after application of biosolids) (Peyton et al., 2016), which is too short a period to determine if such changes occur.

Another factor influencing the persistence of organic and synthetic compounds is the manner in which they are applied to the soil. Al-Rajab et al. (2015) reported that dissipation of PPCPs and antimicrobials, including TCS and TCC, increase over a duration of several months if they are placed below the soil surface (i.e. through ground injection systems). Therefore, the surface runoff of TCS and TCC in the current study may have been further reduced if the biosolids were applied in the soil subsurface.

3.3 Impact of the experiment results on reuse of biosolids in agriculture

The current study forms part of a larger study in which the metal content of biosolids from a selection of WWTPs were evaluated (Healy et al., 2016a), and the loss of nutrients, metals and pathogens in surface runoff (Peyton et al., 2016) and the uptake of metals by ryegrass following their land application was quantified (Healy et al., 2016b). For the water quality
parameters analysed in these studies, the environmental impacts on receiving waters arising from the landspreading of biosolids are no different to those arising from the landspreading of, for example, dairy cattle slurry (Brennan et al., 2012). It was also found that the metal content of ryegrass in biosolids-amended plots and control plots were similar (Healy et al., 2016b).

While the metal, TCS and TCC contents of the biosolids in the WWTPs examined in our studies were below the concentrations measured elsewhere, there may be a possibility that this may increase from one season to the next. In addition, until threshold values, based on human or ecological risk, are set, there is no clear basis for determining if the concentrations of TCS and TCC, as measured in the current study, are safe. Furthermore, the current study only examined two types of antimicrobials, which is only a small fraction of the total number of contaminants that may be present in biosolids. Parameter testing of this type is extremely expensive, so the costs of routinely testing sludge for all possible contaminants would be prohibitive. While most commentators have stated that the risk to human health following dietary intake of organic contaminants from crops grown on biosolids-amended lands is minimal (Verslycke et al., 2016), they acknowledge that a certain amount of uncertainty still exists (Smith, 2009; Oun et al., 2014). On account of these issues, and public perception issues in particular, many countries have prohibited the use of biosolids in agriculture, and even in those countries that permit their use, local restrictions exist (e.g. Bord Bia, 2013). This conservative ethos has a substantial ‘buy-in’ from major industries, who use products sourced from agricultural land, as there would be reputational damage to a brand if it emerged that biosolids, which could potentially contain ECs, were used in the production of their feedstock.
Although legislation has attempted to address public concern by regulating the amount of biosolids applied to land, there are considerable differences in national policy regarding the reuse of biosolids in agriculture. In Europe, the application of biosolids to land is governed by EU Directive 86/278/EEC (EEC, 1986), which is based on the nutrient and metal content of the biosolids (although more stringent guidelines are enforced in some member states). In comparison, in the majority of states of the USA, biosolids are applied to land based on the nitrogen requirement of the crop being grown and not on a soil-based test (McDonald and Wall, 2011). It would be impossible to fully regulate the application rates of all potential contaminants in biosolids, so while legislation has mainly focused on nutrient and metal content of biosolids, the possibility exists that other potentially harmful, unregulated ECs, for which no international standards exist for reuse in agriculture, may accumulate in the soil upon repeated application.

4. Conclusions

There were low concentrations of triclosan and triclocarban in treated sewage sludge across a number of WWTPs of varying PE. In addition, measurements of triclosan and triclocarban in surface runoff following land application were at or below the limits of detection, which indicates that there appears to be no risk of acute biological effects as a result of runoff.

There is a need to fully quantify the concentration of pharmaceuticals and other emerging contaminants in treated municipal wastewater, particularly in countries that recycle a high percentage of sludge produced in municipal sewage treatment plants in agriculture. To fully characterise all existing known, as well as emerging contaminants is cost prohibitive.
Therefore, any potential economic and practical gains arising from the recycling of sewage sludge in agriculture need to be considered alongside cost and public health issues.

Acknowledgements

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References


Table 1. Triclosan and triclocarban concentrations (µg g⁻¹ dry weight) in national studies of biosolids produced in municipal wastewater treatment plants.

<table>
<thead>
<tr>
<th>Reference</th>
<th>Country</th>
<th># WWTPs examined</th>
<th>Mean concentration (µg g⁻¹)</th>
<th>Maximum concentration (µg g⁻¹)</th>
</tr>
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<tr>
<td></td>
<td></td>
<td></td>
<td>Triclosan</td>
<td>Triclocarban</td>
</tr>
<tr>
<td>McClellan and Halden (2010)</td>
<td>USA</td>
<td>94</td>
<td>12.6</td>
<td>36</td>
</tr>
<tr>
<td>Subedi et al. (2015)</td>
<td>India</td>
<td>5</td>
<td>1.2</td>
<td>7.0</td>
</tr>
<tr>
<td>Chu and Metcalfe (2007)</td>
<td>Canada</td>
<td>4</td>
<td>4.2</td>
<td>4.3</td>
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<tr>
<td>Guerra et al. (2014)</td>
<td>Canada</td>
<td>6</td>
<td>6.8</td>
<td>2.9</td>
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<tr>
<td>Subedi et al. (2014)</td>
<td>S. Korea</td>
<td>40</td>
<td>3.1</td>
<td>6.9</td>
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<tr>
<td>This study</td>
<td>Ireland</td>
<td>16</td>
<td>0.61</td>
<td>0.08</td>
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Table 2. Studies examining the surface losses of triclosan and triclocarban from land applied biosolids.

<table>
<thead>
<tr>
<th>Reference</th>
<th>Type of sludge used</th>
<th>Compound in biosolids (µg g⁻¹)</th>
<th>Application rate (expressed as dry matter)</th>
<th>Concentration in surface water (ng L⁻¹)</th>
<th>Time of detection (days post application)</th>
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<tr>
<td>Edwards et al. (2009)</td>
<td>Anaerobically digested</td>
<td>14</td>
<td>~8 Mt ha⁻¹</td>
<td>240</td>
<td>&lt;LOQ</td>
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<tr>
<td>Gottschall et al. (2012)</td>
<td>Anaerobically digested</td>
<td>10.9</td>
<td>~22 t ha⁻¹</td>
<td>73</td>
<td>40</td>
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<tr>
<td>Topp et al. (2008)</td>
<td>Not stated</td>
<td>2.6 t ha⁻¹</td>
<td>92,500 L ha⁻¹</td>
<td>258</td>
<td>1</td>
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<tr>
<td>This study</td>
<td>Anaerobically digested</td>
<td>0.27</td>
<td>&lt;90</td>
<td>&lt;6</td>
<td>15</td>
</tr>
<tr>
<td></td>
<td>Thermally dried</td>
<td>4.9</td>
<td>&lt;6</td>
<td>15</td>
<td>15</td>
</tr>
</tbody>
</table>

Values in this study were below the limits of detection (90 ng L⁻¹ for triclosan and 6 ng L⁻¹ for triclocarban). First row refers to anaerobically digested sludge, second row refers to thermally dried sludge.
Table 3. Concentrations of triclosan and triclocarban in applied biosolids to field plots (µg g$^{-1}$; ‘Influent’) and average concentrations of triclosan and triclocarban in surface runoff (µg L$^{-1}$) from field plots. LOD (limit of detection) = 0.09 µg L$^{-1}$ (TCS) and 0.006 µg L$^{-1}$ (TCC) in this study.

<table>
<thead>
<tr>
<th></th>
<th>Triclosan</th>
<th></th>
<th></th>
<th>Triclocarban</th>
<th></th>
<th></th>
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<tbody>
<tr>
<td></td>
<td>TCS in applied sludge dose</td>
<td>µg g$^{-1}$</td>
<td>µg L$^{-1}$</td>
<td>TCC in applied sludge dose</td>
<td>µg g$^{-1}$</td>
<td>µg L$^{-1}$</td>
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<tr>
<td>TD</td>
<td>4.9</td>
<td>&lt;LOD</td>
<td>&lt;LOD</td>
<td>0.05</td>
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<tr>
<td>LS</td>
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<td>&lt;LOD</td>
<td>Not measured</td>
<td>0.02</td>
<td>&lt;LOD</td>
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<tr>
<td>AD</td>
<td>0.27</td>
<td>&lt;LOD</td>
<td>&lt;LOD</td>
<td>&lt;LOD</td>
<td>&lt;LOD</td>
<td>&lt;LOD</td>
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</table>
Figure 1. Triclosan and triclocarban concentrations (µg g\(^{-1}\)) in treated sludge from 16 wastewater treatment plants in Ireland, ranging (numerically in ascending order) from a population equivalent (PE) of 2.3 million to 6,500. Two forms of treatment of sludge are carried out in one WWTP: anaerobic digestion (1a) and thermal drying (1b). WWTPs with no concentrations shown are WWTPs in which triclosan or triclocarban were below the limits of detection (TCS, 0.006 µg g\(^{-1}\); TCC, 0.0024 µg g\(^{-1}\)).