



Assessment of leachable and persistent dissolved organic carbon in sludges and biosolids from municipal wastewater treatment plants

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ABSTRACT

Environmental regulation of organic pollutants has not kept pace with the growth in the number and diversity of legacy and emerging organic substances now in use. Simpler and cheaper tools and methodologies are needed to quickly assess the organic pollutant risks in waste materials applied to land such as municipal wastewater treatment sludges and biosolids. This study attempts to provide these, using an approach that consists of chemical leaching and analysis of dissolved organic carbon and determination of its biodegradability by measuring persistent dissolved organic carbon. Primary and secondary sludges, dewatered sludge cake, and anaerobically and thermally treated biosolids obtained from various types of municipal wastewater treatment plants were used in the study. The study found little variability in the levels of dissolved organic carbon leached from primary sludges obtained from different municipal wastewater treatment plants but found significant differences for secondary sludges based on levels of nitrification at the municipal wastewater treatment plants. As predicted treated biosolids leached less dissolved organic carbon than untreated dry sludges but had relatively higher proportions of persistent or poorly biodegradable dissolved organic carbon. Across all tested sludges and biosolids persistent dissolved organic carbon ranged from 14 to 39%, with biosolids that have undergone anaerobic digestion and thermal treatment more likely to contain greater relative proportion of persistent dissolved organic carbon than untreated sludges. The approach presented in this study will be useful in assessing the effectiveness of current and widely employed sludge treatment methods in reducing persistent organic pollutants in biosolids disposed on land.

1. Introduction

The circular economy concept aims to conserve finite non-renewable resources in a cycle of production, to waste, and back to production. A common application of the concept is the use of municipal wastewater treatment plant (MWWTP) residues such as fresh sewage sludge or biosolids (e.g., treated sewage sludge) as soil amendments to provide nutrients and improve soil quality for agricultural and horticultural purposes and to improve soil productivity. While amending soils with MWWTP residues has been widely practised, concern has often been raised about the potential risks to soil and water quality (Singh and Agrawal, 2008; Carbonell et al., 2009; Geissen et al., 2015; Brown et al., 2020; Collivignarelli et al., 2020). MWWTP are the primary pathway for many organic contaminants from various sources, such as pharmaceuticals and personal care products (PPCP), to reach the environment. Some contaminants are degraded by chemical and biological processes during wastewater treatment, but many of these contaminants can

partition to the solid phase through sorption processes, resulting in their accumulation in the sludges and subsequently on soils where the sludges or biosolids are applied. These substances include not only lipophilic compounds that would be expected to partition out of the liquid phase of wastewater, but also a wide array of organic micropollutants, both halogenated and non-halogenated, lipophilic and hydrophilic, some with bioaccumulative potential, at appreciable concentrations, representing a diverse cocktail of potential pollutants (Venkatesan and Halden, 2014). Many PPCPs and persistent organic pollutants (POPs) have been detected in MWWTP residues, however, there has been minimal study of the potential for these substances to leach into the environment from amended soils (Kinney et al., 2006; Radjenović et al., 2009; Monteith et al., 2010; Jelic et al., 2011; Liu and Wong, 2013; Narumiya et al., 2013; Venkatesan and Halden, 2014; Huber et al., 2016; Rigby et al., 2020; Tasselli and Guzzella, 2020).

The current regulatory framework for land application of MWWTP residues in most developed countries focuses on trace metals, odour and

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pathogens with less consideration given to the presence of organic micropollutants. There are several reasons for this. The sheer number of possible organic contaminants raises practical difficulties in their individual identification, quantification and monitoring due to the high time, cost, and technical expertise required (Diamond et al., 2011; Anumol et al., 2015; Geissen et al., 2015; Petrie et al., 2015). There are also challenges in keeping pace with the study of the potential environmental or human health impacts of both legacy and emerging compounds, transformation by-products and mixtures of compounds, for which toxicological data may not currently be available. In addition, replacements for regulated toxic substances such as some per- and polyfluorinated alkyl substances (PFASs), polybrominated diphenyl ethers (PBDEs) and others are emerging over time, with a significant time lag between production, use and regulation of new chemicals (Hwang et al., 2012; Gao et al., 2020; Semerád et al., 2020). Current regulations in the US and Canada for the application of biosolids to land exclude organic pollutants from consideration due to assumptions that concentrations of individual contaminants are likely below possible levels of harm, and treatment processes are probably adequate to reduce organic pollutants to acceptable levels. This approach excludes the consideration of compound mixtures, the relative ability for current MWWTP processes to remove recalcitrant organic compounds, and the cumulative effects of regular application over time.

Some regulatory regimes provide only generic guidelines for organic pollutants in biosolids, specifying that those applying biosolids to land take reasonable actions to reduce harm to health and the environment. However, there is little guidance on how this should be achieved, or how risk reduction can be measured. A paradox appears to exist in the consideration of regulatory regimes for wastewater effluents compared to wastewater solids. Effluents are heavily monitored, and treatment processes are designed to enhance the removal of pollutants to meet quality standards before discharging to the environment. Future regulation of wastewater effluents is likely to include an increasing number of organic compounds, which could lead to treatment plants designing treatments that decrease effluent concentrations by increasing sorption to solids, transferring the pollutant burden to the sludge or biosolid matrix. For example, the use of granular activated carbon as a sorbent is one option for reducing concentrations of pharmaceuticals in effluent. This treatment will not degrade POPs but instead move them into the solid matrix, where they will not be subject to the same regulatory controls as they would be for effluents.

In the absence of regulatory drivers for MWWTP to reduce POP loads in sludges and biosolids, there is little incentive to investigate or adapt treatment processes to quantify or reduce POP burden in sludges and biosolids prior to application to land. Regulators are thus left without the evidence base with which to set regulatory limits, and to date there are no agreed indicators that provide an overall quantification of organic pollutant risk. The lack of tools for assessing overall organic pollutant burden is a serious barrier to understanding environmental risks in biosolids.

This study proposes the measurement of leachable (or desorbed) dissolved organic carbon (DOC) from sludges and biosolids, and quantification of the persistent fraction of DOC (PDOC), to be used as an indicator of the mobile persistent organic pollutant load. Total organic carbon (TOC) has long been considered a useful parameter for estimating organic loading into the environment and assessing wastewater treatment performance (Aziz and Tebbutt, 1980; Fadini et al., 2004; Dubber and Gray, 2010). TOC has also been used widely in the pharmaceutical manufacturing industry to detect residues of pharmaceutical products in production systems and to screen for hydrocarbon pollution on industrial sites, ground and surface water and soils (Spruill, 1988; Schreiber et al., 1999; Nam et al., 2008). The use of TOC to understand organic pollution in biosolids is complicated by the presence of innocuous organic matter and microorganisms. However, measurements of DOC and a quantification of its PDOC component can help to improve understanding of the content, and potential for leaching of persistent

organic compounds from land application of sludges and biosolids. The comparison of DOC and PDOC from sludges from various types of MWWTPs and across their stages of treatment can provide insights into the relative importance of unit treatment operations and processes in the fate of organic compounds. This study therefore aims to utilise these tools to assess the effect of different treatment stages and parameters on the accumulation of persistent organic compounds in various sources. Characterising the biodegradability of the leachable fraction of MWWTP sludges from various treatment plant types and stages will thus help to inform treatment and disposal strategies.

2. Material and methods

2.1. Site selection

Four MWWTP of various sizes and treatment types located in the East of Scotland were identified as sources for sludge and biosolid collection in this study (Table 1). Plants with varying levels of aeration were included (e.g., nitrifying, partially nitrifying and non-nitrifying). Of the plants, only two (Sites 1 and 2) included a primary settlement stage from which fresh sludge solids were collected. Secondary sludge was collected from all four plants comprising two nitrifying (Sites 2 and 3), one non-nitrifying (Site 1) and one partially nitrifying (Site 4) fresh sludges. Additionally, sludge cake, referred to in this paper as biosolids, comprised of thickened and dewatered mixtures of sludge residues, were collected from Sites 3 and 4. Finally, sludge pellets, (e.g., biosolids that have undergone anaerobic digestion (AD) and drying at 120 °C), were obtained from Site 1. For the biosolids, chemical thickening was achieved using polyacrylamide prior to dewatering. A certified reference material (CRM 055, Lot LRAA8035, Sigma-Aldrich RTC), representing a domestic sewage sludge after final processing and thermal stabilisation, with certified TOC (29.9% Wt%) was also assessed in this study for comparison with other thermally treated biosolids.

2.2. Sample collection and preparation

Sludge samples were collected in sterile amber 1 L wide mouth containers that were sealed, placed in cool boxes and immediately returned to the laboratory. A minimum of 2 L of sludge was collected from each sampling point. Sludge samples from primary and secondary treatment stages were placed in a laboratory refrigerator ($4^{\circ} \pm 2^{\circ}\text{C}$) overnight to allow solids to settle. Supernatant was decanted and discarded. The remaining settled solids were transferred onto foil-lined trays in layers less than 1 cm depth to air dry at room temperature ($20\text{--}24^{\circ}\text{C}$) to a constant mass. Sludge cake was also placed in trays and allowed to air dry under the same conditions. Samples were kept in the dark and turned daily with a stainless-steel spatula to assist drying. Time to achieve air drying varied by sludge type from 7 to 14 days. Air-dried sludge was removed from the foil trays and placed into foil lined containers and stored in the refrigerator ($4^{\circ} \pm 2^{\circ}\text{C}$). The particle size after drying for most dried sludge samples was $<9.5\text{ mm}$ as per specified in the leaching method described below except for the biosolids (e.g., sludge pellets and sludge cake), for which particle size reduction (gentle crushing with mortar and pestle) was used to reduce any large particles to $<9.5\text{ mm}$ diameter. Moisture content and volatile solid content were determined on a sub-sample following air drying for each sampling location using USEPA method 1684 (United States Environmental Protection Agency, 2001) prior to the start of experimental work.

2.3. Leaching procedure

Solid waste leaching procedures were reviewed for their suitability for sludge and biosolid samples and for potential interference in determination of TOC in leachate (United States Environmental Protection Agency, 1994; Washington State, 2003; Morissette et al., 2015; Tiwari et al., 2015). The USEPA synthetic precipitation leaching procedure

Table 1
Description of wastewater treatment plants.

Plant ID	Catchment description	Treatment stages sampled			Treatment type		Details of treatment processes
		Primary	Secondary	Sludge cake or pellets	Nitrifying system	Non-nitrifying system	
Site 1	Large urban catchment (237,000 pe) receiving wastewater from storm drainage, domestic, commercial, and industrial premises including pharmaceutical manufacturing, a large hospital, and two universities.	✓	✓	✓		✓ (HRT 4 h)	Grit removal (6 mm screen), primary settlement, secondary aeration followed by settlement. Sludge treatment by AD (mesophilic process, 35 °C), pH adjustment, dewatering (belt press) and thermal drying at 120 °C to create a sludge pellet biosolid.
Site 2	Small community catchment (2893 pe) receiving wastewater from storm drainage, domestic premises and a small number of commercial premises (e.g., restaurants, hotel, shops).	✓	✓			✓ (HRT 20 h, SRT ~ 25 d)	Primary settlement followed by secondary treatment in aerated oxidation ditch. Sludge is taken offsite for disposal (no dewatering). No final biosolids are produced at this plant.
Site 3	Medium sized urban catchment (21,364 pe) receiving wastewater from domestic and commercial premises, including hotels, restaurants, a large university, and a community hospital.		✓	✓		✓ (HRT 18–27 h, SRT ~6 d)	No primary settlement. The first stage of treatment is in an oxidation ditch dosed with return activated sludge, followed by secondary aeration (extended aeration), and final settlement. Sludge is dewatered using polyacrylamide dosing and centrifugation.
Site 4	Medium sized urban catchment (21,800pe) receiving wastewater from storm drainage, domestic, commercial, and industrial premises, and a small community hospital.		✓	✓		partially nitrifying (HRT 4+ h)	No primary settlement. Influent is introduced into a cyclic activated sludge system (CASS) in the form of a sequencing batch reactor (SBR) with four tanks. Polyacrylamide is used for sludge dewatering.

pe = population equivalent, ✓ indicates treatment stages sampled, HRT = hydraulic retention time, SRT = solids retention time.

(SPLP) SW 846 Test Method 1312 (United States Environmental Protection Agency, 1994) was selected as the basis for evaluating leaching (desorption) of DOC in this study with minor modifications including a scaled down sample mass and leaching fluid volumes and exclusion of zero headspace vessels required for the determination of volatile organics, which were not assessed in this experiment.

A stock solution of concentrated sulphuric acid and nitric acid at a 60:40 vol/vol ratio was prepared using sulphuric acid (2.5 M) and nitric acid (2.5 M) (both Fisher, Laboratory Reagent grade) and added dropwise to a volume of ultrapure water until pH reached a value of 4.20 ± 0.05 pH units (Hach Sension 3 pH meter). The leaching fluid was then used directly for leaching experiments at room temperature. Initially a 20:1 liquid to mass ratio of leaching fluid to dried sludge or biosolid sample was prepared, as prescribed in the SPLP method, by adding approximately 2.5 g dry weight (dw) to each tube with 50.0 mL of leaching fluid. Sample mass was recorded to the nearest 0.0001 g, and dry weight (dw) calculated based on moisture content assessed using standard gravimetric methods. Initial experimental work found that a 2.5 g sample size resulted in solutions with DOC concentrations that significantly exceeded the analytical range of the analyser. A reduced sample mass of 1.0 g was used for subsequent samples to reduce dilution requirements prior to analysis. Replicate samples were prepared for each sludge or biosolid sample and the reference sludge (CRM 055) mentioned in 2.1. Process blanks (leaching fluid only) were included with each batch to check for sources of contamination.

Sample tubes were tightly capped and loaded onto the mixing apparatus to be turned end-over-end at a rotation of 30 ± 2 rpm for 18 ± 2 h at room temperature (23 ± 2 °C). At the end of the rotation procedure, samples were removed from the apparatus, and centrifuged for 5 min at 300 rpm to aid settlement of solids. Sample pH was recorded following centrifuging using a handheld pH meter (Hach, Sension 3). Leachates were filtered using 0.45 µm pore size filters (PALL GN-6 Metricel Grid, 47 mm) directly into a sterile amber coloured sample tube and capped. The leachates were stored in a refrigerator at 4 ± 2 °C (for analysis within 24 h) or in a freezer at -18 ± 2 °C (for analysis that would take place more than 24 h later).

2.4. Biodegradation procedure

To determine the PDOC, a 28-day biodegradation test was applied based on Organisation for Economic Development (OECD) Guideline for Testing of Chemicals 301, which is used to assess the relative biodegradability of organic compounds in an aerobic aqueous medium (Kowalczyk et al., 2015). An aliquot of filtered leachate obtained from the leaching test was added by pipette to a sterile 250 mL glass sample bottle, with the exact volume added recorded. The quantity of sample added was based on the known starting concentration of the undiluted sample DOC to ensure adequate DOC in the starting sample to observe degradation over 28 days. Parallel inoculum and feed water blanks were used to determine DOC in the test matrix from the inoculum and check for sources of contamination. The aerated feed water containing the microbial consortium, pH buffer and micro-nutrients was added to make each sample solution up to a volume of 250 mL. The feed water was produced in a 5 L batch comprised of 5 L of ultrapure water (Puracel PURITE Select) aerated for a minimum of 2 h before use with nutrients and buffer components added (phosphate buffer (pH 7.2), magnesium sulphate, calcium chloride, ferric chloride).

Sample bottles were covered with foil and placed in a dark incubator at 20 °C. To observe the pattern of biodegradation, subsampling occurred at intervals leading up to the 28-day end point, as specified in OECD method 301. Samples were taken after 1, 2, 7, 16, and 28 days. The day 1 and day 2 sampling events were chosen to observe the predicted rapid degradation of readily degradable material. Subsequent sampling events on day 7 and day 16 were chosen to identify if more resistant DOC would begin to decline, potentially due to microbial adaptation over time (Scott and Jones, 2000; Martin et al., 2017; Poursat et al., 2019). For each subsampling event, sample bottles were removed from the incubator and stirred with a metallic stir bar. An automatic pipette was used to extract a subsample, which was filtered directly through a 0.45 µm filter paper into a clean amber coloured centrifuge tube. Sample bottles were then covered and returned to the incubator until the next sub-sampling event. The filtered subsamples were labelled with sampling time and date and placed in a refrigerator at 4 ± 2 °C for analysis within 24 h, or in a freezer at -18 ± 2 °C for analysis beyond 24 h. Dissolved oxygen (DO) concentrations were measured at the start and end of the biodegradation test to ensure aerobic conditions were

maintained (Hach, Sension 6 laboratory DO meter).

2.5. Dissolved organic carbon analysis

Determination of DOC was performed using an OI Analytical model 1010 Wet Oxidation Total Organic Carbon (TOC) Analyser fitted with model 1051 auto-sampler using the persulfate oxidation method (OI Analytical, 2003). All reagents were produced using ultrapure water (Puracel, PURITE Select 18 MΩ). Prepared reagents included sodium persulfate, Na₂S₂O₈ 100 g.L⁻¹; (ACROS Organics, 98+) and phosphoric acid (5% vol/vol ACS Reagent Grade - 85% H₃PO₄). Calibration standards were potassium hydrogen phthalate (KHP) at 1, 10, 25, 50 and 100 mg.L⁻¹ prepared by dilutions of 1000 mg.L⁻¹ stock solution (BDH AnalaR). Nitrogen gas (BOC, > 99.98% purity, 50–60 psi) was used for reagent purging.

Results were reported as mg.L⁻¹ with dilution factors applied using the instrument software. Values were converted to mg.kg⁻¹ dw using initial sample mass, extraction volume, and moisture content using Equation (1):

$$DOC = DOC_i \left[\frac{V_i}{W_w(1 - M_o)} \right] \quad (1)$$

where:

DOC = mobile dissolved organic carbon (mg.kg⁻¹).

DOC_i = TOC concentration measured in filtered leachate, with dilution factor (mg.L⁻¹).

V_i = sample volume (L).

W_w = wet weight (mg).

M_o = moisture content of the sample, as expressed in decimal format.

For PDOC experiments, the determination of DOC was performed as above on day 1, 2, 7, 16 and 28. The relative percent biodegradation (D_t) was calculated for days 1–28 based on the starting DOC concentration (C₀) in the original leachates, corrected for DOC concentration in the feedwater blanks (C_{bl}) as in Equation (2).

$$D_t = \left[1 - \frac{C_t - C_{bl(t)}}{C_0 - C_{bl(0)}} \right] \times 100 \quad (2)$$

where:

D_t = % biodegradation at time t; C₀ = mean concentration of DOC in the sample (mg.L⁻¹ DOC) at time 0; C_t = mean concentration of DOC in the sample (mg.L⁻¹ DOC) at time t; C_{bl(0)} = mean concentration of DOC in the blank (mg.L⁻¹ DOC) at time 0; C_{bl(t)} = mean concentration of DOC in the blank (mg.L⁻¹ DOC) at time t; To test for statistical differences between sludge types, analysis of variance (p < 0.05, one-way ANOVA),

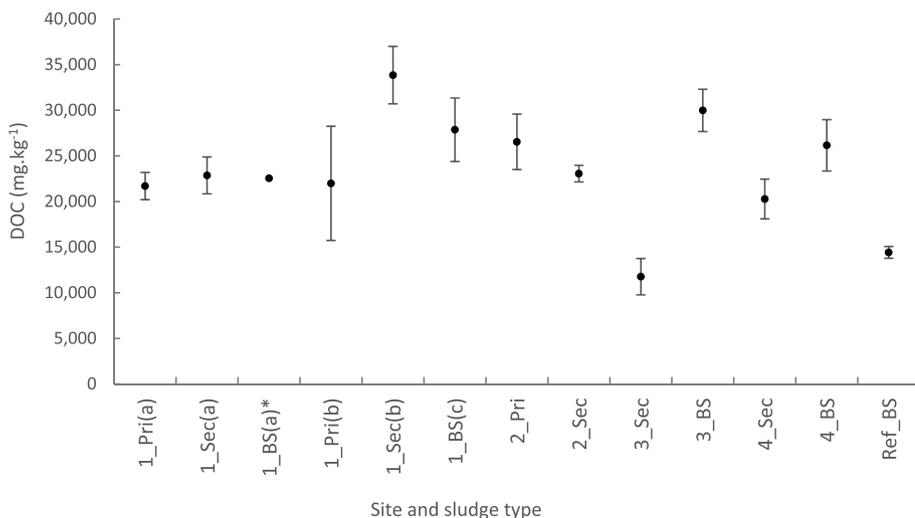


Fig. 1. Mean DOC by treatment plant and treatment stage (error bars showing 2 × SD). Pri – primary sludge; Sec = secondary sludge; BS = treated biosolid; Ref = reference sludge, CRM 055. a = 1st sample; b = 2nd sample collected one month later; c = sample collected one year earlier. *Due to analytical error, one replicate sample for Site 1 biosolid (first sampling) was excluded from calculations, hence only two replicates were used to calculate mean DOC for 1_BS(a), which is provided for illustrative purposes only excluding SD.

multiple comparisons and non-parametric tests were used. Statistical analysis software IBM SPSS (version 25, 2017) was used for the evaluation of results.

3. Results

3.1. Desorbed dissolved organic carbon (DOC)

Mean DOC (mg.kg⁻¹ dw) ranged from 11760 to 33853 mg.kg⁻¹ across sludge and biosolid types. The mean DOC values for each MWWTP and treatment stage are shown in Fig. 1. The results indicate a significant difference in DOC based on nitrification status (p = 0.018). More DOC was obtained from sludges from the non-nitrifying plant as compared to the nitrifying and partially nitrifying plants (Site 1 - non-nitrifying; Sites 2 and 3 - nitrifying, and Site 4 partially nitrifying). DOC from nitrifying plants was significantly less than from the non-nitrifying plant (p = 0.016), however there was no significant difference between partially nitrifying plants and other plants (p > 0.05).

3.2. Persistence of leached DOC (PDOC)

The rates of degradation of DOC obtained from the various sources, as assessed by the DOC concentrations obtained in the biodegradation experiments are shown in Figs. 2–4. All samples were blank corrected for their respective batch blanks. A similar pattern of degradation was observed with rapid degradation in the first two days in all assays. This suggests that under aerobic conditions, the microbial consortia present consume readily degradable carbon quickly, however the percentage of DOC consumed in the first two days varied by site and sample type.

Fig. 2 presents the biodegradation profiles for primary sludge extracts. Site 2 displays more rapid degradation in the first two days, compared to Site 1, indicating more readily biodegradable material (as a percentage of total DOC) in Site 2 compared to Site 1 primary sludge. Site 2 represents the smallest of the treatment plants, serving a small village and selection of commercial premises, compared to Site 1, which was the largest of the treatment plants with a much more diverse catchment including municipal, commercial, industrial, and hospital inputs, which could be sources of POPs. The profile of both samples collected from Site 1 over a gap of one month show a similar pattern of biodegradation.

Fig. 3 presents the biodegradation profiles for secondary sludges from all sites. For secondary sludges rapid degradation is also indicated in the first two days and slows by day 7. Secondary sludge from Site 3, a nitrifying plant, exhibited a lower initial decrease in DOC in the first two days, and a lower overall rate of biodegradation after 28 days, followed

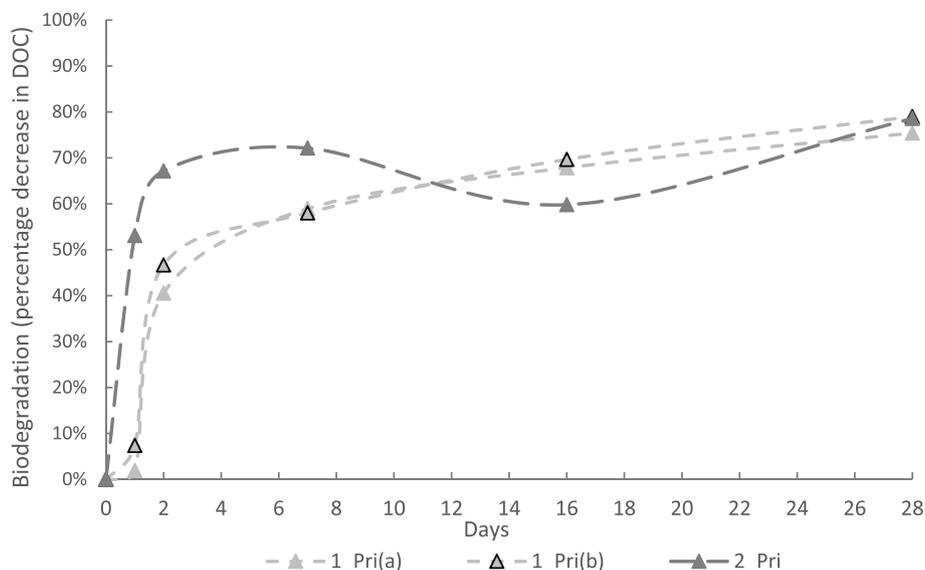


Fig. 2. Biodegradation profiles (primary sludge, mean reduction in DOC %). Pri – primary sludge; a = 1st sample; b = 2nd sample collected one month later.

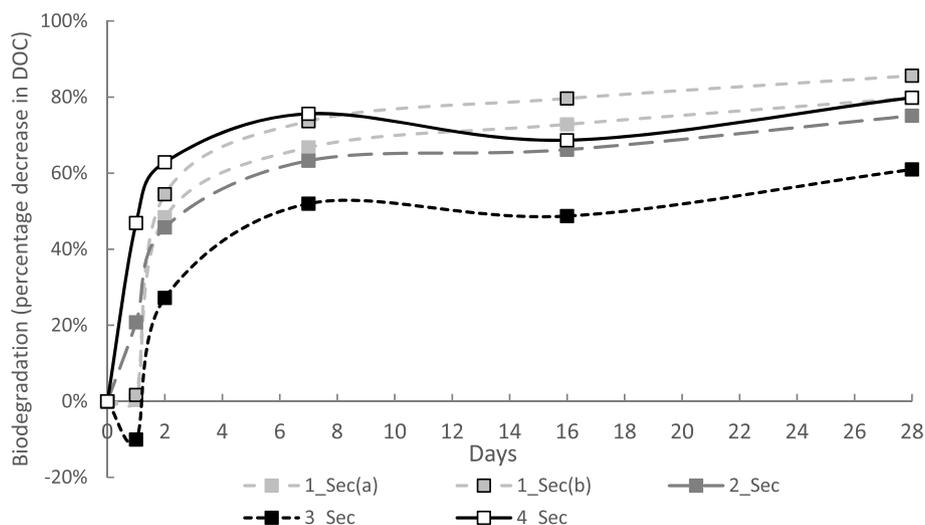


Fig. 3. Biodegradation profiles (secondary sludge, mean reduction in DOC %). Sec = secondary sludge; a = 1st sample; b = 2nd sample collected one month later.

by secondary sludges from another nitrifying plant (Site 2) and the partial-nitrifying plant (Site 4). This is an indication that a greater proportion of biodegradable organic compounds may have already been broken down within the plants receiving lower organic pollution or operated with longer aeration times. In contrast, secondary sludges from the non-nitrifying plant (Site 1) demonstrate a higher initial decrease in DOC in the first two days, and a higher overall level of biodegradation after 28 days. A consistent biodegradation profile was observed for secondary sludges from Site 1 collected one month apart.

Fig. 4 presents the biodegradation profiles for biosolids from Sites 1 and 3 and the reference sludge. For the biosolids, all samples again show a rapid rate of biodegradation in the first two days, which then slows, with a more gradual reduction in DOC to Day 28. The two biosolid samples from Site 1 collected with one year apart show a similar pattern and overall degradation on Day 28. The biosolid from Site 3 is sludge cake that had undergone thickening with a polymer and dewatering. In contrast the biosolids from Site 1 had undergone same thickening and dewatering as those from Site 3 but was additionally treated by anaerobic digestion and drying. The reference sludge was treated by thermal treatment. The profiles for the treated biosolids are more gradual compared to that observed for either the primary or secondary sludges.

Although the pattern of biodegradation in the first two days is similar to that observed in Figs. 2 and 3, the relative reduction in DOC is somewhat lower indicating there may be a smaller proportion of readily degradable DOC available. The rate of degradation in the days that follow may be indicative of a relatively greater proportion of poorly degradable DOC remaining, potentially due to the types of treatment that they had been subjected to (e.g., anaerobic digestion and drying), which already consumed readily degradable material. In contrast to the pattern observed in Figs. 2 and 3, there appears to be a slight increase in degradation from Day 16–28. This may indicate that the microbial consortia are adapting to degrade resistant compounds in the absence of easily biodegradable substrates (Urase and Kikuta, 2005). Over time the biotransformation of some compounds may also release more readily biodegradable sources, which may explain the non-linear degradation pattern observed (Blair et al., 2015).

3.3. Persistent DOC (PDOC) after 28 days

For all samples, the total quantity of DOC was reduced significantly after the 28-day aerobic biodegradation test with mean PDOC and percentage of the initial DOC remaining after 28 days illustrated in Fig. 5.

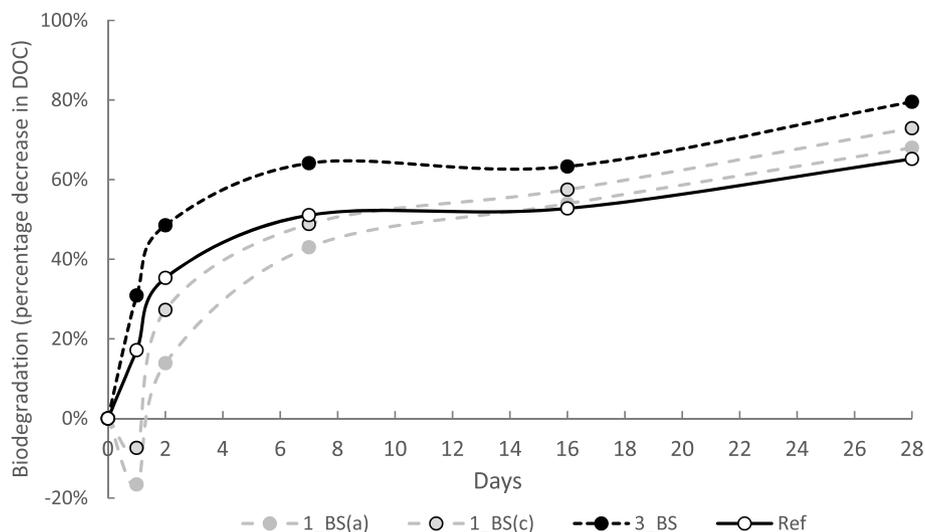


Fig. 4. Biodegradation profiles (treated biosolids, mean reduction in DOC %). BS = treated biosolid; Ref = Reference sludge, CRM 055; a = 1st sample; c = 2nd sampled collected one month later.

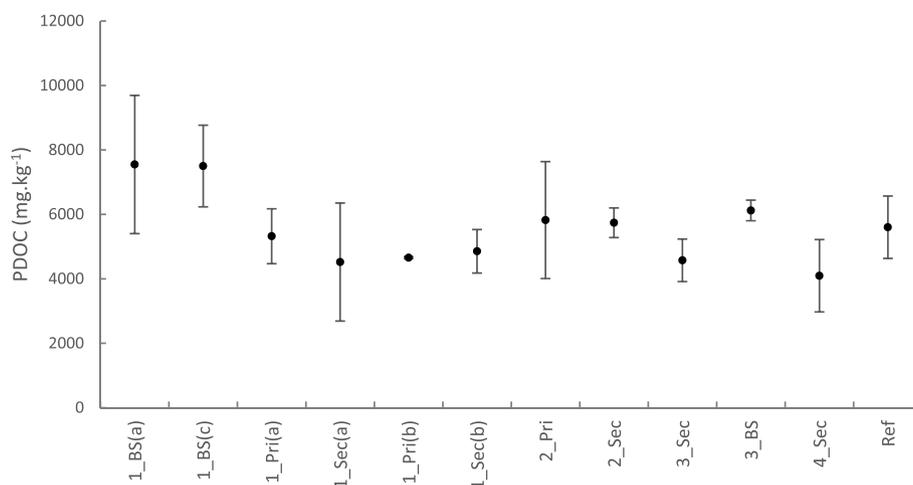


Fig. 5. Mean PDOC for all sites (mg.kg^{-1}) after 28-day aerobic biodegradation test (error bars representing $2 \times \text{SD}$). Pri – primary sludge; Sec = secondary sludge; BS = treated biosolid; Ref = Reference sludge, CRM 055. a = 1st sample; b = 2nd sample collected one month later; c = Sample collected one year earlier.

Between 61 and 86% of the desorbed organic carbon in the samples tested was biodegraded after 28 days. Final PDOC concentrations ranged from 4096 mg.kg^{-1} to 7547 mg.kg^{-1} across all sludge and biosolid samples. The biosolids from Site 1 had the highest concentration of PDOC remaining, with similar results obtained for biosolids collected in both sampling events for this site. The original material was treated by anaerobic digestion and thermal treatment. Biosolids from Site 3 had the second highest PDOC concentrations. This biosolid had not undergone additional treatment (e.g., AD or thermal treatment). For secondary sludges, the final Day 28 PDOC concentrations at Sites 1 and 3 were similar (mean DOC of 4520 and 4854 mg.kg^{-1} for Site 1 for the two samples collected within a gap of one month compared to 4574 mg.kg^{-1} for Site 3). This suggests that although overall biodegradation levels are different, the absolute quantity of persistent material remaining is similar. Although the endpoint of the test was set at 28 days, it is possible that microbial consortia present may continue to degrade resistant DOC over time.

A statistical comparison of mean PDOC identified significant differences across the category of process stage (primary, secondary and biosolids; $p < 0.001$ for ANOVA and $p = 0.001$ non-parametric Kruskal-Wallis test). The multiple comparison tests indicate that there is no significant difference between total PDOC levels in primary and

secondary sludge samples, but there is a difference between primary sludge and biosolids ($p = 0.009$) and secondary sludge and biosolids ($p < 0.001$). A comparison of results for secondary sludges shows significant differences based on degree of nitrification ($p < 0.05$ for ANOVA and $p = 0.032$ for non-parametric Kruskal-Wallis test). The multiple comparison tests also show that there is a significant difference between nitrifying and both non-nitrifying ($p = 0.01$) and part-nitrifying ($p = 0.001$) sites but no difference between non-nitrifying and part-nitrifying sites ($p = 0.31$).

4. Discussion

4.1. DOC

The fate of organic pollutants in treatment works includes sorption to particulate matter, sedimentation, volatilisation and biotransformation in the primary treatment stage, and air stripping volatiles and biodegradation in the activated sludge stage (Byrns, 2001). This assumes that organic pollutants in the primary treatment stage are largely available for sorption to solids, and in the secondary treatment stage, are largely available for biodegradation. However, the potential for sorption to dissolved/colloidal matter and subsequent organic pollutant transport

in the aqueous phase suggest that contaminant partitioning between various wastewater treatment stages may not be so straightforward (Barret et al., 2010). Among the sludges and biosolids tested in this study, the level of leachable DOC was of a similar magnitude, of between 20,000 and 30,000 mg kg⁻¹. For fresh sludges collected from primary settlement tanks, there was not a statistically significant difference in DOC, although only two treatment plants were tested in this study.

Differences were observed between secondary sludges from nitrifying vs non-nitrifying plants indicating that treatment process parameters influence leachable DOC. The nitrifying plants generally have longer hydraulic aeration (or retention) times and/or receive wastewaters containing lower levels of readily biodegradable organic pollutants, compared to the non-nitrifying plants. The process conditions in nitrifying plants are likely to result in higher breakdown of biodegradable organic carbon than in non or partially nitrifying plants (Xu et al., 2016). Aerobic processes have been found to improve removal of some trace organic compounds in wastewater treatment processes and enhanced retention time may facilitate this biological breakdown (Semblante et al., 2015).

The study found that the DOC from biosolids that had undergone treatment varied by treatment type. The biosolids that had undergone minimal processing (e.g., chemical thickening and dewatering only) from Sites 3 and 4 had higher levels of leachable DOC than those from Site 1, which had undergone additional treatment in the form of anaerobic digestion and drying, and from the reference sludge (CRM 055), which had undergone thermal treatment. The biosolids overall had a higher mean DOC than the sludges, except for the reference sludge, which had the lowest DOC overall. This suggests that thermal treatment may bring about a loss of leachable DOC, however the specific details of the source material for this CRM and treatment parameters (e.g., temperature) were not available. Comparing the mean DOC for the reference sludge of 14422 mg kg⁻¹ with the certified total organic carbon content of the material (29.9 wt%), the quantity of DOC desorbed represents 4.8% of the total carbon present in the sample. In comparison, Wijesekara et al. (2017) estimated DOC from biosolids (MWWTP sludges after aerobic treatment and anaerobic digestion) using an alternative method (shaking of samples with water for 30 min at 30 rpm, followed by 30 min centrifuging), were in the range of 2815 mg kg⁻¹ to 5635 mg kg⁻¹ for samples with TOC (Wt%) of 45.3% and 35.2% respectively (Wijesekara et al., 2017). These results are between 2 and 12-fold lower than the range of DOC measured in this study (11760 mg kg⁻¹ to 33853 mg kg⁻¹ corresponding to leachable DOC of 0.6% and 1.6% of TOC (Wt%) respectively). The significantly reduced shaking time used by these authors (30 min vs 18 h in this study) may have contributed to the reported differences (Wijesekara et al., 2017). Ashworth and Alloway (2004) used a method similar to the SPLP method used in this study (1:5 ratio of sludge to water, shaken for 24 h) with the primary differences being the shaking speed (14 rpm vs 32 rpm) and the extraction fluid (neutral water vs pH adjusted) (Ashworth and Alloway, 2004). The study estimated the quantity of DOC leached from anaerobically digested sludges, with a 27 wt% carbon content to be about 4395 mg kg⁻¹, or approximately 1.6% of TOC (Ashworth and Alloway, 2004). This result is also lower than those obtained in this study, possibly due to the reduced shaking speed the authors had adopted in their studies.

Understanding the leaching characteristics of DOC from biosolids can provide some information on the potential for pollutant transfer more generally. Organic matter content has been found to be an important feature of soils and biosolids in determining leaching potential of pollutants including PPCPs and metals, for which sorption to organic matter may influence mobility (Alloway and Jackson, 1991; Edwards et al., 2009; Gibson et al., 2010). For example, Dizer et al. (2002) found that applying biosolids to land may cause both soil contamination and transport of endocrine disrupting compounds to surface and/or groundwater as contaminants move from biosolids into the environment (Dizer et al., 2002).

4.2. PDOC

The biodegradation study aimed to quantify the leachable organic carbon that was readily biodegradable, and the quantity that remained recalcitrant after 28 days. The study indicated that rapid biodegradation occurs within the first two days of incubation and slowed significantly from about 7 days onwards, indicating most biodegradable carbon is lost rapidly, leaving more recalcitrant substances behind. This suggests that 7 days may be a good predictor of the 28-day test and could potentially reduce the time required to assess relative levels of PDOC in a MWWTP residue. The study found that the majority (61–86%) of DOC leached from biosolids was biodegraded after 28 days, however the proportion varied between sludges and biosolids. In this study, no significant difference between the total PDOC concentration in primary and secondary sludge samples was observed. In contrast, a significantly higher PDOC fraction was found in the biosolids. This may be due to additional treatments that biosolids have been exposed to, such as anaerobic digestion and high temperature, which may have had a greater impact in the reduction of those organic compounds that are more amenable to biodegradation, leaving more recalcitrant substance behind. While treatment of WWTP sludges by AD may be perceived to be effective for reducing organic pollutant burden, the effect may vary by compound type and pre-treatment processes, which can influence the sorption of POPs to sludge particles. AD has been found to provide very-high removal efficiency (e.g., >80%) for some pharmaceuticals including sulfamethoxazole and 17 α -estradiol, and high removal (e.g., >60%) for galaxolide, tonalide and diclofenac (Carballa et al., 2007), and while moderate degradation of low molecular weight PAHs may be achieved, high molecular weight PAHs are less readily degraded (Barret et al., 2012). On the other hand, AD processes have been found to increase concentrations of some compounds such as estrogens, due to destruction of solids, and desorption of compounds (Marti and Batista, 2014). Overall, there is generally poor reduction in the most persistent compounds in typical mesophilic or thermophilic processes (Semblante et al., 2015).

The biosolid from Site 3 (sludge cake) had one of the highest percentages of biodegradable carbon, in comparison to the biosolids from the other site, however it had not undergone additional treatment and had a higher starting concentration of leachable DOC in comparison. The remaining PDOC was higher in this sludge cake than for secondary sludge collected from this site indicating a higher proportion of recalcitrant carbon. Torri et al. (2003) estimated that 29–45% of the carbon in biosolids spread to land was recalcitrant, with the recalcitrant fraction including fatty-acids, n-alkanes, steroids, POPs, stable sterols and potentially a wide array of unknown persistent organic compounds (Torri et al., 2003). The results from this study, while only considering the dissolved and leachable portion, show some agreement with this estimate. The dissolved and leachable portion, however, may be the most concerning for understanding environmental pollution risks, given that this fraction represents the mobile pollutants that may distribute further into the environment. The persistent fraction is also of interest given the potential to accumulate over time. Evidence of non-biodegradable organic compounds remaining after sludge application has been reported in the literature. Eljarrat et al. (2008) found that PBDEs in biosolid amended soils were present at concentrations of 21–690 ng g⁻¹ dw with an indication that the compounds may be accumulating over time (Eljarrat et al., 2008). These compounds may also be metabolised in the environment, potentially de-brominating into more bioavailable congeners. Elsewhere, some POPs (PCDD and PCDF) have been found to persist beyond 260 days of monitoring (Wilson et al., 1997) and compounds such as carbamazepine have been found to be resistant to degradation and show the potential to accumulate over time (Gibson et al., 2010). Further study is needed to understand the potential accumulation and/or mobility of these compounds following land application.

Although the endpoint for the biodegradation test was fixed at 28

days, it is possible that degradation of some substances could continue to occur. Observation of the biodegradation profile for primary and secondary sludges provided limited indication of an increasing rate of biodegradation after 7 days. The slope of the profile for biosolids after 7 days was steeper however, which may be an indication of adaptation of the microbial consortium resulting in further biodegradation or biotransformation processes. The literature provides evidence that persistent organics can degrade over a longer timeframe. For example, Wilson et al. (1997) found that for biosolids applied to soils, some compounds such as PCBs, chlorophenols and volatile organic compounds took 128 days to reach background levels after 128 days; however, some compounds persisted (PCDD and PCDF) beyond 260 days of monitoring (Wilson et al., 1997).

4.3. Limitations of this study

This study faced some limitations. Notably, results from the leaching tests may have been influenced by the particle size of sample sludges and biosolids. While the upper limit of particle size for samples were within the specification of the method used (<9.5 mm), there was no lower limit applied, so some variation existed in the range of particle sizes used across samples. The use of sample replicates for each sample accounted for some of the variability. The only sample that was in powder form, as provided by the supplier, was the CRM. As observed this sample had the lowest SD for DOC, indicating that greater homogeneity likely reduced variability in DOC results. This form of biosolid however is unlikely to be representative of how sludges and biosolids are applied to land in practice, with the sludge pellets and dried sludge, as used in the test, more representative of real-world sample sizes. This study was also limited to a small number of available functional wastewater treatment plants for sample collection. Further work could be performed with a wider range of plant types with different operating conditions and catchment profiles. Another limitation of this study is the lack of reporting on chemical analysis of the leachates to measure specific organic pollutant concentrations. A subsequent study using the leachates from this study applied further analytical techniques (UV-Vis and FTIR) to provide broad general indicators of the types of compounds or chemical structures present in the leachate mixture and will be reported in a subsequent manuscript.

5. Conclusions

This study has provided a methodology for the quantification of leachable DOC from MWWTP sludges and biosolids. This study contributes towards a greater understanding of the nature and occurrence of leachable organic compounds and provides a simple tool for assessing their fate, informing environmental risk assessment. The approach presented here allows for a non-specific method for characterising the mobile and persistent organic pollutant burden of sludges and biosolids applied to land that overcomes some of the analytical and resource barriers that currently exist.

The study also provides valuable information on the effect of wastewater treatment unit operations and processes on the occurrence and nature of leachable DOC. Key findings suggest that:

- nitrification in secondary treatment processes can decrease biodegradable DOC in secondary sludges.
- anaerobic digestion and thermal treatment can reduce DOC, with the latter likely to provide greater reduction than the former.
- biosolids that have undergone anaerobic digestion and thermal treatment are more likely to contain greater levels of PDOC than untreated sludges. This suggests that the current and widely applied treatment methods for biosolids prior to application to land are not appropriate for reducing the environmental risks from POPs.
- most biosolids still contain an appreciable concentration of persistent DOC, suggesting that additional treatment such as nitrification,

AD, and thermal treatment may only reduce biodegradable DOC significantly and result in substrates with more concentrated levels of PDOC.

The use of the PDOC measurement could be important as wastewater treatment plants face more stringent environmental regulation of organic pollutants in effluents. Treatment processes that improve effluent quality could potentially adversely influence sludge quality. The general findings of the research collectively provide evidence that sludges and biosolids could present a risk of environmental transfer of POPs and leaching tests can be effectively applied to quantify DOC while biodegradation tests can be applied to quantify PDOC. The combined approach provides an alternative bulk quantitative assessment that could be used to estimate pollutant burden or assess the effectiveness of treatment processes. This applies contaminated land assessment approaches in reverse by assessing the level of possible contamination before a material is applied to land rather than assessing the land contamination in-situ. Further studies of this nature could be performed with additional characterisation of leachates, including toxicity testing, to identify if correlations exist between observed POP concentrations or adverse effects and PDOC concentrations.

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Author contributions

Juliette O'Keeffe performed writing – original draft, conceptualization, methodology, formal analysis, investigation. Joseph Akunna performed writing – review and editing; conceptualization, supervision.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.jenvman.2022.114565>.

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