Environmental behaviour of pharmaceuticals, personal care products and endocrine disrupting compounds following land application of biosolids

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ABSTRACT

The reuse of biosolids through application onto agricultural land has been shown to provide plants with additional nutrients and organic carbon and improve moisture retention in soils. This practice can however be a route of entry into the environment for numerous contaminants that may be contained within the biosolids. The work presented in this thesis aims to gain a better understanding of the environmental behaviour of pharmaceuticals and personal care products (PPCPs) and endocrine disrupting compounds (EDCs) following the addition of biosolids to land. This work involved initially conducting an aquatic hazard assessment for PPCPs and EDCs following biosolids addition to land. Following this, seven compounds were selected, 4-nonylphenol (4NP), 4-t-octylphenol (4tOP), bisphenol A (BPA), triclosan (TCS), 17β-estradiol (E2), estrone (E1), estriol (E3) and 17α-ethinylestradiol (EE2), for an Australian biosolids survey. Four of these compounds were then chosen (i.e. 4NP, 4tOP, BPA and TCS) for a series of experiments assessing their dissipation (i.e. decreases in concentration) following the addition of biosolids to soil in the laboratory and in the field, as well as the suitability of using spiking experiments (i.e. spiking elevated concentrations of compounds into a soil and biosolids sample) to predict the persistence of these compounds following biosolids addition. Finally, the yeast estrogen screen (YES) bioassay was conducted on several soil sample extracts from the field trial to determine if estrogenic activity could be measured in soils following biosolids addition.

The results from the hazard assessment showed that the majority of PPCPs and EDCs that have been detected in biosolids pose low hazard to adjacent aquatic ecosystems. However, there were ten compounds that posed a high hazard and therefore warrant further investigation. These compounds were the fragrance compounds, tonalide and galaxolide, the estrogen compounds, 17β-estradiol and 17α-ethinylestradiol, the antibiotic compounds

ciprofloxacin, doxycycline and norfloxacin and the antimicrobial agents triclosan and triclocarban. The survey of Australian biosolids detected concentrations of 0.35 to 513 mg/kg for 4NP, 0.05 to 3.08 mg/kg for 4tOP, < 0.01 to 11.2 mg/kg for TCS, < 0.01 to 1.47 mg/kg for BPA and < 0.05 to 0.37 mg/kg for E1. The remaining compounds, E2, E3 and EE2, were below the limit of detection (i.e. 45 µg/kg) in all samples. These concentrations were similar to those that have been measured internationally.

The dissipation of the compounds 4NP, 4tOP, BPA and TCS was assessed over 32 weeks in the laboratory, following the addition of biosolids to a soil. The dissipation of 4NP, BPA and TCS followed a biphasic pattern which consisted of a dissipating fraction and a recalcitrant fraction. When the dissipation rates of the same four compounds were assessed under field conditions, 4NP and 4tOP dissipated 10- to 20-times slower in the field and BPA dissipated 2.5-times slower compared to the laboratory-based dissipation rates. The compound TCS, however, showed no dissipation in the field, however, in the laboratory-based study approximately 30% to 50% dissipation was observed. These results showed that there was the potential for PPCPs and EDCs to accumulate in agricultural soils and that laboratory studies overestimated dissipation rates.

The suitability of using spiking experiments to predict the dissipation of compounds following the addition of biosolids to a soil was assessed. This was tested using two methods: (i) spiking isotopically labelled surrogate compounds (i.e. BPA-d_{16} and TCS-$^{13}C_{12}$) into a biosolids amended soil and, (ii) spiking elevated levels of the same compound (i.e. non-labelled 4NP and 4tOP) into a biosolids amended soil and comparing the dissipation rates and patterns with those of the same compounds indigenous to the biosolids. Overall, it was determined that degradation experiments that involved spiking, yielded both faster rates of dissipation (up to 5-times faster) and, particularly in the case of

BPA, variations in the pattern of dissipation, in terms of the presence of a recalcitrant fraction. It was concluded that spiking experiments were not suitable to predict the dissipation of compounds following land application of biosolids.

Finally, estrogenic activity was measured in extracts of agricultural soil that had received biosolids, for at least the initial four months of the field trial. Overall this activity was low, however, it was still present at a level that may pose a high hazard to aquatic ecosystems (based on the results of the hazard assessment conducted earlier as part of this project).

The results presented in this thesis indicate that there is a need for further research with regards to the risks associated with PPCPs and EDCs in biosolids, relating to both their mobility and persistence. The results presented show that the biosolids matrix and the specific field conditions of application should be taken into consideration when determining the environmental behaviour of these compounds. It is also likely that the overall conclusions of the current project will apply to other groups of organic compounds present in biosolids. The data provided in this thesis will assist in future hazard and risk assessments and management of organic contaminants in biosolids applied to agricultural soils.

STATEMENT OF DECALRATION

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THESIS STRUCTURE

The experimental chapters in this thesis are all written as journal articles. Since journal articles must be self-contained there is some degree of repetition in this thesis

Chapter 1 discusses the potential environmental risks associated with pharmaceuticals and personal care products (PPCPs) and endocrine disrupting compounds (EDCs) following the land application of biosolids. It highlights several important factors that need to be considered in order to assess these risks, including the influence of waste water treatment plant catchment characteristics and waste treatment processes in the final concentrations, the expected dissipation of the compounds in the environment (from both degradation and mobility) and the environmental toxicity.

Chapter 2 outlines the hazard posed to aquatic ecosystems from PPCPs and EDCs following the addition of biosolids to land, by predicting runoff water concentrations and comparing these to aquatic toxicity data.

Chapter 3 presents the data from an Australian biosolids survey, which was conducted to obtain data on the concentration in biosolids of several selected PPCP and EDCs (4-nonylphenol, 4-t-octylphenol, bisphenol A, triclosan, 17β-estradiol, estrone, estriol and 17α-ethinylestradiol) in representative Australian biosolids.

Chapters 4 and 5 examine the dissipation of 4-nonylphenol, 4-t-octylphenol, bisphenol A and triclosan following the addition of biosolids to a soil, under laboratory and field conditions, respectively.

Chapters 6 and 7 examine the standard method of "spiking" contaminants into soil and biosolids for its suitability in predicting the persistence of compounds when they are added to a soil by biosolids addition.

Chapter 8 determines the presence of estrogenic activity in soil extracts from a soil where biosolids have been applied and aged in the field.

Chapter 9 summarises and discusses the findings from this thesis and makes several recommendations for future research arising from the experimental work presented.

Chapter 1

Review of literature

CONTENTS

1.1. INTRODUCTION

As the world‟s population expands and cities become denser, the appropriate treatment and disposal of waste products is becoming a matter of increasing concern. This thesis considers the possible environmental implications following the agricultural land application of some of these waste products.

Wastewater refers to a combination of liquid and solid waste that passes into a wastewater treatment plant (WWTP) via the sewage system and generally contains both domestic and industrial waste. Wastewater that has passed through a WWTP produces two waste streams: (i) an aqueous waste stream, and (ii) a solid waste stream. The aqueous end-product of wastewater treatment is generally released into rivers, lakes and oceans. The solid waste stream, following treatment to reduce water and pathogens, is referred to as „biosolids‟, which will be the primary focus of this thesis.

In the past, biosolids were primarily disposed of through incineration, landfill and/or dumping at sea. These methods are becoming less favourable, however, as they tend to be reasonably expensive and are no longer considered to be environmentally sustainable. In more recent years, one of the main methods of disposal has been through the application of biosolids to agricultural soils to aid in the growth and the yield of plants and crops. Land application of biosolids is currently considered by researchers and governments in many countries, including Australia, to be the most sustainable means of disposal and provides the following possible benefits:

i. increased soil fertility by way of increasing available nutrients, therefore reducing the need for fertilisers (Sommers, 1977; Schowanek et al., 2004);

ii. improved soil water holding capacity, porosity and aggregate stability due to the addition of organic matter to soils (Albiach et al., 2001; Schowanek et al., 2004); and,

iii. increased plant growth and productivity (Kelling et al., 1977; Fresquez et al., 1990; Al-Mustafa et al., 1995; Cooper, 2005)

Despite the numerous benefits of applying biosolids to agricultural land, such reuse of waste products does need to be monitored carefully due to the potentially high levels of contaminants that may be present in the biosolids. As a result of the wide range of input sources into WWTPs, there can be a relatively diverse mixture of contaminants that may be present in biosolids. Since land application of biosolids is a route of entry into the environment for potentially damaging contaminants, the risks associated with the practice need to be considered. An understanding of these potential risks will ensure the necessary management of this practice, and in turn minimise adverse environmental effects.

In Australia, federal and state-based environmental and primary industry agencies have developed guidelines to control the land application of biosolids, including the Natural Resource Management Ministers Council (NRMMC, 2004); the Environmental Protection Agency of New South Wales, Australia (EPA NSW, 1997); the South Australian Environmental Protection Agency (SA EPA, 1997); the Department of Primary Industry, Water and Environment (DPIWE, 1999); the Western Australia Department of Environmental Protection (WA DEP, 2002); and the Environmental Protection Agency of Victoria (EPA Victoria, 2004). These guidelines provide concentration limits for a selection of chemicals in biosolids that must not be exceeded if they are to be applied to various land uses, including agriculture. In all jurisdictions, maximum limit values are provided for a range of metals, including, arsenic, cadmium, chromium, copper, lead,

mercury, nickel, selenium and zinc, due to their potentially high concentrations in biosolids and their possible adverse environmental effects.

Some biosolids guidelines (e.g. EPA Victoria, 2004) also provide limit values for some groups of organic contaminants, including dichlorodiphenyltrichloroethane (DDT) and its derivatives, polychlorinated biphenyls (PCBs), and organochlorine pesticides. The potential risk from many other groups of organic contaminants are not as well understood, including the groups referred to as pharmaceuticals and personal care products (PPCPs) and endocrine disrupting compounds (EDCs) which have received increasing interest recently.

The terms PPCPs and EDCs are used to refer to a very broad range of organic compounds. Pharmaceuticals constitute a large group of biologically active chemicals that have been developed to cure disease, fight infection and/or reduce symptoms of human illness. Within the category of pharmaceuticals there are two main divisions: (i) antibiotics, which have a biological effect designed to fight infections; and (ii) medications (both prescription and non-prescription), which are used mainly to reduce symptoms (Diaz-Cruz et al., 2003). The main input sources for pharmaceuticals into WWTPs are via: (i) human excretion (of parent compounds and metabolites); (ii) disposal of unused products down toilets or drains; and (iii) trade and industrial waste from pharmaceutical production (Daughton & Ternes, 1999).

Personal care products include a diverse range of organic compounds that are the chemical constituents of materials used for personal care, including, soaps, shampoos, cosmetics and sun screens. A considerable portion of research into this particular category of organic compounds to date is centred on antimicrobial agents, such as triclosan and triclocarban

(e.g. Ying et al., 2007; Wu et al., 2009a) and fragrance compounds, such as polycyclic and nitro musks (e.g. Difrancesco et al., 2004; Yang & Metcalfe 2006).

The term EDC is used to refer to a number of chemicals that are present in the environment and have been shown to mimic or antagonise the actions of steroid hormones in organisms (Jobling et al., 1998). The work presented in this thesis will deal solely with those compounds that are known to interfere with estrogen receptors. These estrogenic EDCs can be divided into two main categories: (i) estrogen compounds, which occur both naturally (e.g. 17β-estradiol and its metabolites estrone and estriol), and synthetically in the female contraceptive pill (e.g. 17α-ethinylestradiol); and (ii) xenoestrogens which are non-estrogen compounds that are able to interfere with estrogen receptors, including, for example: nonylphenol and nonylphenol ethoxylates; octylphenol and octylphenol ethoxylates; and bisphenol A. Many persistent organic pollutants are also considered to be estrogenic EDCs, e.g. PCBs and phthalates, however these will not be addressed in this thesis.

1.2. ASSESSING RISKS OF PPCPs AND EDCs IN BIOSOLIDS

In order to assess the potential risks that may be posed to the environment from PPCPs and EDCs following the land application of biosolids, several factors need to be considered. These include: (i) the concentrations of PPCPs and EDCs in the biosolids; (ii) the dissipation of these compounds through degradation or mobilisation; and (iii) their environmental toxicity (both terrestrial and aquatic). The following sections provide a summary of the literature related to each of these three factors.

1.2.1 Concentration of PPCPs and EDCs in biosolids

The concentrations of PPCPs and EDCs that are likely to be present in biosolids is difficult to predict and can be influenced by a number of factors, such as the particular catchment of a WWTP and the treatment processes used within a WWTP (Schowanek et al., 2004). The following section will provide a brief summary of the general effects of these above factors, while Chapter 2 of this thesis will provide a list of specific PPCPs and EDCs and the associated range of concentrations that have been detected in biosolids.

1.2.1.1. The effects of catchment location of a WWTP

The location of a WWTP and the characteristics of its catchment can potentially lead to differences in the types and concentrations of PPCPs and EDCs that may be present in biosolids. WWTPs that mainly serve industrial areas are likely to contain higher levels of chemicals that are used for industrial purposes. These chemicals include, for example, the xenoestrogen surfactant metabolite compounds 4-nonylphenol and 4-t-octylphenol and bisphenol A, which is used in the production of plastics. In contrast, a WWTP that serves mainly a domestic catchment area is more likely to contain higher levels of human pharmaceuticals, compounds found in personal care products and human hormones (Campbell-Board, 2005).

A specific example of the differences resulting from variations in catchment location is a study conducted in New Zealand investigating the presence of organic contaminants in biosolids from a range of influent sources (Campbell-Board, 2005). Half of the WWTPs sampled served predominantly a domestic area while the other half served mainly an industrial area. The average concentration of 4-nonylphenol in biosolids that were sourced from a WWTP with predominantly industrial inputs was 600 mg/kg (range 27 to 1800 mg/kg), whereas, in the biosolids that were sourced from a WWTP with predominantly

domestic inputs, the concentrations were considerably lower at 76 mg/kg on average (ranging from 10 to 180 mg/kg).

1.2.1.2. The effects of different wastewater treatment processes

Wastewater treatment usually comprises a variety of processes, and the effectiveness of these will influence the concentrations of PPCPs and EDCs that are found in the resultant biosolids. In general, the two main treatment processes for producing biosolids from sewage sludge are aerobic and anaerobic digestion. Both aerobic and anaerobic digestion use bacteria to break down the organic matter, however, they differ both in the amount of oxygen that is present and the types of bacteria that are present and metabolically active. These different treatment processes have their advantages and their disadvantages and can ultimately lead to differences in the contaminant loads in the final biosolids product. Aerobic treatment has been shown to result in faster degradation rates for many groups of organic compounds. On the other hand, anaerobic treatment can be more cost and space effective, but may result in less complete degradation of contaminants when compared to aerobic treatment (e.g. Chang et al., 2005a; Chang et al., 2005b).

1.2.2. Dissipation of PPCPs and EDCs following land application of biosolids

Another important factor to consider with regard to potential risks that may be posed to the environment from PPCPs and EDCs in biosolids involves the dissipation of compounds contained within the biosolids following the application of biosolids to land. This dissipation generally occurs via two main routes, either degradation of the compounds in the soil or mobilisation and transport of the compounds, normally associated with water. Each of these is discussed below.

1.2.2.1 Degradation of PPCPs and EDCs in soils

One of the main functions of biosolids guidelines is to ensure that contaminants do not accumulate in soils at concentrations that would cause adverse environmental or human health effects from the application or re-application of biosolids. The likelihood and the extent of a chemical persisting in soil is dependent on the particular chemical as well as the environmental conditions, including, for example, the temperature, rainfall and soil properties. Most of the research conducted in this area tends to be compound specific and research findings can also be influenced strongly by the variations in experimental conditions that are used. Due to this, results are often conflicting and broad conclusions are difficult to make. In addition, very few studies have measured the degradation of PPCPs and EDCs that are indigenous to biosolids and instead, in many cases, have involved the compounds being spiked into a soil or soil and biosolids sample. It is currently unknown if the practice of spiking yields similar results to those of compounds that are contained within biosolids at the time of land application.

In general, pharmaceutical compounds have been shown to exhibit a high level of persistence in soils. For example, a study that examined the presence of pharmaceuticals (e.g. erythromycin, carbamazepine, fluoxetine and diphenhydramine) in a soil for five months following irrigation with effluent water revealed concentrations ranging from 0.02 to 15 μg/kg (Kinney et al., 2006). Persistence of antibiotic compounds in soils has also been observed; for example, tetracyclines and sulphonamides have been detected in soils up to seven months after the application of animal manures (Hamscher et al., 2002; Christian et al., 2003). The extended persistence of pharmaceuticals in the environment is possibly to be expected as they are compounds that are specifically designed to be stable in human bodies in order to elicit their desired effect (Loffler et al., 2005).

There are however, some studies on specific pharmaceutical compounds that have found low levels of persistence. One such compound is the anti-inflammatory pharmaceutical naproxen, which tends to be rapidly mineralised in soils (Topp et al. 2008a) with degradation half lives ranging from 3 to 7 days (Monteiro & Boxall, 2009). A „half life" refers to the time required for the concentration of a compound to decrease by 50%. In general, estrogen compounds (such as 17β-estradiol and 17α-ethinylestradiol) have also been shown to degrade rapidly in soils, with reported half lives of less than 7 days (e.g. Colucci et al., 2001; Colucci & Topp, 2001; Ying & Kookana, 2005)

In terms of the antimicrobial agents, comparatively more research has been conducted on triclosan than on triclocarban; however, both compounds have been shown to degrade only under aerobic soil conditions (McAvoy et al., 2002; Ying et al., 2007). Reported half life values for triclosan in spiked aerobic soils, under laboratory conditions, have been reported to range from 13 to 58 days (Ying et al., 2007; Wu et al., 2009a; Xu et al., 2009). Triclocarban has a greater persistence in soils, with a reported half life value of 108 days (Ying et al., 2007).

The degradation of many fragrance compounds in spiked soils has been assessed with variable results. For example, Difrancesco et al. (2004) found that several nitro and polycyclic musk compounds (e.g. tonalide, galaxolide, musk ketone, musk xylene, acetyl cedrene and cashmeran) persisted in soils for at least three months. Moreover, two of these compounds (i.e. tonalide and musk ketone) remained in the soil for up to 12 months. Other studies have found quite different results, however. For example, a study, which examined the concentrations of polycyclic musk compounds, galaxolide and tonalide, in a soil following the addition of biosolids found that these compounds degraded rapidly over a six week period (Yang & Metcalfe, 2006).

Research into the degradation of organic compounds (including PPCPs and EDCs) in biosolids or biosolids-amended soils has also shown that, in some cases, degradation is incomplete. A glasshouse trial measuring the degradation of 4-nonylphenol, which was conducted by Brown et al. (2009), reported half life values of 16 to 23 days; however, after 45 days, 15 to 30% of the initial compound was still present in the soil. A degradation study conducted on the same compound over a longer period of time (i.e. 105 days) found that, although there was an initial rapid degradation of the compound, there was a non-degrading or recalcitrant fraction of 26 to 35% which remained (Sjostrom et al., 2008). Wu et al. (2009b) also found a similar non-degrading fraction following the addition of six antibiotic compounds to a digested biosolids sample. Although it is unclear what mechanism is responsible for this pattern of degradation, this finding does raise concerns with relation to the potential accumulation of compounds in soils following repeat applications of biosolids.

1.2.2.2. Mobilisation of PPCPs and EDCs in soils

The second main route of dissipation of PPCPs and EDCs is mobilisation or transport of the compounds involving water. In more detail, following the addition of biosolids to land, there is the potential for contaminants within the biosolids to be mobilised with water and to migrate into the adjacent waterways via surface runoff or leaching. This offsite migration of organic compounds from land that has been applied with biosolids can occur via two main routes: (i) movement in the dissolved phase following desorption (i.e. when the compound has been released from the soil); and (ii) movement of the particle or colloid-bound compounds through soil erosion. Physicochemical properties of individual compounds and environmental factors will each influence the degree of mobilisation of a compound in soil following biosolids application.

Physicochemical properties of individual compounds have been used to predict the likelihood of a compound being mobilised in runoff or leachate (Wilson et al., 1996). These properties include water solubility, vapour pressure, the octanol-water partition coefficient (K_{OW}) and the organic carbon water partition coefficient (K_{OC}) (Wilson et al., 1996). The most important of these properties are water solubility and the two partition coefficients (i.e. K_{OW} and K_{OC}). In recent years, some attempt has been made to categorise the likely mobility of compounds in the soil on the basis of their physicochemical properties. Table 1 illustrates one of these approaches (Wilson et al., 1996) to categorising the mobility of chemicals in soil based, in particular, on their K_{OW} and K_{OC} values.

Table 1-1: Ranking of mobility of chemicals in soil based on K_{OW} (unitless) and K_{OC} values from Wilson et al. (1996)

Ranking	K_{OW}	K_{OC} (cm^3/g)
Very mobile	< 1.2	0 – 50
Mobile	1.2 – 23	50 – 150
Medium mobility	23 – 245	150 – 500
Low mobility	245 – 6000	500 – 2000
Slight mobility	-	2000 – 5000
Immobile	> 6000	> 5000

In addition to the properties of the specific compounds, there are also numerous environmental factors that are likely to modify the amount of leaching or surface run-off that will occur, including:

1. frequency and intensity of rainfall – higher rainfall leads to a greater chance of off-site movement;
2. soil types – more porous soils (e.g. those containing high levels of sand compared to clays) will experience increased movement; and

3. slope of land – highly sloping land promotes greater volumes of run-off than flat land where the water is more likely to percolate down through the soil profile.

Recent research shows that PPCPs and EDCs have the potential to be mobilised with runoff water in detectable concentrations. For example, Pedersen et al. (2005) collected samples of runoff water from fields that had been irrigated with wastewater. These samples were then analysed for a suite of PPCPs and EDCs. Numerous compounds were detected in the dissolved phase in runoff water (i.e. in the filtered samples) with concentrations ranged from low ng/L concentrations for the estrogen compounds 17β-estradiol and estrone (at 3 ng/L and 52 ng/L, respectively) to high ng/L range concentrations for some pharmaceuticals, including, the antiepileptic drug carbamazepine and the muscle relaxant carisoprodol (at concentrations of 320 to 440 ng/L and 680 ng/L respectively). Other compounds that were detected in runoff water in this study included the fragrance compounds, galaxolide and tonalide.

The mobility of PPCPs and EDCs from areas that have specifically received the addition of biosolids has also been assessed. A study that used biosolids spiked with elevated concentrations of PPCPs and EDCs found that runoff from an initial rain event one day post-application contained a range of PPCPs (including, e.g., acetaminophen, ibuprofen, naproxen, carbamazepine, gemfibrozil and triclosan), with triclosan and carbamazepine still being detectable in runoff water 266 days post-application (Topp et al. 2008b). Following this study, Sabourin et al. (2009) conducted another related study where biosolids, which had *not* been spiked with PPCPs, were applied to and incorporated into the soil. Rainfall was simulated at 5 time intervals from 1 to 36 days post biosolids application. Runoff that was collected from the simulated rainfall events showed varying concentrations of PPCPs, ranging up to 110 ng/L for triclosan. Overall, however, the

majority of compounds analysed (i.e. triclocarban, triclosan, sulfamethoxazole, ibuprofen, naproxen and gemfibrozil) showed a low level of mobility, with less than 1% being exported in runoff water at four sampling times over the 36 days. These absolute concentrations need to be related to aquatic toxicity values, however, in order to assess the potential hazard or risk.

The method used for the addition of biosolids to the soil has also been shown to affect the runoff concentrations of PPCPs and EDCs. For example, Topp et al. (2008b) found that the addition of biosolids to a soil via subsurface injection at a depth of 10 cm effectively eliminated the surface runoff of pharmaceuticals. In contrast, when the biosolids were applied to the surface of the soil and incorporated to a depth of 15 cm, numerous pharmaceuticals were detected in the runoff.

The mobility of antibiotics, primarily veterinary antibiotics, has also been investigated in several studies. A „lysimeter" study was conducted by Kay et al. (2005) to observe the fate of three veterinary antibiotics from three different groups: sulphonamides, tetracyclines and macrolides. A lysimeter study involves the use of a soil core in the field and is often used to investigate leaching behaviour of compounds in soils. Only the sulphonamide antibiotic, sulphachloropyridaze (SCP), was detected in the leachate, although at a concentration considerably less than the concentration that was applied to the top soil at the commencement of the study (i.e. < 0.1%). These findings were taken to indicate limited downward movement of these antibiotics. Rabolle and Spliid (2000) similarly found that the tetracycline antibiotic, oxytetracycline, was also immobile in the leachate studies that they carried out.

1.2.3. Environmental toxicity of PPCPs and EDCs

In order to assess the risks that PPCPs and EDCs pose to the environment following land application of biosolids, an understanding of the concentrations that may lead to adverse effects on exposed organisms is required. Following the land application of biosolids, both terrestrial and aquatic ecosystems could be exposed to PPCPs and EDCs. These will each be discussed below with relation to PPCPs and EDCs.

1.2.3.1. Terrestrial toxicity

The terrestrial toxicity of PPCPs and EDCs is of particular importance, as this is the environmental compartment that will be directly exposed to contaminants following the addition of biosolids to land. The information available on the terrestrial toxicity of PPCPs and EDCs is, however, sparse.

Some concerns surround the presence of antimicrobials and antibiotics in biosolids as these compounds are designed to be toxic to microorganisms, and are therefore likely to pose a potential risk to soil microbial communities. In terrestrial toxicity tests conducted on the antimicrobial agent, triclosan, some negative responses, as discussed below, have been observed; however these negative effects only tend to occur at relatively high concentrations or show rapid recovery rates. For example, the effect of triclosan on soil respiration and nitrogen cycling was investigated in two soils (a sandy acidic soil and a clay rich alkaline soil) by Waller and Kookana (2009). Respiration in the sandy soil was not found to be affected at concentrations up to 100 mg/kg, whereas, in the clay soil, a decrease in respiration was observed at 50 mg/kg. This study reported that the nitrification process was adversely affected in both of the soils that were tested; however, this negative effect was more pronounced in the sandy soil where it was noticeable at concentrations of 5 mg/kg. Adverse affects of triclosan on soil respiration were also observed by Liu et al.

(2009) at concentrations from 1 mg/kg; however, in this study, the affected microbial communities showed rapid recovery.

There are some reported toxicity data for triclosan to terrestrial plants as part of the National Industrial Chemicals Notification and Assessment Scheme (NICNAS) recent report assessing the potential environmental risks from this compound (NICNAS, 2008). The most sensitive plant tested was the Cucumber, which showed a lowest observed effect concentration (LOEC) of 45 µg/kg after a 21 day exposure using sand as a test media.

Terrestrial toxicity testing of the effects of antibiotics on soil microorganisms also shows that there is the potential for adverse effects at environmentally relevant concentrations. Effective doses of antibiotics for inhibiting soil microbial activities by 10% have been shown to range from total antibiotic concentrations of 0.003 to 7.35 mg/kg (Thiele-Bruhn & Beck, 2005). However, similar to the terrestrial tests discussed above for triclosan, microbial communities have been shown to recover quite rapidly following initial toxicity caused by antibiotics at these concentrations (Thiele-Bruhn & Beck, 2005). There is also some concern surrounding the potential for antibiotic resistance in soil bacteria following the addition of biosolids; however, early research does indicate that land application of biosolids does not increase antibiotic resistant bacteria above background soil levels (Brooks et al., 2007).

As for other possible PPCPs and EDCs, the presence of the surfactant metabolite, nonylphenol in biosolids has been found to produce some adverse effects. For example, there have been reports of adverse effects on the life history of the parthenogenetic earthworm, *Dendrodaena octaedra* (Widarto et al., 2004). In more detail, increasing concentrations of nonylphenol were found to have a negative effect on the growth rate of

juveniles and the percent of worms producing cocoons. However, other traits of the life history of the parthenogenetic earthworm that were measured in that study, including, population growth, number of cocoon produced per worm, time to first reproduction, adult survival and juvenile survival were not found to be affected by increases in soil nonylphenol concentrations.

The toxicity of nonylphenol in soils to the soil invertebrate, collembolan, *F. fimetaria,* in the presence and the absence of sludge has also been observed using different exposure scenarios (Scott-Fordsmand & Krogh, 2004). These scenarios included soil that was spiked with nonylphenol, sludge that was spiked with nonylphenol and then mixed with soil, and sludge that was spiked with nonylphenol prior to being made into pellets and applied to the soil. Several endpoints were measured in this study, including mortality, growth and reproduction. Reproduction appeared most sensitive to the presence of nonylphenol in the soil. In general, the toxicity values ranged from 6 to 91 mg/kg when expressed as the concentration required to cause 10% inhibition (EC10).

1.2.3.2. Aquatic toxicity

Aquatic ecosystems may also become exposed to PPCPs and EDCs that are contained within biosolids as a result of runoff or leaching from biosolids-amended land. The study of the effects of EDCs on aquatic organisms is an area of research that has received considerable interest recently, and this interest has been far more extensive than the extent of terrestrial toxicology research.

The most potent of the EDCs tend to be the natural and the synthetic estrogen compounds due to their potential to exert deleterious effects in the ng/L range (e.g. Mills & Chichester, 2005). The presence of estrogens in waterways has been linked with the partial

feminisation of male fish, described as intersex, and increased levels of the yolk precursor protein, vitellogenin (Vtg), with effects being observed following exposure to concentrations as low as 10ng/L of the synthetic estrogen, 17α-ethinylestradiol (Orn et al., 2006). High Vtg concentrations in fish as a result of their exposure to 17α-ethinylestradiol have also been found to lead to kidney failure and, in turn, mortality in fish in some cases (Zillioux et al., 2001). Other examples of adverse effects of estrogen compounds on aquatic organisms include effects on gonopodium development in male mosquitofish (Rawson et al., 2006) and on gamete quality and gamete maturation in rainbow trout (Lahnsteiner et al., 2006). Both studies involved exposure to 17β-estradiol. In comparison, many of the xenoestrogen compounds, for example, bisphenol A and nonylphenol, tend to be only weakly estrogenic when compared to the estrogen compounds (Jobling & Sumpter, 1993; Jobling et al., 1996; Fukuhori et al., 2005). These subtle changes in fish morphology, as a result of exposure to these EDCs over a long time frame, could lead to quite significant changes in populations of aquatic organisms.

The acute toxicity of a range of pharmaceuticals (including, clofibric acid, carbamazepine, ibuprofen, diclofenac, naproxen, captopril, metformin, propranolol, and metoprolol) has also been examined (Cleuvers, 2003). In this study, effects were examined on: cladoceran, *Daphnia magna*; the chlorophyte, *Desmodesmus subspicatus*; and the macrophyte, *Lemna minor*. Diclofenac (an anti-inflammatory drug) and propranolol (a beta blocker drug) were found to be the most toxic with EC50 values (i.e., the concentration of a contaminant that causes a 50% effect) of 7.5 mg/L to both *Lemna minor* and *Daphnia magna* (Cleuvers, 2003). More subtle, sub-lethal or longer-term chronic effects (e.g. cellular responses in fish and, in particular, oxidative metabolism in liver cells that could possibly lead to oxidative damage) have also been observed following exposure to such pharmaceuticals (Gagne et al., 2006).

Acute aquatic toxicity testing of antibiotics has revealed effects in the mg/L range, similar to those observed for non-antibiotic pharmaceuticals (Isidori et al., 2005). However, in tests of chronic effects, antibiotics have also been shown to be bioactive at concentrations in the order of µg/L. For example, exposure to the antibiotic erythromycin has been shown to cause chronic toxicity to a range of aquatic organisms at concentrations ranging from 20 to 940 µg/L (Isidori et al., 2005).

Finally, considerable work has been conducted on the aquatic toxicity of triclosan. This is because the input of this chemical into waterways through the release of effluent is an area of considerable concern. Triclosan has been found to cause toxic effects to aquatic organisms at very low concentrations. EC50 values for *Daphnia magna* exposed for 48-hours can be as low as 390 µg/L (Orvos et al., 2002), and, for the medaka fish, 96-hour LC50 values for 24-hour old larvae have been reported at 620 µg/L (Ishibashi et al., 2004). Reproduction effects have also been observed in exposed organisms. In a study using medaka fish, hatchability was found to be decreased and time to hatching of fertilised eggs that were exposed to 313 µg/L of triclosan for 14 days was delayed (Ishibashi et al., 2004). These results indicate that this particular compound has the potential to exhibit fairly substantial toxic effects to aquatic organisms at low and environmentally relevant concentrations.

1.3. AIMS OF THIS STUDY

The above discussion of the literature on PPCPs and EDCs in biosolids, and their possible effects in the environment, indicates that, although some research has been conducted, the majority of results to date are compound specific and there remain substantial research gaps in the field. This doctoral thesis aims to address several of these knowledge gaps. In particular, the specific aims of this thesis are to:

(i) quantify the hazard posed by PPCPs and EDCs to aquatic ecosystems by the application of biosolids to agricultural land based on an international review of the literature (Chapter 2);

(ii) determine the concentration of selected PPCPs and EDCs in Australian biosolids and examine the factors that affect the concentrations (Chapter 3);

(iii) determine the persistence of selected PPCPs and EDCs in a representative South Australian agricultural soil following the application of biosolids under both laboratory and field conditions (Chapters 4 and 5);

(iv) determine if the standard experimental practice of „spiking" chemicals into biosolids amended soils produces comparable degradation results to those obtained for „indigenous" compounds that are found to commonly occur within biosolids (Chapters 6 and 7); and

(v) determine if land application of biosolids produces detectable levels of estrogenic activity in a soil (Chapter 8).

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Chapter 2

Aquatic hazard assessment for pharmaceuticals, personal care products and endocrine disrupting compounds from biosolids-amended land

STATEMENT OF AUTHORSHIP

Aquatic hazard assessment for pharmaceuticals, personal care products and endocrine disrupting compounds from biosolids-amended land

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Abstract

Reuse of biosolids on agricultural land is a common practice. Following the application of biosolids to land there is potential for contaminants in the biosolids to migrate off-site via surface runoff and/or leaching and pose a hazard to aquatic ecosystems. The aim of this screening level assessment study was to determine the relative hazard posed to aquatic ecosystems by pharmaceuticals, personal care products and endocrine disrupting compounds that have been detected and quantified in biosolids. This involved estimating maximum possible runoff water concentrations of compounds using an equilibrium partitioning approach and comparing these to the lowest available aquatic toxicity data using the hazard quotient (HQ) approach. A total of 45 pharmaceuticals, personal care products and endocrine disrupting compounds have been detected in biosolids. Ten of these compounds (tonalide, galaxolide, 17β-estradiol, 17α-ethinylestradiol, ciprofloxacin, doxycycline, norfloxacin, ofloxacin, triclosan and triclocarban) posed a high (HQ > 1.0) hazard to aquatic ecosystems relative to the other compounds. This hazard assessment indicated that further research into potential off-site migration and deleterious effects on aquatic ecosystems is warranted for the ten organic contaminants identified, and possibly for chemicals with similar physicochemical and toxicological properties, in biosolids-amended soils. As many antibiotic compounds (e.g., ciprofloxacin, norfloxacin and ofloxacin) have ionic properties, the methods used may have overestimated their predicted aqueous concentrations and hazard. Further research that includes site-specific variables, e.g., dilution factors in waterways, rain intensity, slope of land, degradation and the use of management strategies such as buffer zones, are likely to decrease the hazard posed by these high hazard compounds.

CONTENTS

2.1. INTRODUCTION

Application of biosolids to agricultural land as a replacement or supplement for agricultural fertilisers is widely practiced. This practice can provide plants with additional nutrients and improve soil structure and water holding capacity. Biosolids also contain a broad range of inorganic and organic contaminants therefore, the risks associated with these entering the environment following land application need to be evaluated.

Pharmaceuticals and personal care products (PPCPs) and endocrine disrupting compounds (EDCs) have received considerable attention recently as "contaminants of emerging concern". PPCPs are compounds that are used in everyday life through ingestion of drugs and use of products of personal care, for example, soaps and toothpaste. Endocrine disrupting compounds are both estrogen compounds (natural and synthetic) which are excreted by humans and xenoestrogen compounds, for example, the surfactant metabolite 4-nonylphenol, which has been shown to interfere with estrogen receptors in non-target organisms (Mills & Chichester, 2005). These groups of compounds are of environmental concern as they tend to be biologically active compounds and can therefore exert a response in non-target organisms. PPCPs and EDCs enter wastewater treatment plants via residential, commercial and industrial inputs, where depending on their hydrophobicity, they can be concentrated in biosolids rather than in the aqueous waste stream of the treatment process. These compounds can then enter the environment through biosolids land application, where they can potentially pose risks to both terrestrial and aquatic ecosystems. Aquatic ecosystems may be exposed to contaminants from biosolids through off-site migration bound to particulate matter or in the aqueous phase through surface runoff or leaching. In the aquatic environment PPCPs and EDCs have been shown to exhibit varying degrees of toxicity. Some examples are, the antidepressant drugs fluoxetine and the antibiotic ciprofloxacin, which have been reported to show aquatic

toxicity at 5 µg/L (Foran et al., 2004 and Halling-Sorenson et al., 2000, respectively). A higher degree of aquatic toxicity has been seen for triclosan, an antimicrobial agent found in many personal care products, exhibiting toxicity at 0.5 µg/L (Orvos et al., 2002). The most harmful compounds from these groups tend to be the estrogen compounds, most specifically, the synthetic estrogen 17α-ethinylestradiol (EE2). EE2 has been reported to have a no observed effect concentration (NOEC), which typically corresponds to 10 to 30% effect (Moore & Caux, 1997), as low as 0.03 ng/L (Metcalfe et al., 2001).

A key factor in determining the potential aquatic risks associated with compounds that are applied to land with biosolids is understanding their ability to be mobilised with water. Most of the studies on the mobility and transport of PPCPs and EDCs in soils have been conducted as controlled laboratory or lysimeter experiments and have shown varying results which tend to be compound specific. In a large scale field experiment, Pedersen et al. (2005) found many PPCPs and EDCs in surface runoff water from fields that had been irrigated with sewage treatment plant effluent. More recently, Topp et al. (2008) used simulated rainfall experiments to study the mobility of PPCPs from sites where biosolids spiked with elevated concentrations of PPCPs had been applied and incorporated. Surface runoff caused by an initial rain event one day post-application contained a range of PPCPs (including, acetaminophen, ibuprofen, naproxen, carbamazepine, gemfibrozil and triclosan), with triclosan and carbamazepine still detectable in runoff water 266 days post-application. Following this, Sabourin et al. (2009) conducted a study where biosolids, which had not been spiked with PPCPs, were applied and incorporated into land. Rainfall was simulated at 5 time intervals from 1 to 34 days post biosolids application. Runoff collected from the simulated rainfall events showed varying concentrations of PPCPs with up to 110 ng/L for the antimicrobial agent triclosan. In this field study, the majority of compounds analysed, i.e. triclocarban, triclosan, sulfamethoxazole, ibuprofen, naproxen

and gemfibrozil, showed a low level of mobility with less than 1% being exported in runoff water.

Although research has shown that there is the potential for PPCPs and EDCs to migrate off-site with runoff water following land application of biosolids it would be a vast undertaking to experimentally assess the risks associated with all these compounds that have been identified in biosolids. This is primarily due to the wide range of properties of the compounds and the lengthy analytical procedures required. To identify specific compounds of concern as a priority for experimental assessment, a hazard assessment can be used as an initial screening tool. A commonly used method for this is the hazard quotient (HQ) approach of Urban & Cook (1986) which has been used by many regulatory agencies including the USEPA. This approach involves comparing predicted maximum environmental concentrations with the lowest reported concentrations that exert toxicity to produce a HQ value. This value can then be used to determine if harmful effects are possible and ultimately be used as a tool to rank compounds in terms of the relative hazard that they pose.

The aim of this study was to estimate the maximum hazard posed to freshwater ecosystems by PPCPs and EDCs from biosolids amended land, and subsequently identify and prioritise those compounds that warrant further investigation. The study dealt exclusively with compounds dissolved in the aqueous phase in runoff water however, it is acknowledged that particulate-bound compounds may also be transported off-site and contribute to deleterious effects. Dissolved contaminants are likely to be more bioavailable to aquatic organisms, as well as pose a more immediate hazard to aquatic ecosystems following a rain event and were therefore chosen as the focus of this study. In addition, as this study is an initial screening type assessment, more complex environmental variables,

for examples, rain intensity and duration and slope of land will not be considered, however, it is acknowledged that these will play a role in a "real world" scenario.

2.2. MATERIALS AND METHODS

The assessment conducted in this study required:

i. the calculation of maximum concentrations of PPCPs and EDCs present in soil following land application of biosolids;
ii. the prediction of maximum concentrations of PPCPs and EDCs present in runoff water following a rain event;
iii. a preliminary "worst case scenario" hazard assessment to identify the hazard posed by each compound; and,
iv. an additional hazard assessment for compounds classed as posing a high hazard.

2.2.1. Calculation of maximum soil concentrations of compounds

A literature search was conducted to determine the PPCPs and EDCs that have been detected and quantified in biosolids. The mean, median and range of the reported concentrations were calculated for each compound (Table 2-1). As biosolids are applied to the surface of soil and then incorporated, the maximum concentration of PPCPs and EDCs in soil was therefore calculated by,

maximum soil conc. = conc. in biosolids x (mass biosolids / mass soil)

where, the mass biosolids is the mass of biosolids applied to land and the mass soil is the mass of soil into which the biosolids are incorporated. For the mass of biosolids a value of 40 dry t/ha was used. This is the maximum permissible application rate for a typical Australian lagoon biosolids based on its nitrogen concentration (Heemsbergen et al. 2007).

The mass soil value used was 1300 t/ha, which is based on incorporation depth of 100 mm and a soil bulk density of 1.3 g/cm^3 (SA EPA, 1997).

2.2.2. Prediction of maximum runoff water concentrations

The maximum soil concentrations of each of the compounds were then used to calculate the maximum runoff water concentrations. Maximum runoff water concentrations were calculated using partition coefficient (K_d) data. K_d values for each compound were calculated from the octanol-water partition coefficients (K_{OW}) via the intermediate step of predicting the organic carbon-water partition coefficient (K_{OC}). Predicted K_{OC} values were used in this assessment as experimental K_{OC} values were only available for a limited number of the PPCPs and EDCs identified in biosolids. The relationship between log K_{OW} and log K_{OC} for various groups of organic compounds has been represented by numerous equations. Equation 2-1 (Kenaga & Goring, 1980) was selected for this study as it yielded more accurate predictions of log K_{OC} values compared to other equations (Briggs, 1981; Schwarzenbach et al., 2003) for compounds where experimental K_{OC} values were available (data not presented). In addition, equation 2-1 was derived from a relatively large data set of 108 compounds which had a wide range of properties.

$$\log K_{OC} = 0.544 \log K_{OW} + 1.377 \qquad (2\text{-}1)$$

Following estimation of K_{OC}, K_d was determined as $K_d = K_{OC} \times f_{oc}$, where the fraction of organic carbon, f_{oc}, was assumed to be 0.016 which is approximately the 40th percentile of the f_{oc} of 14 agricultural soils used in the Australian National Biosolids Research Program (Broos et al., 2007) and provides a conservative estimate of K_d.

Table 2-1: Summary of pharmaceuticals and personal care products, and endocrine disrupting compounds that have been detected in biosolids, their concentrations in biosolids, octanol-water (K_{OW}), predicted organic carbon-water (K_{OC}) partition coefficients and calculated distribution coefficients (K_d)

Chemical group	Compound	Type/use of chemical	Biosolids concentration (µg/kg)*			Log K_{OW}	Log K_{OC}	K_d
			mean	median	range			
Antibiotics	ciprofloxacin	antibiotic	3960	2650	500 – 11700	0.28	1.53	0.54
	doxycycline	antibiotic	430	nd	nd – 1500	-0.02	1.37	0.37
	erythromycin	antibiotic	5.5	nd	nd – 41	3.06	3.04	17.6
	norfloxacin	antibiotic	3490	2450	100 – 11100	-1.03	0.82	0.10
	ofloxacin	antibiotic	580	300	nd – 2000	-0.39	1.16	0.23
	sulfamethoxazole	antibiotic	20.7	nd	nd – 160	0.89	1.86	1.16
	trimethoprim	antibiotic	3.0	nd	nd – 22	0.91	1.87	1.19
Endocrine disrupting compounds	17α-ethinylestradiol	estrogen	4.01	1.31	0.42 – 17	3.67	3.37	37.8
	17β-estradiol	estrogen	13.5	7	0.31 – 49	4.01	3.56	54.9
	estrone	estrogen	10.9	nd	nd – 150	3.13	3.08	19.2
	bisphenol A	plasticizer	1220	630	100 – 4600	3.32	3.18	24.4
	diphenyl ether	heat transfer / fragrance	99 600	99 600	99 600	4.21	3.67	74.4
	4-nonylphenol	surfactant metabolite	102 000	51 700	606 – 438 000	6.75	5.05	1790
	4-t-octylphenol	surfactant metabolite	945	1010	167 – 2400	3.70	3.39	39.3

Table 2-1 Continued

Chemical group	Compound	Type/use of chemical	Biosolids concentration (µg/kg)*			Log K_{OW}	Log K_{OC}	K_d
			mean	median	range			
Personal care products	acetophenone	fragrance	375	82	nd – 2300	1.58	24	2.76
	acetyl cedrene	fragrance	20 200	20 200	9000 – 31 300	5.78[a]	4.52	531
	cashmeran	fragrance	328	287	47.2 – 1450	5.2[b]	4.21	257
	celestolide	fragrance	187	108	0.07 – 1100	6.6[b]	4.97	1480
	d-limonene	fragrance	227	175	nd – 1070	4.57	3.86	117
	galaxolide	fragrance	14 100	10 700	13 – 177 000	5.90[b]	4.59	618
	indole	fragrance	4460	3170	980 – 10 600	2.14	2.54	5.56
	musk ambrette	fragrance	1.3	nd	nd – 33	4.17	3.65	70.7
	musk ketone	fragrance	16.4	nd	nd – 163	4.3[b]	3.71	83.2
	musk moskene	fragrance	0.86	nd	nd – 36	na	-	-
	musk xylene	fragrance	9.5	nd	nd – 121	4.45	3.80	100
	phantolide	fragrance	374	180	0.41 – 1800	6.7[b]	5.02	1680
	tonalide	fragrance	9310	3540	32 – 427 000	5.7[b]	4.48	481
	traseolide	fragrance	369	300	nd – 1000	8.1[b]	5.7	9720
	triclocarban	antimicrobial	3900	4200	370 – 5970	4.90	4.04	176
	triclosan	antimicrobial	4280	2280	443 – 21 700	4.76	3.97	148

Table 2-1 Continued

Chemical group	Compound	Type/use of chemical	Biosolids concentration (µg/kg)*			Log K_{OW}	Log K_{OC}	K_d
			mean	median	range			
Pharmaceuticals	acetaminophen	analgesic	414	70	nd – 4540	0.46	1.63	0.68
	albuterol	asthma medication	90.4	nd	nd – 850	0.64	1.73	0.85
	caffeine	stimulant	204	74	nd – 1200	-0.07	1.34	0.35
	carbamazepine	anti-epileptic	149	20	0.01 – 1730	2.45	2.71	8.20
	codeine	analgesic	3.1	nd	nd – 22	1.19	2.02	1.69
	dehydronifedipine	anti-anginal	6.5	nd	nd – 26	na	-	-
	diltiazem	anti-anginal	8.5	nd	nd – 59	2.79	2.89	12.6
	diphenhydramine	sedative	609	180	15 – 7000	3.27	3.16	22.9
	fluoxetine	anti-depressant	124	25	nd – 1500	4.05	3.58	60.9
	gemfibrozil	lipid regulator	140	nd	nd – 1190	4.77	3.97	150
	ibuprofen	anti-inflammatory	1990	1990	0.006 – 4000	3.97	3.54	55.1
	miconazole	antifungal	175	100	nd – 460	6.25	4.78	957
	naproxen	anti-inflammatory	511	511	0.001 – 1020	3.18	3.11	20.5
	salicylic acid	analgesic	6874	6874	0.002 – 13 748	2.26	2.61	6.46
	warfarin	anti-coagulant	26	13	nd – 92	2.60	2.79	9.90

* biosolids concentration data obtained from: Herren & Berset 2000; Khan & Ongerth 2002; M[c]Avoy et al. 2002; Ternes et al. 2002; Bester 2003; Golet et al. 2003; Stevens et al. 2003; Difrancesco et al. 2004; Kupper et al. 2004; Braga et al. 2005; Campbell-Board, 2005 Lindberg et al. 2005; Miao et al. 2005; Kinney et al. 2006; Lindberg et al. 2006; Chu & Metcalfe 2007; [a] Difransesco et al. 2004; [b] Peck & Hornbuckle 2004; nd indicates not detected.

The ratio of partitioning of each compound between the bound phase and aqueous phase in the soil (M_b / M_{soln}) at equilibrium, per given volume (i.e. 1 cm^3) was determined from Equation 2-2. This equation was obtained by rearranging the equation used to experimentally determine K_d (OECD, 2000),

$$M_b / M_{soln} = (K_d.M_s) / V_0 \quad (2\text{-}2)$$

where M_b is the mass of the compound bound to the solid phase at equilibrium (µg), M_{soln} is the mass of the compound in the aqueous phase at equilibrium (µg), K_d is the soil-solution partition coefficient, M_s is the mass of soil in 1 cm^3 (g dry weight) (i.e. 1.3 g) and V_0 is the volume of aqueous phase in 1 cm^3 soil (mL) (i.e. 0.5 mL). These values were selected to represent the saturation point of a standard soil.

Equation 2-3 was then used to determine the mass of each compound in the aqueous phase (M_{soln}) in the same given volume,

$$M_{soln} = M_0 / [(M_b / M_{soln}) +1] \quad (2\text{-}3)$$

where, M_0 is equal to the total mass (µg) of test substance in 1 cm^3 soil (i.e. 1.3 g soil) which was calculated from the maximum soil concentration of each of the compounds that had been previously determined. M_{soln} therefore related to the mass in solution in 0.5 mL of water (i.e. 1 cm^3 of soil at saturation). This value was then converted for each compound into an aqueous concentration in µg/L which was used in the following hazard assessment as the maximum runoff water concentration.

2.2.3. Preliminary hazard assessment

The preliminary hazard assessment was conducted in order to rank the PPCPs and EDCs based on a "worst case scenario". Therefore, the maximum predicted concentrations of each compound in runoff water following land application of biosolids were compared with the most sensitive aquatic toxicity data using the HQ approach (Urban & Cook, 1986).

Aquatic toxicity data for each compound were collated from the USEPA ECOTOX database (USEPA, 2009) as well as from the literature. Toxicity data collected included the concentrations that caused up to a 50% sub-lethal or lethal effect (i.e. ≤ EC50 and ≤ LC50 respectively) and "no observed effect concentrations" (NOECs). Although the use of NOEC data in hazard assessments has been questioned (Moore & Caux, 1997), NOEC values in some cases provided the most sensitive toxicity data in some relatively small datasets, and therefore they were included. Only toxicity data that measured ecologically relevant endpoints (e.g. lethality, immobilisation, reproduction and growth inhibition) (Warne, 1998; ANZECC and ARMCANZ, 2000) were used. However, as EDCs modify the endocrine system, particularly reproduction, endpoints that related to changes in physical sexual characteristics were considered ecologically relevant. The most sensitive (i.e. lowest) toxicity datum point for each compound was then selected to calculate the HQ using Equation 2-4.

$$HQ = \text{maximum aqueous concentration} / \text{lowest aqueous toxicity value} \qquad (2\text{-}4)$$

All compounds were then placed into hazard categories based on their HQ value. If the HQ obtained for a compound was greater than 1.0 then toxicity would be expected and the

compound was therefore categorised as posing a high hazard. The other hazard categories used were moderate (HQ = 0.5 – 1.0) and low (HQ < 0.5) hazard.

2.2.4. Additional hazard assessment for high hazard compounds

An additional, more environmentally realistic, hazard assessment was conducted on compounds that were classed as posing a high hazard in the preliminary hazard assessment (i.e. when HQ > 1.0). This used the concentration that should theoretically protect 95% of aquatic species (i.e. PC95, which is equivalent to the concentration hazardous to 5% of species, HC5).

To obtain the PC95 for each of the compounds, the BurrliOZ species sensitivity distribution (SSD) method (Campbell et al., 2000) was applied to NOEC and EC/LC10 to EC/LC25 data. All EC/LC50 data were converted to EC/LC10 data by dividing by 5 (ANZECC and ARMCANZ, 2000; Warne, 2001). Using BurrliOZ (Campbell et al., 2000) requires toxicity data from a minimum of five species belonging to at least four taxonomic groups (e.g. ANZECC & ARMCANZ, 2000; Warne, 2001). The use of chronic toxicity data was preferred to that of acute data, as the former is more representative of concentrations which will cause adverse effects from long-term exposure. For some compounds, however, the use of only chronic data reduced the data sets to below this minimum data requirement of BurrliOZ, or removed the most sensitive data. In these cases, acute data were converted to chronic data following the calculation of an acute to chronic ratio (ACR) using the rules specified in Warne (2001) and used to derive the Australian and New Zealand water quality guidelines (ANZECC and ARMCANZ, 2000). In cases where an ACR could not be determined, a default ACR of 10 was used. After the acute to chronic conversions, compounds that still did not have enough data to fulfil the minimum data requirements for SSD analysis were excluded.

The BurrliOZ SSD method (Campbell et al., 2000), fits a Burr Type III distribution to the toxicity data. In this method, a single toxicity value is used to represent the toxicity of a compound to each species. A single toxicity datum point was determined for each species using the following rules:

i. if there was only one datum point for a species then this value represented the toxicity for that species; or
ii. if there were two or more data points for a species for a single toxicity endpoint then the geometric mean of those values was calculated and used to represent the toxicity for that species; or,
iii. if there were two or more data points for multiple toxicity endpoints then the geometric mean was determined for each endpoint and the lowest geometric mean used to represent the toxicity for that species.

The hazard quotient method was then used to calculate a HQ95 value by dividing the maximum aqueous concentration by the PC95. The HQ values based on PC95 data are believed to be more environmentally realistic, as they use all the available toxicity data rather than only the lowest toxicity value, and therefore should supersede those obtained in the preliminary hazard assessment (i.e. HQ).

2.3. RESULTS

The list of PPCPs and EDCs detected in biosolids globally and their mean, median and range of concentrations are shown in Table 2-1. Physicochemical data including log K_{OW}, predicted log K_{OC} and K_d values for each of the compounds are also provided in Table 2-1. Overall there were 45 PPCPs and EDCs that have been detected in biosolids at a wide range of concentrations, from low µg/kg concentrations for the natural and synthetic

estrogens to high mg/kg concentrations for the surfactant metabolite 4-nonylphenol. There were 16 compounds from personal care products (that had median biosolids concentrations ranging from non-detection to 20 200 µg/kg), 15 pharmaceutical compounds (median biosolids concentrations ranged from non-detection to 6870 µg/kg), 7 antibiotic compounds (median biosolids concentrations ranged from non-detection to 2650 µg/kg) and 7 endocrine disrupting compounds (median biosolids concentrations ranged from non-detection to 51 700 µg/kg). These 45 compounds had a wide range of calculated K_d values. In general, the antibiotics had the lowest K_d values (e.g. norfloxacin = 0.10) and the fragrance compounds had the highest (e.g. traseolide = 9720).

The predicted maximum soil concentration, the ratio of the mass in the bound phase to the mass in solution (M_b / M_{soln}), the percentage of the total mass that will desorb into the aqueous phase of the soil, and the maximum aqueous concentration for each of the compounds found in biosolids are shown in Table 2-2. The predicted maximum soil concentrations of the compounds in soil after biosolids application ranged over four orders of magnitude from 0.52 µg/kg for the synthetic estrogen compound 17α-ethinylestradiol to 13 500 µg/kg for 4-nonylphenol. The majority of the compounds have M_b / M_{soln} values that are greater than 50, indicating that a low percentage of the total mass of these compounds is likely to be found in the soil aqueous phase. In fact, approximately 47% of all the PPCPs and EDCs are classed as being immobile (Table 2-2) with less than 1% of the total compound predicted to desorb into the aqueous phase in the soil, and approximately another 24% of all compounds were classed as having low mobility (Table 2-2), where less than 10% is predicted to desorb.

Table 2-2: Maximum soil concentrations, predicted partitioning and maximum run-off water concentrations of pharmaceuticals and personal care products, and endocrine disrupting compounds from biosolids amended land

Chemical Group	Compound	Predicted maximum soil conc (µg/kg)	M_b / M_{soln}	% of compound in solution	Predicted maximum run-off concentration (µg/L)	Mobility[#]
Antibiotics	ciprofloxacin	360	1.41	41.5	389	M
	doxycycline	46.2	0.97	50.9	61.2	HM
	erthromycin	1.26	45.8	2.14	0.07	LM
	norfloxacin	342	0.27	78.6	698	HM
	ofloxacin	61.5	0.61	62.2	99.5	HM
	sulfamethoxazole	4.92	3.02	24.9	3.18	MM
	trimethoprim	0.68	3.10	24.4	0.43	MM
Endocrine disrupting compounds	17α-ethinylestradiol	0.52	98.3	1.01	0.01	LM
	17β-estradiol	1.51	150	0.66	0.03	IMM
	estrone	4.62	50.0	1.96	0.24	LM
	bisphenol A	142	63.4	1.55	5.71	LM
	diphenyl ether	3070	193	0.51	40.0	IMM
	4-nonylphenol	13 500	4660	0.02	7.52	IMM
	4-t-octylphenol	73.8	102	0.97	1.86	IMM

Table 2-2 Continued

Chemical Group	Compound	Predicted maximum soil conc (µg/kg)	M_b / M_{soln}	% of compound in solution	Predicted maximum run-off concentration (µg/L)	Mobility[#]
Personal care products	acetophenone	70.8	7.17	12.2	22.5	MM
	acetyl cedrene	963	1380	0.07	1.81	IMM
	cashmeran	44.6	668	0.15	0.17	IMM
	celestolide	33.8	3860	0.03	0.02	IMM
	d-limonene	32.9	3.4	0.33	0.28	IMM
	galaxolide	5450	1610	0.06	8.81	IMM
	indole	326	14.5	6.47	54.8	LM
	musk ambrette	1.02	184	0.54	0.01	IMM
	musk ketone	5.02	216	0.46	0.06	IMM
	musk moskene	1.11	na	na	na	na
	musk xylene	3.72	261	0.38	0.04	IMM
	phantolide	55.4	4370	0.02	0.03	IMM
	tonalide	13100	1250	0.08	27.3	IMM
	traseolide	30.8	2.53×10^4	0.004	0.003	IMM
	triclocarban	184	459	0.22	1.04	IMM
	triclosan	669	385	0.26	4.50	IMM

Table 2-2 Continued

Chemical Group	Compound	Predicted maximum soil conc (µg/kg)	M_b / M_{soln}	% of compound in solution	Predicted maximum run-off concentration (µg/L)	Mobility[#]
Pharmaceuticals	acetaminophen	140	1.76	36.2	131	M
	albuterol	26.2	2.21	31.2	21.2	M
	caffeine	36.9	0.91	52.4	50.3	HM
	carbamazepine	53.3	21.3	4.48	6.20	LM
	codeine	0.68	4.40	18.5	0.33	MM
	dehydronifedipine	0.80	na	na	na	na
	diltiazem	1.82	32.6	2.97	0.14	LM
	diphenhydramine	215	59.6	1.65	9.25	LM
	fluoxetine	46.2	158	0.63	0.75	IMM
	gemfibrozil	36.7	390	0.26	0.24	IMM
	ibuprofen	123	143	0.69	2.21	IMM
	miconazole	14.2	2490	0.04	0.01	IMM
	naproxen	31.5	53.2	1.84	1.50	LM
	salicylic acid	423	16.8	5.62	61.8	LM
	warfarin	2.83	25.7	3.74	0.28	LM

na, not available; # HM, highly mobile (> 50% of compound in solution); M, mobile (30 – 50% of compound in solution); MM, medium mobility (10 – 30% of compound in solution); LM, low mobility (1 – 10% of compound in solution); IMM, immobile (< 1% of compound in solution).

It should be noted that the concentrations in the solid and aqueous phases do not directly correspond to the percentage of each compound in the two phases presented in Table 2-2. The reason for this is that the total mass of each of the compounds is not partitioning between equal masses of soil and water, based on the assumptions of a soil bulk density of 1.3 g/cm^3 and a porosity of 50%, that is, within 1 cm^3 of soil there is 1.3 g of soil and 0.5 mL of water when all pores are saturated. Therefore, based on these calculations, the compounds are actually concentrated in the aqueous phase. For compounds that have very high proportions of the total compound that will partition into the aqueous phase, for example norfloxacin, the concentration in the aqueous phase is higher than the total predicted soil concentration (Table 2-2).

2.3.1. Preliminary hazard assessment

Aquatic toxicity data were available for the majority of compounds that had been reported in biosolids (Table 2-1). No aquatic toxicity data was available for: acetyl cedrene, cashmeran, musk moskene, phantolide, traseolide, albuterol, codeine, dehydronifedipine, diphenhydramine and miconazole. For the compounds for which toxicity data were available, an indication of the reliability of each data set is provided (i.e. the total number of data points, the number of species and the number of taxonomic groups that are represented) and details of the most sensitive toxicity data for each compound are provided in Table 2-3. The most sensitive toxicity data provided in Table 2-3 for the pharmaceuticals gemfibrozil, ibuprofen and naproxen is for a morphological (feeding) endpoint. Although this endpoint may have low ecological relevance, it provided the most sensitive toxicity data for these compounds.

Table 2-3: Most sensitive toxicity data for pharmaceuticals and personal care products, and endocrine disrupting compounds found in biosolids

Chemical group	Compound	No. data	No. sp.	No. tax groups[#]	Most sensitive toxicity data				
					Species	toxicity (µg/L)	measure	endpoint	Duration (days)
Antibiotics	ciprofloxacin	23	6	5	*M. aeriginosa* (blue green algae)	5[a]	EC50	growth	3
	doxycycline	14	1	1	*L. gibba* (duckweed)	54[b]	EC10	weight	7
	erythromycin	20	7	4	*P. subcapitata* (green algae)	20[c]	EC50	growth	3
	norfloxacin	15	1	1	*L. gibba* (duckweed)	206[b]	EC10	growth	7
	ofloxacin	35	9	6	*S. leopolensis* (blue green algae)	5[d]	NOEC	growth	4
	sulfamethoxazole	34	10	8	*S. leopolensis* (blue green algae)	5.9[d]	NOEC	growth	4
	trimethoprim	6	3	3	*S. capricornutum* (green algae)	110000[a]	EC50	population	3
Endocrine disrupting compounds	17α-ethinylestradiol	376	43	8	*O. latipes* (medaka higheyes)	0.00003[e]	NOEC	intersex	85
	17β-estradiol	38	10	3	*O. mykiss* (rainbow trout)	0.00042[f]	NOEC	reproduction	35
	estrone	1	1	1	*A. tonsa* (copepod)	410[g]	EC50	development	5

Table 2-3 Continued

Chemical group	Compound	No. data	No. sp.	No. tax groups[#]	Most sensitive toxicity data				
					Species	toxicity (µg/L)	measure	endpoint	Duration (days)
Endocrine disrupting compounds	bisphenol A	62	16	8	*S. maximus* (flounder)	59[h]	NOEC	intersex	21
	diphenyl ether	12	6	3	*D. magna* (water flea)	670[i]	LC50	mortality	2
	4-nonylphenol	306	41	8	*C. tentans* (midge)	42[j]	NOEC	mortality	20
	4-t-octylphenol	26	9	3	*A. tonsa* (copepod)	13[g]	EC50	development	5
Personal care products	acetophenone	16	3	3	*T. pyriformis* (ciliate)	42756[k]	IC50	population	2
	celestolide	6	2	1	*N. spinipes* (copepod)	30[l]	NOEC	development	7 - 8
	d-limonene	27	4	3	*P. promelas* (fathead minnow)	702[m]	LC50	mortality	4
	galaxolide	19	6	4	*N. spinipes* (copepod)	7[l]	NOEC	development	7 - 8
	indole	5	3	3	*D. magna* (water flea)	1000[n]	LC50	mortality	2
	musk ambrette	1	1	1	*D. magna* (water flea)	620[o]	EC50	immobilisation	2

Table 2-3 Continued

Chemical group	Compound	No. data	No. sp.	No. tax groups[#]	Most sensitive toxicity data				
					Species	toxicity (µg/L)	measure	endpoint	Duration (days)
Personal care products	musk ketone	19	4	2	*D. rerio* (zebrafish)	33[p]	NOEC	survival	2
	musk xylene	7	1	1	*D. rerio* (zebrafish)	10[p]	NOEC	survival	2
	tonalide	22	6	4	*A. tonsa* (copepod)	7[q]	EC10	development	5
	triclocarban	84	13	4	*A. bahia* (opossum shrimp)	0.056[r]	NOEC	reproduction	28
	triclosan	85	17	8	*S. subspicatus* (green algae)	0.5[s]	NOEC	growth	4
Pharmaceuticals	acetaminophen	16	6	3	*D. magna* (water flea)	9200[t]	EC50	immobilisation	2
	caffeine	22	6	4	*A. salina* (brine shrimp)	52730[u]	LC50	mortality	1
	carbamazepine	32	11	8	*C. dubia* (water flea)	25[d]	NOEC	reproduction	7
	diltiazem	4	2	2	*D. magna* (water flea)	8200[v]	EC50	immobilisation	4
	fluoxetine	27	2	2	*O. latipes* (medaka fish)	5[w]	NOEC	reproduction	28

Table 2-3 Continued

Chemical group	Compound	No. data	No. sp.	No. tax groups[#]	Most sensitive toxicity data				
					Species	toxicity (µg/L)	measure	endpoint	Duration (days)
Pharmaceuticals	gemfibrozil	13	3	3	*H. attenuate* (cnidaria)	100[x]	NOEC	morphology (feeding)	4
	ibuprofen	16	3	3	*H. attenuate* (cnidaria)	100[x]	NOEC	morphology (feeding)	4
	naproxen	14	3	3	*H. attenuate* (cnidaria)	1000[x]	NOEC	morphology (feeding)	4
	salicylic acid	22	6	4	*L. minor* (duckweed)	30000[q]	NOEC	population	7
	warfarin	12	6	2	*I. punctatus* (channel fish)	34.3[z]	LC50	mortality	4

based on definitions provided by Warne (1998); a Halling-Sorenson et al. 2000; b Brain et al. 2004; c Isidori et al. 2005; d Ferrari et al., 2004; e Metcalfe et al. 2001; f Lahnsteiner et al. 2006; g Andersen et al. 2001; h Larsen et al. 2006; i LeBlanc 1980; j Kahl et al. 1997; k Schultz et al. 1995; l Breitholtz et al. 2003; m Geiger et al. 1990; n Maas 1990; o Schramm, et al. 1996; p Carlsson & Norrgren 2004; q Wollenberger et al., 2003; r EPA/OTS, 1992; s Orvos et al. 2002; t Kuhn et al. 1989; u Wilkins & Metcalfe 1993; v Kim et al. 2007; w Foran et al. 2004; x Quinn et al. 2008; y Wang & Lay 1989; z Mayer & Ellersieck 1986

For the compounds that had toxicity data, the amount of available toxicity data was highly variable. It ranged from one toxicity datum point for musk ambrette and estrone to 376 data points for 43 species and 8 taxonomic groups for the synthetic estrogen 17α-ethinylestradiol (Table 2-3). For the compounds with toxicity data, the median was 19 toxicity values for six species from three taxonomic groups. The endocrine disrupting compounds 17α-ethinylestradiol and 17β-estradiol were the most harmful with NOEC values of 0.03 ng/L and 0.42 ng/L respectively to fish. In contrast, the antibiotic trimethoprim was the least harmful with its lowest value being an EC50 of 110 mg/L to a green alga.

Using the predicted maximum aqueous concentrations and the most sensitive toxicity data from Tables 2-2 and 2-3 respectively, the HQ values were calculated and the distribution of these values is shown in Figure 2-1. The range of HQ values was 3.9×10^{-6} for the antibiotic trimethoprim to 457 for the synthetic estrogen 17α-ethinylestradiol. The horizontal lines in Figure 2-1 indicate where the HQ value is equal to 0.5 and 1.0, and therefore they identify the low, moderate and high hazard cut-off points. Overall, 24 compounds were classed as posing a low hazard, one posed a moderate hazard and ten compounds (galaxolide, tonalide, triclocarban, triclosan, ciprofloxacin, doxycycline norfloxacin, ofloxacin, 17α-ethinylestradiol and 17β-estradiol) posed a high hazard.

2.3.2. Additional hazard assessment

For the ten compounds that were classed as posing a high hazard by the preliminary hazard assessment, an additional hazard assessment was conducted where less conservative assumptions were made and HQ95 values were calculated (Table 2-4). For doxycycline and norfloxacin, HQ95 values could not be calculated because all the toxicity data points available were from one species and therefore the minimum data requirements

to conduct an SSD were not met and PC95 values could not be calculated. In some cases, (ciprofloxacin, galaxolide, ofloxacin, and tonalide) the PC95 values were lower than the most sensitive toxicity data points used previously, which resulted in higher estimates of hazard (i.e., the HQ95 > HQ). For the remaining four compounds (17α-ethinylestradiol, 17β-estradiol, triclosan and triclocarban), the PC95 values were larger than the most sensitive species data points and resulted in lower estimates of hazard (i.e., the HQ95 < HQ). For all compounds identified by the initial hazard assessment as posing a high hazard and for which a HQ95 value could be calculated, the hazard classification remained as high.

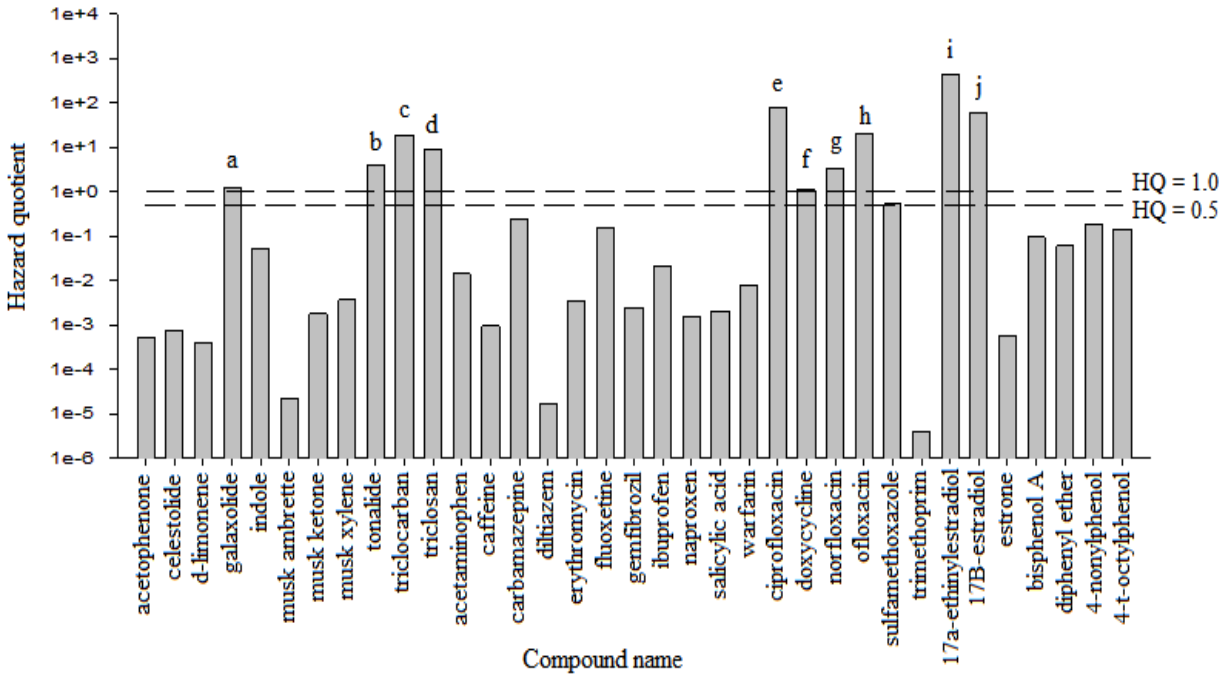

Figure 2-1: Distribution of hazard quotient values for pharmaceuticals, personal care products and endocrine disrupting compounds in biosolids. Compounds marked with letters indicate compounds that were classed as high hazard where the HQ values are: a (galaxolide) = 1.26; b (tonalide) = 3.90; c (triclocarban) = 18.5; d (triclosan) = 9.01; e (ciprofloxacin) = 77.8; f (doxycycline) = 1.13; g (norfloxacin) = 3.39; h (ofloxacin) = 19.9; i (17α-ethinylestradiol) = 457; j (17β-estradiol) = 61.6

Table 2-4: Concentrations that should theoretically protect 95% of aquatic species (PC95) for the high hazard compounds, the corresponding hazard quotient values (HQ95) and the HQ values from the preliminary hazard assessment

Chemical group	Compound	PC95 (ug/L)	HQ95	HQ
Antibiotics	ciprofloxacin	1.46	267	77.8
	doxycycline	na	na	1.13
	norfloxacin	na	na	3.39
	ofloxacin	2.21	45.3	19.9
Endocrine disrupting compounds	17β-estradiol	0.0007*	39.0	61.6
	17α-ethinylestradiol	0.0003	45.7	457
Personal care products	galaxolide	3.73	2.36	1.26
	tonalide	3.48	9.54	3.90
	triclosan	0.59	7.64	9.01
	triclocarban	0.07	14.9	18.5

*calculated from data from 3 taxonomic groups; na (not available) – indicates insufficient toxicity data to conduct an SSD

2.4. DISCUSSION

Through collating the available literature this study identified 45 PPCPs and EDCs that have been detected in biosolids globally. However, it should be noted that with advancements in analytical techniques for these types of compounds, this list is likely to expand. The log K_{OW} values of these chemicals range from -1.03 to 8.1 and therefore these compounds are likely to have a diverse range of environmental behaviours. Greater than 70% of the PPCPs and EDCs detected in biosolids were predicted to be immobile or to have low mobility in soil, and greater than 45% of the compounds were predicted to have less than 1% of the total compound partitioning into the aqueous phase in soil. Experimental work conducted by Sabourin et al., 2009 supporting these predictions found that several of these compounds, triclocarban, triclosan, gemfibrozil and ibuprofen, showed low transport with less than 1% mobile with runoff water following land

application of biosolids. In fact, the experimental concentrations measured in runoff water in the above study were, in the majority of cases, less than those that were predicted in this hazard assessment for compounds common to both studies (i.e. caffeine, acetaminophen, triclosan, triclocarban, sulfamethoxazole, ibuprofen and naproxen). This is to be expected for two reasons: first due to the conservative assumptions used throughout this hazard assessment and second that the actual amount desorbed is likely to be lower than estimated due to non-singular (hysteretic) sorption-desorption isotherms (i.e. desorption coefficients higher than sorption coefficients). However, for gemfibrozil and carbamazepine the measured concentration in runoff (Sabourin et al., 2009) was higher than predicted by the current project by a factor of approximately two and four respectively. There are no obvious reasons for this disparity in measured and predicted aqueous concentrations for these two chemicals.

The preliminary hazard assessment conducted in this study found that approximately 69% of the compounds that could be assessed were classed as posing a low hazard to aquatic ecosystems (Figure 2-1). This indicates that even at the maximum concentrations that these compounds have been reported in biosolids, they are not present at concentrations sufficient to adversely affect aquatic ecosystems even given the conservative assumptions of the preliminary hazard assessment. There were a total of ten compounds for which the preliminary hazard assessment could not be completed, due to a lack of available aquatic toxicity data and/or partition coefficient data. Many of these compounds were, however, fragrance compounds that tend to have relatively high log K_{OW} values, ranging from 5.2 to 8.1 (cashmeran and traseolide respectively) and therefore are likely to be bound strongly to the solid phase in soils and have low aqueous concentrations which would in turn lower the hazard that they pose to aquatic systems. A study by Difrancesco et al. (2004) produced results that are consistent with this hypothesis. They did not detect any fragrance

compounds in leachates from a laboratory experiment where biosolids (not spiked with additional compounds) had been mixed with soils. Given the above it is likely that aqueous concentrations of these compounds will be low, but without the toxicity data for the compounds, the hazard cannot be determined.

As discussed previously Sabourin et al. (2009) found measured aqueous concentrations of gemfibrozil and carbamazepine were two and four times larger than those estimated in the present study. Taking these aqueous concentrations into account resulted in the low hazard classification of gemfibrozil not changing while the hazard classification for carbamazepine increased from low to moderate.

Ten compounds were classed as posing a high hazard following the preliminary hazard assessment. All the high hazard compounds were antibiotics, antimicrobials, estrogens or fragrance compounds, more specifically, polycyclic musks. The antimicrobial agents (triclocarban and triclosan), estrogens (17α-ethinylestradiol and 17β-estradiol) and polycyclic musks (galaxolide and tonalide) were all classed as being immobile or having low mobility (Table 2-2). In fact, the only compound of the high compounds that was not classed as immobile was 17α-ethinylestradiol where it was predicted that 1.01% of the total compound would partition into the aqueous phase in soils, which is extremely close to the immobile cut-off of 1%. In the case of the antimicrobial agents (triclosan and triclocarban) and polycyclic musk compounds (galaxolide and tonalide), their high hazard is driven by a combination of high initial biosolids concentrations and high aqueous toxicity. In comparison, for the estrogen compounds, the high hazard is driven by the fact that they exert deleterious effects at concentrations (i.e. 0.03 and 0.42 ng/L for 17α-ethinylestradiol and 17β-estradiol respectively) below concentrations at which they are predicted to occur in water. For example, 17β-estradiol has been found at concentrations

of approximately 3 ng/L in runoff water from agricultural land that has been irrigated with effluent water (Pedersen et al., 2005), while the lowest effect concentration reported is 0.03 ng/L.

The four high hazard antibiotic compounds were predicted to be mobile or highly mobile in soils (Table 2-2) as a result of their relatively low log K_{OW} values. This is the primary reason why they are classed as posing a high hazard. The aquatic toxicity values for these high hazard antibiotic compounds ranged from 5 to 206 μg/L (Table 2-3). Although the mobility of the antibiotic compounds in this study was predicted to be high (e.g. up to 78.6% mobile for norfloxacin), this is likely to be an overestimation as many of these compounds tend to be ionic and the model used in this study was based on the partitioning behaviour of neutral compounds between soil organic carbon (i.e. K_{OC}, K_d and f_{oc}) and soil pore water. Hydrophobic-independent mechanisms such as soil cation exchange, cation bridging on clay surfaces, surface complexation and hydrogen bonding have all been found to be involved in the sorption of many antibiotics to soil (Tolls, 2001). More specifically, these types of interactions have been shown (Blackwell et al., 2007; Stoob et al., 2007; Zhang & Dong, 2008) to strongly influence the sorption of the antibiotics that posed a high hazard or to antibiotics that are in the same class as those. As a result of this, the aqueous concentrations predicted by the current study are likely to overestimate the actual aqueous concentrations. Thus, the results of the hazard assessment for these ionic compounds is conservative (i.e., errs on the side of protecting the environment) as was the initial aim. These additional mechanisms are likely to vary considerably with different soil types and conditions and are poorly understood and therefore difficult to predict. Although there is some evidence that the aquatic hazard posed by these antibiotics has been overestimated, these compounds may still warrant further investigation, as some studies have shown antibiotics to be present in surface run-off (e.g. Davis et al., 2006).

The hazard assessment based on PC95 values estimated by the SSD method could be calculated for eight of the ten high hazard compounds from the preliminary hazard assessment. The exceptions to this were norfloxacin and doxycyline, which did not meet the minimum toxicity data requirements of the SSD method. For several of the compounds, the concentration that was predicted to protect 95% of aquatic species was lower than the minimum toxicity value available in the literature that had previously been used in the preliminary hazard assessment. As calculations of HQ values based on PC95 data (i.e. HQ95) are believed to be more reliable than those calculated in the preliminary hazard assessment (i.e. HQ), these values supersede those obtained previously. In all cases where the PC95 value could be calculated, the hazard classification of all the high hazard compounds did not change. This therefore reinforces the need for further research into the aquatic risks associated with the mobility of these compounds from biosolids amended land. It should be noted however that all the calculated HQ values presented in this study were based on maximum runoff water concentrations which had been calculated from initial maximum biosolids concentrations. If the same PC95 calculations are made based on the median biosolids concentrations, the hazard classification decreased to moderate for triclosan and low for galaxolide and tonalide, indicating that for at least 50% of biosolids, these compounds do not pose a high hazard.

The HQ and HQ95 values produced in this hazard assessment were in some cases relatively high, for example, up to 457 for 17α-ethinylestratiol. These absolute HQ values are likely to be over-estimates of the actual hazard due to the conservative assumptions used throughout the assessment methodology. Two important assumptions in this study were that there was no degradation of the compounds, and no dilution of the runoff entering a waterway. Although some research has been conducted into the degradation of

many PPCPs and EDCs in soils, e.g. triclosan by Ying et al. (2006), the vast majority of these data has been obtained from spiked-degradation experiments. As it is possible that the patterns of degradation observed for organic compounds that are contained within biosolids may vary from those that are spiked into soils, it may be misleading to use these degradation rates in this level of hazard assessment. In addition, degradation rates will vary with site and climatic conditions. The dilution of runoff water was also not considered in this assessment, i.e. no dilution factor was incorporated into the calculations. Therefore, the HQ values obtained throughout this assessment can be used as an initial baseline guide if dilution factors can be estimated for specific sites of concern. For example, if it is estimated that there will be a 10-fold dilution of surface run-off entering a waterway, all HQ values shown in this study will be 10-fold lower. However, it should be noted that site specific dilutions factors will not change the order of priority of the compounds highlighted in this study. In addition to these two assumptions discussed, others used in this study include:

1. the use of maximum biosolids concentrations of the compounds and minimum aqueous toxicity data;
2. the application rate of biosolids is 40 t/ha which is the maximum permissible application rate for a typical Australian biosolids based on its nitrogen concentration; and,
3. the organic contaminants in biosolids are readily available to partition between the biosolids and soil pore-water

There are management strategies in place to further reduce the environmental risk of runoff water from biosolids amended land. Australian guidelines (e.g. EPA NSW, 1997; SA EPA, 1997; DPIWE, 1999; WA DEP, 2002; EPA Victoria, 2004) require that biosolids are applied a minimum distance from adjacent waterways. For example,

biosolids cannot be applied closer than 50m from surface waters in Victoria, Australia (EPA Victoria, 2004). There are also restrictions on the slope of the land and the minimum depth to the water table. All these management strategies will reduce the volume of runoff water that will enter waterways from biosolids amended land and thereby reducing the hazard posed by PPCPs and EDCs to aquatic ecosystems. The extent to which these various management strategies will decrease the hazard posed by organic compounds from biosolids amended land will be site-specific and would therefore be a factor to be addressed in future research focusing on the specific compounds highlighted as a result of this initial screening level assessment.

2.5. CONCLUSION

Overall, this study identified 45 PPCPs and EDCs that had been detected and quantified in biosolids samples globally. Of these 45 compounds, 22% could not be assessed in terms of the hazard they posed to aquatic ecosystems due to a lack of physicochemical data and/or aquatic toxicity data. The majority of PPCPs and EDCs (56%) posed a low or moderate hazard to aquatic ecosystems, indicating that even at the maximum concentrations that these compounds have been detected in biosolids, they are not present at concentrations sufficient to adversely affect aquatic ecosystems. The remaining compounds (22%) posed a high hazard to aquatic ecosystems and included four antibiotic compounds (doxycycline, norfloxacin, ofloxacin and ciprofloxacin), two antimicrobial agents (triclosan and triclocarban), two estrogen compounds (17α-ethinylestradiol and 17β-estradiol) and two polycyclic musk fragrance compounds (tonalide and galaxolide). These groups of compounds should be prioritised in any future research of potential aquatic impacts associated with PPCPs and EDCs in biosolids. However, as the method may have overestimated the hazard posed by the antibiotics they should be given the lowest priority. The absolute HQ values that have been calculated in this study are, however, likely to

overestimate the hazard posed and should therefore be used as a guide. This is due to the conservative assumptions used throughout and the management strategies that are in place to reduce risks associated with land application of biosolids. However, these modifying factors will not affect the relative ranking of the compounds.

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Chapter 3

Concentrations of 4-t-octylphenol, 4-nonylphenol, triclosan, bisphenol A and estrogens in Australian biosolids and an analysis of factors that affect these concentrations

Abstract

Pharmaceuticals and personal care products (PPCPs) and endocrine disrupting compounds (EDCs) are groups of organic contaminants that have been detected in biosolids around the world. In this study, 14 biosolids samples were collected from 13 Australian wastewater treatment plants (WWTPs) to determine concentrations of eight PPCPs and EDCs: 4-t-octylphenol (4tOP), 4-nonylphenol (4NP), triclosan (TCS), bisphenol A (BPA), estrone (E1), 17β-estradiol (E2), estriol (E3) and 17α-ethinylestradiol (EE2). Concentration data were compared to other research and evaluated to determine if differences were observed in samples from WWTP with varying parameters (i.e. stockpiling, treatment, biosolids drying and WWTP location). Only 4tOP, 4NP, TCS, BPA and E1 were detected. Their concentrations ranged from 0.05 to 3.08 mg/kg, 0.35 to 513 mg/kg, < 0.01 to 11.2 mg/kg, < 0.01 to 1.47 mg/kg and < 45 to 370 μg/kg, respectively. Overall, 4NP, TCS and BPA concentrations in Australian biosolids were lower than global averages (by 42%, 12% and 62%, respectively) and higher for 4tOP (by 25%), however, of these differences only that for BPA was statistically significant. The European Union limit value for NP in biosolids is 50 mg/kg, which 4 of the 14 samples in this study exceeded. Different concentrations of 4NP, 4tOP, TCS and BPA were observed in WWTPs with differing parameters (i.e. stockpiled < non-stockpiled; aerobically treated < anaerobically; belt filter press dried < solar < centrifuge; and regional centre < capital city). The data provided from this study will assist in future hazard and risk assessments and management of organic contaminants in biosolids.

CONTENTS

3.1. INTRODUCTION

Pharmaceuticals and personal care products (PPCPs) and endocrine disrupting compounds (EDCs) are two groups of organic contaminants that have received interest recently due to their potential release into the environment following wastewater treatment and the potential for subsequent environmental risks. Environmental research into PPCPs and EDCs has predominantly focussed on their removal from the aqueous phase during wastewater treatment (e.g. Zorita et al., 2009) and their potential deleterious effects to aquatic organisms when released in effluents (e.g. Batty & Lim, 1999; Castro et al., 2007). The removal of PPCPs and EDCs from the aqueous phase occurs via degradation, as a result of treatment processes, or through sorption to the solid waste phase, referred to as biosolids. The levels of PPCPs and EDCs that are found in biosolids may also be of environmental concern, as in many countries, including Australia, biosolids are applied to land as a supplement or replacement for inorganic fertilisers.

Numerous PPCPs and EDCs have been identified in biosolids (e.g. Ternes et al., 2002; Braga et al., 2005; Kinney at al., 2006; Chu & Metcalfe, 2007), however, the potential environmental risks that these compounds pose varies. Eight PPCPs and EDCs were selected for the current study because of environmental concerns, including their potential to cause adverse impacts to aquatic (Langdon et al., in press) and/or terrestrial ecosystems (Waller & Kookana, 2009). The compounds selected were the EDCs 4-nonylphenol (4NP), 4-t-octylphenol (4tOP) and bisphenol A (BPA), the antimicrobial agent triclosan (TCS) and the natural and synthetic estrogenic compounds 17β-estradiol (E2), estrone (E1), estriol (E3) and 17α-ethinylestradiol (EE2).

The surfactant metabolites 4NP and 4tOP and the industrial chemical BPA are all compounds that have been found to mimic natural hormones and interfere with estrogen

receptors in non-target organisms (Jobling & Sumpter, 1993; Jobling et al., 1996; Fukuhori et al., 2005). The compound 4NP tends to be very prevalent in biosolids at concentrations ranging from 600 – 438 000 µg/kg (Kinney et al., 2006). This finding is consistent with the widespread use of the parent alkylphenol ethoxylate compounds in many industrial and domestic surfactant products (Ying et al., 2002). In comparison, the parent compounds that ultimately degrade to 4tOP are used to a lesser extent in surfactant products, resulting in lower biosolids concentrations of this compound, with reported ranges from 167 – 2400 µg/kg (Kinney et al., 2006). The compound BPA, which is used in the production of polycarbonate plastics, epoxy resins and flame retardants (Staples et al., 1998), has been detected in biosolids at a similar range of concentration of 100 – 4600 µg/kg (Kinney et al., 2006).

Triclosan is a commonly used antimicrobial agent found in many domestic personal care products (e.g., soaps, detergents, surface cleaners, disinfectants, cosmetics and other topical personal care products, pharmaceuticals and oral hygiene products), with published concentrations in biosolids ranging from 90 µg/kg (Ying & Kookana, 2007) to 21 740 µg/kg (Campbell-Board, 2005). As TCS is used specifically for its antibacterial properties, its subsequent release into the environment may lead to toxicity to non-target organisms, with a specific risk to micro-organisms. In the recent Targeted National Sewage Sludge Survey (TNSSS), conducted by the United States Environmental Protection Agency (USEPA), TCS was detected in 94% of the samples at concentrations ranging from 0.43 to 133 mg/kg (USEPA, 2009).

The naturally occurring estrogen compound E2, its metabolites E1 and E3, and the synthetic estrogen compound EE2 (the active compound used in the female contraceptive pill) mainly enter the environment via WWTPs, following excretion from humans. These

compounds have received considerable attention recently as they are highly potent compounds and can produce estrogenic responses in non-target organisms at trace concentrations, in the ng/L range (Mills & Chichester, 2005). In the TNSSS, the three naturally occurring estrogens, E1, E2 and E3, were detected in 71%, 13% and 21% of sludge samples with the lowest overall concentrations being for E3 (7.6 to 232 µg/kg) and the highest being for E1 (26.7 to 965 µg/kg) (USEPA, 2009). Other published biosolids concentration values for E1 and E2 range from 12 to 150 µg/kg and 0.31 to 49 µg/kg, respectively (Ternes et al., 2002; Braga et al., 2005; Kinney et al., 2006). In comparison EE2 has been detected in biosolids samples at considerably lower concentrations ranging from 0.42 to 17 µg/kg (Ternes et al., 2002; Braga et al., 2005), and in the TNSSS it was below the limit of detection (LOD) (i.e. < 21 µg/kg) in all samples that were analysed.

The aim of this study was to conduct a survey of Australian biosolids to obtain data on concentrations of 4tOP, 4NP, TCS, BPA, E1, E2, E3 and EE2 and to compare these to previous Australian and global concentration data, as well as threshold limits where available. The data was also assessed to determine if there were any relationships between concentrations of the compounds and other parameters including biosolids treatment, method of drying, stockpiling and the location of the WWTPs (i.e. in capital cities or regional centres).

3.2. MATERIALS AND METHODS

3.2.1. Biosolids sample collection and preparation for analysis

A total of 14 different biosolids samples, each collected as four replicates, were obtained between January and March 2009 from 13 WWTPs located in all six Australian states and the Northern Territory. Personnel at each WWTP collected the four replicates in pre-cleaned 250 mL glass jars with Teflon-lined lids. At the time of sampling, the personnel

filled out an information sheet providing a description of the treatment processes used on the samples. After collection, all samples were placed in insulated containers with ice packs and sent by overnight courier to the laboratory where they were immediately placed in a freezer at -18°C. All samples were then freeze dried, homogenised using a mortar and pestle and sieved to < 2 mm.

3.2.2. Sample extraction and GCMS analysis

All replicates of the 14 different biosolids samples were extracted and prepared for analysis of the eight target compounds, 4tOP, 4NP, TCS, BPA, E1, E2, E3 and EE2. All glassware used for extraction and preparation of the samples had been pre-cleaned by solvent rinsing and baking at 350°C. One day prior to sample extraction, 1 g of each biosolids sample was weighed into a glass tube (i.e. one tube for each replicate). For quality assurance, one of the replicates from each WWTP was duplicated and a method blank was run with each batch of samples. The method blank was an empty glass tube (i.e. containing no biosolids), which was run through the entire extraction and preparation concurrently with the biosolids samples. This was done to ensure that there was no contamination in any of the solvents or sample preparation steps. Two randomly selected samples from each batch were also spiked with labelled surrogates in methanol (i.e. 4nNP-d_8, TCS-$^{13}C_{12}$, BPA-d_{16}, E1-d_4, E2-d_4, EE2-$^{12}C_2$) that were used to determine recoveries. Following surrogate spiking, samples were left overnight in the dark for extraction the following day. Each sample was extracted three times. Each extraction involved adding 10 mL of methanol and acetone (1:1) to the sample and placing the sample in an ultrasonic bath for 10 minutes. After ultrasonication, the sample was centrifuged at 630 × g for 20 minutes and the supernatant decanted into a 500 mL clean glass amber bottle. The subsequent two supernatants were added to the same amber bottle after extraction and centrifugation. The extracts were diluted to 500 mL with MilliQ (MQ) water. The diluted

extracts were loaded onto Oasis HLB® solid phase extraction (SPE) cartridges which had been preconditioned with 5 mL of methanol and equilibrated with 5 mL of MQ water. Sample loading onto cartridges was done using a vacuum manifold at a rate of approximately 2 mL/min. Each SPE cartridge was then washed with 5 mL of MQ water and then dried thoroughly under vacuum. The target compounds were eluted off each SPE cartridge using 3 × 2.5 mL methanol, followed by 3 × 2.5 mL acetone and 3 × 2.5 mL ethyl acetate. Each eluted sample was then blown to dryness under a gentle stream of N_2 gas and reconstituted in 4 mL of methanol. From the 4 mL sample, a 1 mL subsample was taken and blown to dryness using N_2 gas. The sample was then reconstituted in 400 µL of pyridine and 100 µL of the silylation agent *N,O*-bis-(trimethylsilyl)-trifluoroacetamide (BSTFA) + 1% trimethyl-chlorosilane (TMCS) and placed on a dry heating block at 75°C for 1 hr (based on the method of Shareef et al., 2006). This process induces a reaction that converted all target compounds to their respective trimethylsilyl derivatives to increase their suitability for analysis using gas chromatography (GC). Following the derivatization, anthracene d_{10} was added to each sample as an instrument internal standard (IS) prior to GC analysis. A flowchart of the sample extraction and preparation is provided in Appendix A.

For analysis, 2 µL of each sample was injected into an Agilent 6890 Series GC system, fitted with a DB-5MS (30 m × 0.25 mm internal diameter) capillary column with a 0.25 µm film thickness, that was interfaced with an Agilent 5973 Network Mass Spectrometer (MS). The oven temperature was programmed at 75°C for 1 minute, ramped at 10°C / minute to 150°C, then at 15°C / minute to 280°C and remained at this temperature until the completion of the run time of 32 minutes. Helium was used as the carrier gas at a linear flow rate. The MS was operated in electron impact ionisation (EI) mode at 70 eV. Table 3-1 shows the typical retention times of each of the compounds and

the target ion and qualifier ions used for quantification. The relative response factors, which were determined based on the IS, were used to determine the concentrations of each of the compounds in the samples. All samples were adjusted for extraction recoveries based on the concentration of the labelled surrogates in the previously spiked samples. Table 3-1 indicates which labelled surrogates were used for the recovery adjustment of each target compound. The limit of LOD and limit of quantification (LOQ) for each of the compounds was determined as 3-times and 10-times the signal to noise ratio, respectively, and are reported in Table 3-1.

3.2.3. Data interpretation and statistical analysis

The concentrations obtained for each replicate were used to determine the average and range of concentrations for each of the target compounds for the different biosolids samples. For samples where a compound was not above the LOD in all replicates, only the replicates with detectable concentrations were used to determine the average values.

Concentration data for compounds that were detected in all biosolids samples were used to determine if there were any relationships with various parameters that included the effect of extended sample storage (i.e. stockpiling), the treatment and drying used on the samples, and the location of the WWTP from which the sample was collected (i.e. capital city or regional centre). To determine if there were significant ($p < 0.05$) effects of each of the parameters on the concentrations of the compounds, a repeated measures general linear model (GLM) in PASW® Statistics 17 was used. Prior to these statistical analyses, all concentration data were converted using a logarithm to base 10 transformation to normalise the distribution of the data. The repeated measures factor used was the target compounds (i.e. each of the compounds that were detected in all of the samples) and the independent variables were the parameters being tested. For each parameter (with the

exception of drying), there were two factor levels, i.e. stockpiled against non-stockpiled, anaerobic against aerobic treatment (where aerobic treatment included, aerobic digestion, aerobic bioreactors and dissolved air floatation) and capital city against regional centre WWTP locations. For drying, there were three factor levels, belt filter press dried, centrifuge dried and solar dried (e.g. lagoon systems and drying pans). The repeated measures GLMs identified if there were significant ($p < 0.05$) main effects of each of the parameters on the overall concentrations of the target compounds in the samples. In cases where the repeated measures GLM produced a significant ($p < 0.05$) interaction between the target compound concentrations and the parameter being tested (indicating that the effect of the parameter varied between the compounds), univariate GLMs were conducted for each of the compounds individually to determine the effect of the parameter on the individual compounds. For the parameter drying, which had three factor levels, Tukey‟s test was used as a post-hoc assessment to identify the factor levels that were not significantly different from each other. All other parameters consisted of two factor levels (i.e. storage, treatment and location), therefore no post-hoc assessment was required.

Table 3-1: Typical retention times for the internal standard, labelled surrogates and target compounds using gas chromatography mass spectrometry (GCMS) and the corresponding level of detection (LOD) and level of quantification (LOQ).

Compound type	**Compound name**	**Retention time (min)**	**Quantitation ion (*m/z*)**	**Qualifier ion 1 (*m/z*)**	**Qualifier ion 2 (*m/z*)**	**Qualifier ion 3 (*m/z*)**	**LOD (µg/kg)**	**LOQ (µg/kg)**
Internal standard	Anthracene d_{10}	12.31	188	158	94	—		
Labelled surrogates	4nNP-d_8	13.11	185	300	285	—		
	TCS-$^{13}C_{12}$	14.52	206	357	372	322		
	BPA-d_{16}	14.98	368	386	217	—		
	E1-d_4	17.43	346	220	261	246		
	E2-d_4	17.63	420	287	234	220		
	EE2-$^{13}C_2$	18.37	427	232	442	272		
Compounds	4tOP[a]	10.89	207	263	278	—	10	30
	4NP[a]	12.09	207	221	193	179	55	180
	TCS[b]	14.47	200	347	362	310	10	30
	BPA[c]	15.02	357	372	191	—	10	30
	E1[d]	17.43	342	257	244	218	45	150
	E2[e]	17.63	416	285	327	232	45	150
	EE2[f]	18.37	425	285	300	440	45	150
	E3[e]	18.97	311	345	504	386	45	150

Superscripts indicate for each analyte the labeled surrogate that was used to determine recoveries: [a] 4nNP-d_8; [b] TCS-$^{13}C_{12}$; [c] BPA-d_{16}; [d] E1-d_4; [e] E2-d_4; [f] EE2-$^{13}C_2$

3.3. RESULTS AND DISCUSSION

Table 3-2 summarises selected characteristics of each of the biosolids samples collected for this study - including the duration between completion of biosolids treatment and sampling ("age"), the estimated population serviced by the WWTP where the samples were collected, the location (capital city or regional centre) of the WWTP and a brief description of the biosolids treatment processes. Nine of the samples obtained were collected immediately following completion of the biosolids treatment and this is indicated as an age of < 1 day. A further three biosolids samples were aged ≤ 30 days (samples A, B and J) and two samples had been stockpiled on site prior to collection (sample D for one year and sample E for 3-6 years). The estimated population sizes for each of the WWTPs from which samples were obtained ranged from 20 000 to 1.3 million people and there were an equal number of samples obtained from WWTPs that were located in capital cities and regional centres. A range of aerobic and anaerobic treatment processes were used and a range of drying processes, including, belt filter presses, centrifuges and solar drying (e.g. lagoon systems and drying pans).

3.3.1. Data quality assurance and extraction recoveries

The method blanks run with each batch of biosolids samples were below detection for all of the compounds except for 4NP. The concentrations of 4NP in the method blank varied between each run, however ranged from approximately 50 to 200 µg/L in the final solution. These background concentrations of 4NP were subtracted from each of the samples prior to the concentrations being converted to µg/kg. The variation between each of the duplicated samples in the majority of cases was less than 25%. The variation between duplicates was the lowest for 4NP, ranging up to 15%, whereas it was the highest for 4tOP, ranging up to 38%.

Table 3-2: Summary of information collected about the age of the biosolids samples, the waste water treatment plants (WWTPs) that they were collected from, the population size serviced by each WWTP, the location of the WWTP and a brief description of the treatment processes used.

Sample	Age[a] (days)	Population	WWTP location[b]	Treatment description
A	30	45 000	regional	aerobic sludge digestion; dewatered by gravity drainage and then belt filter press
B	17	210 000	capital	belt filter press and thermal hydrolysis; anaerobically digested; centrifuged
C	< 1	70 000	regional	extended aeration; bioreactors (with anoxic and aerobic zones) thickening; dewatered using belt filter press
D	1 yr	1 200 000	capital	activated sludge thickened; anaerobically digested; dried in sludge drying pans (lined with clay)
E	3-6 yrs	40 000	regional	anaerobically digested sludge; dewatered primary lagoon sludge, stockpiled
F	< 1	1 200 000	capital	activated sludge thickened; anaerobically digested; dried in sludge drying pans
G	< 1	24 000	capital	anaerobically digested sludge; dewatered using belt filter press
H	< 1	20 000	regional	aerobic activated sludge treatment; dewatered using polymer and passed over primary belt, lime added
I	< 1	1 300 000	capital	activated sludge; anaerobically digested, dewatered using centrifuge
J	7	135 000	regional	activated sludge; anaerobically digested, dried using lagoon system
K	< 1	na	regional	liquid waste pumped from waste stabilisation ponds; dissolved air flotation tanks used to separate solids
L	< 1	40 000	capital	lime amended, chemically assisted settling of solids, pumped through drum filters
M	< 1	350 000	capital	thickened in dissolved air flotation tanks; mixed with raw sludge; centrifuged; lime added
N	< 1	52 000	regional	aerobically digested sludge; dewatered using belt filter press

[a] duration of time after the completion of treatment that the sample was collected

[b] location of the WWTP in a capital city or a regional centre in Australia

na information not available

The extraction recovery values that were obtained from the spiked surrogate compounds varied considerably between the different biosolids samples, however, the variation between replicates of the same sample was low (all of the recovery data from this study is shown in Appendix B). Overall, the recovery values ranged from 76 to 352% for 4nNP-d_8, 45 to 314% for TCS-$^{13}C_{12}$, 55 to 359% for BPA-d_{16}, 10 to 283% for E1-d_4, 27 to 295% for E1-d_4 and 120 to 382% for EE2-$^{13}C_2$, with average recoveries of 180%, 125%, 125%, 111%, 156% and 230%, respectively. In several cases, for the labelled estrogen compounds (i.e. E1-d_4 and E2-d_4 from sample G and EE2-$^{13}C_2$ from sample G and I), recovery values could not be determined as the concentrations were below the detection limit. Although some of the recovery values obtained in this study are high, a similar range of recoveries was observed in a recent survey of PPCPs in biosolids conducted in the United States (McClellan & Halden, 2010), which reported recovery values ranging from 12 to 493%.

3.3.2. Concentration in biosolids

The estrogen compounds, E2, E3 and EE2 were below the LOD (i.e. 45 µg/kg) in all replicates of all samples. In all 14 biosolids samples, concentrations of 4tOP, 4NP, TCS and BPA were above the LOD, whereas, E1 concentrations were above the LOD in only four samples (F, H, J and L). The averages and ranges of these concentrations are summarised in Table 3-3. The concentrations of the compounds ranged from 0.05 to 5.35 mg/kg for 4tOP, 0.35 to 513 mg/kg for 4NP, < 0.03 (i.e., < LOD) to 11.2 mg/kg for TCS, < 0.03 (i.e., < LOD) to 1.47 mg/kg for BPA and < 0.045 (i.e. < LOD) to 0.37 mg/kg for E1 (Table 3-3). It should be noted, however, that the concentration data provided in Table 3-3 for E1, for samples H, J and L are below the LOQ for this compound and should therefore only be used as an indication of the concentrations of E1 in these samples. Given the lack

of measured E1 concentrations > LOQ in the biosolids, relationships between contaminant concentrations and WWTP parameters were only examined for 4tOP, 4NP, TCS and BPA.

Table 3-3: Summary of concentration data for each of the compounds that were above the limit of detection (LOD). Data shown as an average of the four replicate samples with the range in parentheses

Sample	**Concentration (mg/kg)**				
	4tOP	**4NP**	**TCS**	**BPA**	**E1**
A	0.21 (0.19-0.22)	5.28 (4.92-5.64)	1.15 (1.05-1.25)	0.18 (0.18-0.19)	< LOD
B	2.88 (2.74-3.08)	114 (109-122)	2.77 (2.44-2.93)	1.37 (1.27-1.47)	< LOD
C	0.11 (0.10-0.12)	9.69 (9.18-10.1)	2.57 (2.03-3.60)	0.17 (0.16-0.18)	< LOD
D	0.24 (0.13-0.39)	10.8 (8.83-12.3)	1.32 (1.13-1.48)	0.06 (0.04-0.09)	< LOD
E	0.06 (0.05-0.06)	0.84 (0.67-1.01)	0.22 (0.15-0.29)	0.15 (0.15-0.16)	< LOD
F	2.46 (2.18-2.71)	70.1 (60.9-87.2)	8.49 (7.02-10.2)	0.54 (0.48-0.64)	0.28 (0.17-0.37)
G	5.35 (4.78-5.73)	87.1 (79.7-91.1)	9.89 (8.64-11.2)	0.78 (0.71-0.90)	< LOD
H	0.06 (0.06-0.07)	1.88 (1.70-2.03)	1.83 (1.38-2.76)	0.39 (0.29-0.61)	0.07* (<LOD-0.08)
I	2.78 (2.75-2.83)	464 (418-513)	8.99 (8.18-9.87)	1.03 (0.91-1.13)	< LOD
J	2.94 (2.76-2.89)	10.2 (8.01-15.1)	5.62 (5.02-7.31)	0.10 (0.09-0.14)	0.10* (0.08-0.13)
K	0.09 (0.07-0.11)	0.48 (0.35-0.60)	0.29 (<LOD-0.29)	0.34 (<LOD-0.67)	< LOD
L	0.11 (0.10-0.13)	36.3 (31.9-43.4)	2.74 (2.60-3.01)	0.60 (0.33-0.76)	0.06* (0.05-0.07)
M	0.25 (0.24-0.27)	8.94 (7.57-10.1)	4.74 (3.10-5.47)	0.79 (0.03-1.11)	< LOD
N	0.08 (0.07-0.11)	2.12 (2.05-2.22)	2.19 (2.07-2.34)	0.08 (0.07-0.10)	< LOD
average	1.26	58.7	3.77	0.47	0.13

* sample below limit of quantification (LOQ)

The variation within the replicates from each sample were reasonably low (in 75% of cases, the relative standard deviation, RSD, was ≤ 20%), however there was considerable variability in concentrations between the different biosolids samples. TCS showed the lowest variation between different biosolids samples with a RSD of 84%, whereas 4NP showed the highest variation with a RSD of 208%. For 4tOP, 4NP, TCS and BPA, samples F, G and I overall had the highest concentrations, whereas sample E was consistently low.

Overall, concentrations of 4NP were considerably higher than all the other compounds, with an average concentration of 58.7 mg/kg. This average 4NP concentration is approximately 16-times higher than the next highest compound TCS, which had an average of 3.77 mg/kg. The high concentrations of 4NP measured in biosolids in this study are probably due to the high domestic and industrial use of the parent nonylphenol ethoxylate (NPE) surfactant compounds (Ying et al., 2002).

3.3.3. Comparisons with Australian and global data

Apart from TCS, there is limited data on the concentrations of the compounds measured in this study in Australian biosolids. A study measuring concentrations of TCS in Australian biosolids was conducted by Ying & Kookana (2007), where samples were collected in 2004 and 2005 from 19 WWTP across 4 states, South Australia (SA), Queensland (QLD), Western Australian (WA) and Victoria (Vic) and the Australian Capital Territory. Due to the WWTPs that provided the biosolids not being identified in the current study or that of Ying & Kookana (2007), direct comparisons are not possible between individual WWTPs, however, overall comparisons can be made. The study by Ying & Kookana (2007) measured TCS at concentrations ranging from 0.09 to 16.8 mg/kg with an average concentration of 5.6 mg/kg and a median concentration of 2.3 mg/kg. In the current study,

the median TCS concentration was similar at 2.7 mg/kg, indicating there is little difference in the range of concentrations measured for TCS. However, both the upper limit of the concentration range and the average concentration were lower in the current study at 12.2 mg/kg and 3.8 mg/kg, respectively.

The results from this study can also be compared with the range of concentrations for these compounds that have been measured in biosolids samples globally. Figure 3-1 shows the average and standard error of the concentrations for each of the four compounds detected in biosolids samples A to N and these are compared to the average values for each compound globally (for sources of the global data see Langdon et al., in press). From Figure 3-1 it can be seen that the compounds 4NP, TCS and BPA in this study all have lower averages than the global average. TCS had the smallest difference, being 12% lower than the global average, whereas the differences for 4NP and BPA were much larger being 42% and 62%, respectively. 4tOP was the only compound whose average was higher (by 25%) than the global average. These differences were only significant, however, for BPA ($p = 0.04$), whereas for all other compounds, there is no significant difference between the average from this study and that from the global data (all p-values > 0.32).

The estrogen metabolite compound E1 was detected in four samples (F, H, J and L) in this study, with concentrations of the replicates ranging from 50 to 370 µg/kg, whereas all other samples were below the LOD of 45 µg/kg. The concentrations of E1 measured in sample F are higher than the concentrations measured by Kinney et al. (2006) where the maximum concentration reported was 150 µg/kg. The concentrations of E1 measured in the current study however are within the range of E1 concentrations in biosolids from across the USA in the more recent TNSSS study, which reported concentrations ranging from 26 to 965 µg/kg (USEPA, 2009). The other natural estrogen compounds E2 and E3

and the synthetic estrogen compound EE2 were below the LOD (45 µg/kg) in all samples analysed in the current study. The maximum concentrations of E2 and E3 that were detected in the TNSSS were 355 µg/kg and 232 µg/kg (USEPA, 2009), respectively, which are only marginally higher than the LOQ for the method used in this study. The synthetic estrogen EE2 was not detected in any samples analysed in the TNSSS, however it has been detected up to a concentration of 17 ug/kg (Ternes et al., 2002), which is considerably lower than both the LOQ and LOD for EE2 for the method used in the current study (Table 3-1). These results indicate that in order to obtain significant datasets for estrogens in Australian biosolids a method with increased sensitivity is required.

Currently, the eight compounds that were analysed for in Australian biosolids as part of this study (4tOP, 4NP, TCS, BPA, E1, E2, EE2 and E3) do not require monitoring in biosolids according to the biosolids guidelines from all Australian jurisdictions (e.g. EPA NSW 1997; SA EPA 1997; DPIWE 1999; WA DEP 2002; EPA Victoria 2004; NRMMC, 2004), nor are there any maximum permissible concentrations in soils for these compounds. This is also generally the case internationally, with the exception of 4NP in the European Union (EU). The EU Working Document on Sludge has set a limit value for nonylphenol ethoxylates (NPEs), which comprises the compounds nonylphenol and nonylphenolethoxylates (with 1 or 2 ethoxy groups), of 50 mg/kg (EU, 2000). Four of the fourteen different biosolids sampled in this study (samples B, F, G and I) exceed this EU limit value for NPE in sludge used on land. The high levels of 4NP present in samples B, F, G and I may be partly due to these samples being collected from WWTPs located in capital cities and therefore having high input of industrial chemicals.

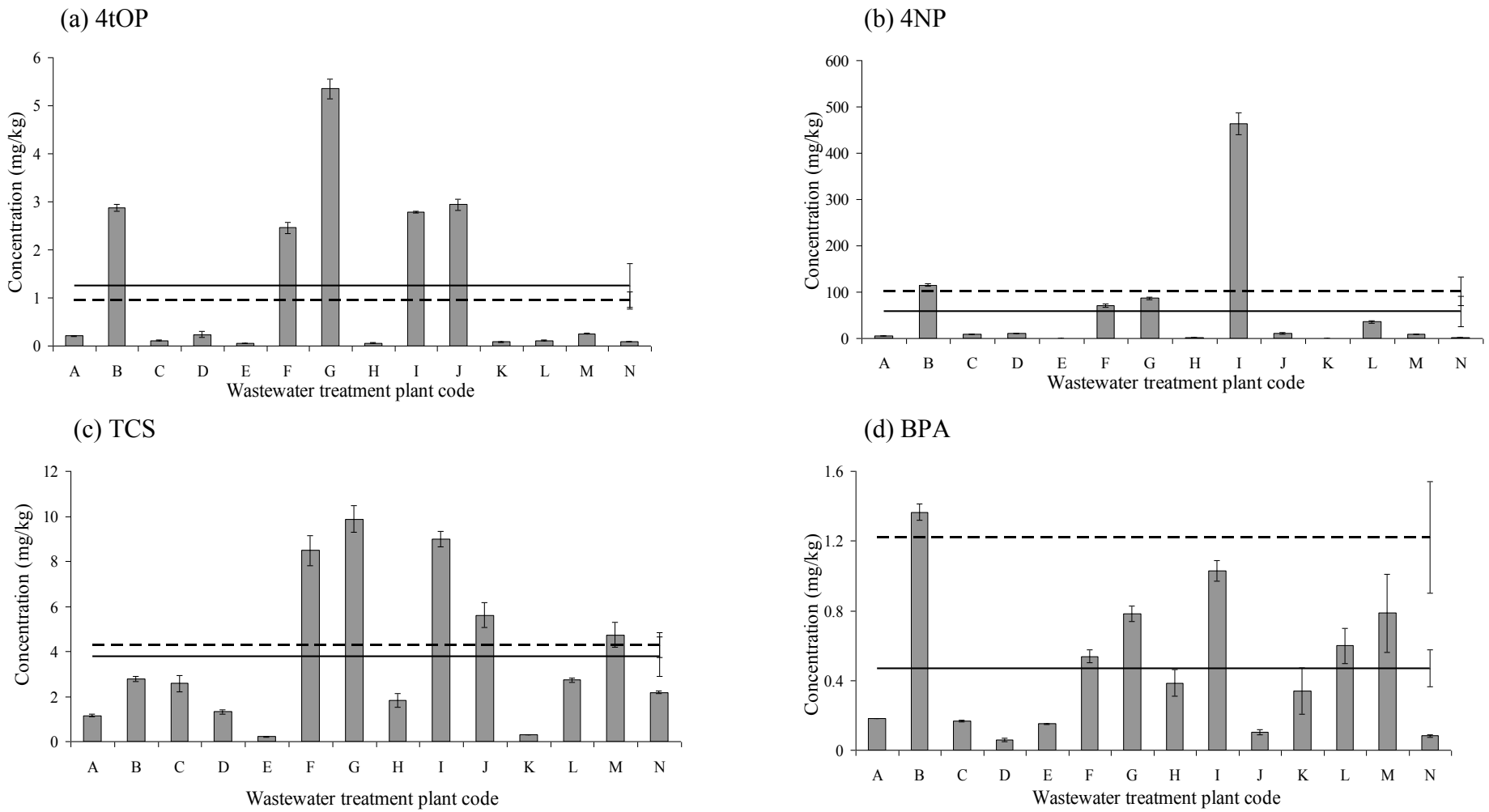

Figure 3-1: The average concentrations of (a) 4tOP, (b) 4NP, (c) TCS and (d) BPA in the 14 biosolids samples analysed in the current study. Error bars indicate the standard error of four replicates. The solid line represents the average across the 14 samples from the current study and the dashed line is the global average as reported in Langdon et al. (in press)

3.3.4. Potential influences of WWTP parameters on concentrations of compounds

The concentrations of the four compounds (i.e., 4tOP, 4NP, TCS and BPA) measured in biosolids samples D and F were compared to determine the effect of ageing due to stockpiling of biosolids. Both of these samples were collected from the same WWTP and had been subject to the same treatment processes, however sample D had been stockpiled for one year whereas sample F was collected immediately following treatment (Table 3-2). For all four compounds the concentrations in the non-stockpiled biosolids were 6- and 10-times higher than those in the stockpiled biosolids and these differences were statistically significant (all p-values < 0.0005). It should be noted, however, that the differences observed between samples D and F may also be due in part to different initial concentrations of these compounds, as although the WWTP and treatment processes were the same, these were different samples in terms of the timing that they entered the WWTP. However, due to the highly significant differences seen between the concentrations of the compounds in these two samples, the data for the samples that had been collected after an extended period of stockpiling (i.e. sample D, stockpiled for 1 year and sample E, stockpiled for 3 to 6 years) were removed from subsequent statistical analyses.

There was a significant ($p < 0.0005$) main effect of treatment type on the concentrations of all four detected compounds, where concentrations were higher in the anaerobically treated biosolids than the aerobically treated biosolids. This result is consistent with other research that indicates that these compounds show minimal degradation under anaerobic conditions (e.g. Brunner et al., 1988; McAvoy et al., 2002; Press-Kristensen et al., 2008). There was a highly significant interaction ($p < 0.0005$) between treatment and compound, as the magnitude of the effect varied between the compounds. The differences in concentration between the treatments were more evident for 4tOP and 4NP which showed

concentrations that were 36% and 27% lower respectively, in the aerobically treated samples than the anaerobically treated samples. In contrast, for TCS and BPA, the concentrations were only 14% and 17% lower respectively, in the aerobically treated biosolids samples.

There were significant differences observed in the concentrations of 4tOP, 4NP, TCS and BPA when the different drying methods were compared. For this parameter, the sample K was removed from the dataset as this sample had not undergone any drying process and was collected from the surface of a dissolved air floatation tank. The results from this analysis showed that there was both a significant main effect of the drying process ($p < 0.0005$) on the concentration of the compounds overall, as well as a highly significant interaction between the compounds and the drying process ($p < 0.0005$). This significant interaction indicated the differences observed for concentrations following different drying methods varied between the compounds. Overall however, the subsets that were derived from the post-hoc tests showed that for all four compounds, samples that had been dried using a belt filter press were in the subset with the lowest concentrations. In comparison, samples that had been centrifuged had the highest concentrations. The significant differences observed for the concentrations in the solar dried samples varied for the different compounds. The solar dried samples showed no significant difference in concentration from the belt filter pressed samples for all the compounds, however, for 4NP and BPA, the solar dried samples were also not significantly different from the centrifuged samples. Therefore, the general trend in concentrations of the compounds across the different drying processes were belt filter press < solar dried < centrifuged. This trend observed for the final concentrations from the different drying processes may be due to variations in the final moisture contents of the biosolids or other factors (e.g. temperature which is likely to be involved in the solar drying more than the other

processes). However, further research would need to be conducted to determine if this is a true cause-effect relationship.

Differences in the final concentrations of all four compounds were observed when WWTPs in capital cities and regional centres were compared. Across all compounds there was a highly significant main effect of location ($p < 0.0005$), whereby concentrations of the compounds in WWTPs located in capital cities was greater than those in regional centres. When tested individually, concentrations of all compounds were significantly different (all p-values ≤ 0.013) between capital city and regional WWTPs, however, the magnitude of the difference varied resulting in a significant ($p < 0.0005$) interaction effect of compound by location. The concentrations of 4tOP, 4NP and BPA were all approximately 23% higher in the biosolids obtained from capital city WWTPs, whereas for TCS it was only 8% higher. One of the likely reasons for the higher concentrations of 4tOP, 4NP and BPA in biosolids from WWTPs located in capital cities is that these compounds can have a high industrial usage and industries tend to be located in capital cities. In comparison TCS, which has predominantly a domestic origin, would not be expected to differ greatly between capital and regional cities which is reflected in the lesser difference observed between the locations. An additional cause of the lower concentrations observed in biosolids from regional centres may be the underlying effect of the treatment and drying processes on the final concentrations of 4tOP, 4NP, TCS and BPA. The WWTPs in the regional centres sampled in this study used predominately (i.e., in 5 out of 7 cases) aerobic treatment, whereas in the WWTPs in the capital cities, predominately anaerobic treatment was used (i.e., in 6 out of 7 cases). In addition, the majority of belt filter press-dried biosolids (i.e., in 4 out of 6 cases), which had significantly lower concentrations of the compounds, were obtained from regional centres, whereas, all the centrifuge dried biosolids, which had significantly higher concentrations

of the compounds, were obtained from capital city WWTPs. The combination of aerobic treatment and belt filter press drying used in the WWTPs sampled in regional centres is likely to have contributed to the significantly lower concentrations in these samples. Therefore, the differences seen between concentrations of the compounds in samples from both locations (i.e. capital vs regional) may be due to variations in input concentrations as well as an underlying effect of the treatment and drying of the biosolids within each WWTP.

3.4. CONCLUSION

Fourteen biosolids samples were collected from 13 WWTPs across Australia to determine levels of eight selected pharmaceutical and personal care products and endocrine disrupting chemicals (i.e., 4-t-octylphenol, 4tOP; 4-nonylphenol, 4NP; triclosan, TCS; bisphenol A, BPA; estrone, E1; 17β-estradiol, E2; estriol, E3; and 17α-ethinylestradiol, EE2). The estrogen compounds E2, EE2 and E3 were below detection in all of the samples, whereas E1 was detected in four of the 14 samples. 4tOP, 4NP, TCS and BPA were detected in all 14 biosolids samples with 4NP detected at the highest concentrations (average of 58.7 mg/kg) and BPA at the lowest concentrations (average of 0.47 mg/kg). The concentrations of BPA were lower in the current study than those that have been measured globally. The concentrations of 4tOP and 4NP in this study were similar to global concentrations, however, in four of the samples, concentrations of 4NP exceeded the EU threshold limit for NPEs in sludge used in land application. The concentrations of TCS in this study were also similar to global concentrations, however, the average concentration was approximately 30% lower than an earlier Australian study. Significant differences in concentrations of the compounds were observed for all the WWTP parameters tested, which included stockpiling (concentrations in stockpiled biosolids were lower than non-stockpiled biosolids), the process used for biosolids treatment

(concentrations in aerobically treated samples were less than anaerobically treated samples), the process used to dry the biosolids (the overall trend of final concentrations was belt filter press < solar dried < centrifuge) and whether the WWTP was from a capital or regional city (with capital city WWTPs having higher concentrations than regional WWTPs). The information generated in this study will assist with future hazard and risk assessments and the management of organic contaminants in biosolids.

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Chapter 4

Persistence of 4-nonylphenol, 4-t-octylphenol, triclosan and bisphenol A following biosolids addition to land. Part A: Dissipation under laboratory conditions

Abstract

The reuse of biosolids through land application is common practice in many countries including Australia, however, there are some potential risks associated with the presence of contaminants within the biosolids. In order to assess the risks associated with this practice, an understanding of the persistence of these contaminants is required. The following study examined the dissipation of four organic contaminants, 4-nonylphenol (4NP), 4-t-octylphenol (4tOP), bisphenol A (BPA) and triclosan (TCS), in soils following the separate addition of two biosolids for a period of 32 weeks. The pattern of dissipation was also assessed to determine if it followed a first-order decay model or if a biphasic model with a recalcitrant fraction better described the data. The time taken for 50% of the initial concentrations of the compounds to dissipate (DT50) in the two biosolids amended soils, based on a standard first-order decay model, was 12 to 25 days for 4NP, 10 to 14 days for 4tOP, 18 to 102 days for BPA and 73 to 301 days for TCS. For 4NP, BPA and TCS, a biphasic model fitted the dissipation data better than the first-order model. The remaining or recalcitrant concentrations of these compounds were 17 to 21%, 24 to 42% and 30 to 51% of the initial concentrations, respectively, which corresponded to 297 – 2480 µg/kg for 4NP, 2.4 – 2.5 µg/kg for BPA and 94 – 108 µg/kg for TCS. The reasons for the presence of a recalcitrant fraction of these compounds are not clear but may be due to the presence of anaerobic zones within biosolids aggregates and/or non-reversible sorption of the compounds. For 4tOP, the first-order model was sufficient in explaining the dissipation, indicating that there was no recalcitrant fraction of this compound. This study showed that the biosolids matrix may influence the rate and pattern of dissipation of organic compounds in soils and that the use of first-order models may underestimate the persistence of some organic contaminants in biosolids amended soils.

CONTENTS

4.1. INTRODUCTION

The reuse of biosolids through application onto agricultural land is a process that can be beneficial to the growth of plants and crops, as well as minimising the need for waste disposal by less sustainable means. Biosolids, however, tend to contain a broad range of organic contaminants (e.g. Kinney et al., 2006; USEPA, 2009; Langdon et al., in press), therefore this practice can be a route of entry for these compounds into the environment. Four specific organic compounds that have received increasing interest recently due to their potential adverse environmental effects, as a result of their toxicity and/or their ability to mimic natural hormones, are the surfactant metabolites, 4-nonylphenol (4NP) and 4-t-octylphenol (4tOP), the plasticiser bisphenol A (BPA) and the antimicrobial agent triclosan (TCS). These four compounds have been detected in biosolids at a range of concentrations, up to 438 000 µg/kg, 2400µg/kg, 4600 µg/kg (Kinney et al., 2006) and 21 700 µg/kg (Campbell-Board, 2005), respectively. When assessing the potential risk that these compounds may pose to the environment following the application of biosolids to land, the time required for the compounds to degrade is an important factor that needs to be considered.

The compounds 4NP and 4tOP are derived from their parent alkylphenol ethoxylate (APE) compounds which are widely used in both domestic and industrial surfactant products (Ying et al., 2002). The most significant environmental concern following the release of 4NP and 4tOP into the environment is that they have been found to mimic natural hormones by interacting with estrogen receptors (e.g., Jobling & Sumpter, 1993; Jobling et al., 1996). The estrogenic activity of these compounds is fairly weak, however, particularly for 4NP, concentrations in biosolids can range up to high mg/kg concentrations (Kinney et al., 2006). The degradation of 4NP in soils has been assessed in some studies by measuring the mineralization of the compound using the ^{14}C-labelled

isotope. The half-lives from these studies range from 1 (Roberts et al., 2006) to 17 days (Topp & Starratt, 2000), with differences likely to be due to variations in soil properties. Slightly slower degradation rates have been observed for this compound in a more recent glasshouse study measuring concentrations, over 45 days, following biosolids addition to soil. The reported half-life values from that study ranged from 16 to 23 days (Brown et al., 2009). At the completion of this 45 day study however, 15 – 30% of the initial 4NP still remained in the soil (Brown et al., 2009). For 4tOP, average half life values of approximately 5 days have been reported from spiked degradation experiments in a range of soils (Ying & Kookana, 2005).

The compounds BPA and TCS have both also been shown to mimic natural estrogens (Fukuhori et al., 2005; Veldhoen et al., 2006; Crofton et al., 2007) and in the case of TCS, a significant level of aquatic and terrestrial toxicity have also been observed (e.g Orvos et al., 2002; Ishibashi et al., 2004; Waller & Kookana, 2009). The reported half-lives for BPA, in various soils, range from 1 to 7 days (Ying & Kookana, 2005; Xu et al., 2009) in spiked degradation experiments. The degradation of TCS has been shown to vary and has been reported to take place relatively quickly, with half-life values of 13 to 18 days (Ying et al., 2007; Xu et al., 2009), however half lives up to 58 days have been reported in another study (Wu et al., 2009a), also from spike degradation experiments.

When conducting degradation or dissipation experiments for organic compounds in soil, the rate is generally presented as a half-life or DT50 value, i.e., the time taken for the initial concentration of a compound to decrease by 50% (by either degradation or dissipation). These values are based on the rate constant derived from the fitting of a standard first-order exponential decay model to the data. DT50 values provide an overall summary of the degradation/dissipation of a compound, which is generally required for

comparison between different studies. The first-order degradation model makes two assumptions: (i) the concentration of the compound approaches zero, therefore, the entire amount of the compound present is subject to degradation, and (ii) the degradation rate is independent of concentration, and therefore the rate can be represented by a single value, i.e. half-life or DT50. Some research has shown however that although a compound might show a small DT50 value in a soil, therefore predicting a fast dissipation rate, accumulation over time can still be observed (Ciglasch et al., 2006). This indicates that the single value of a DT50 may not be appropriate in describing the degradation behaviour of some compounds in soils. It has been highlighted, predominantly in pesticide dissipation research, that a simple first-order degradation model is an oversimplification of a complex system, and the degradation is often more accurately described by biphasic degradation models (e.g. Hill & Schaalje, 1985; Ma, et al., 2004; Sarmah & Close, 2009). The degradation of organic compounds in biosolids or biosolids amended soils has also been shown in some cases to exhibit a biphasic pattern (Hesselsoe et al., 2001; Sjostrom et al., 2008; Wu et al., 2009b), therefore indicating that degradation described by half lives or DT50 values in these systems may be misleading.

Biphasic degradation of organic compounds is often described using a two-compartment model where both fractions of a compound are degrading - one compartment degrading "fast" and the other compartment degrading "slow" (e.g. Hill & Schaalje, 1985; Ma, et al., 2004; Sarmah & Close, 2009). In biosolids, or biosolids amended soils, similar biphasic degradation patterns have sometimes been observed. For example Sjostrom et al. (2008) who assessed the degradation of NP contained within sewage sludge following application to soil, and Wu et al. (2009b) who assessed the sorption and degradation of six antibiotics in a digested biosolids. In both of these studies, although a "slow" degrading fraction was

present, the degradation rate constant of this fraction was either zero or not significantly different from zero, indicating that this fraction was non-degrading or recalcitrant.

The aim of this study was to determine the rate of dissipation of 4NP, 4tOP, TCS and BPA which were native to biosolids (i.e. non-spiked), following addition to a soil, under controlled laboratory conditions over a period of 32 weeks (i.e. 224 days). By conducting the study under controlled laboratory conditions, with constant temperature and moisture, any external influences caused by variations in climatic conditions were removed. The pattern of dissipation of the compounds was also assessed to determine if it was consistent with simple first-order model or a biphasic model indicating the presence of a recalcitrant fraction. This study was conducted as part of a larger study which also examined the same aims under field conditions. This study is the first of a two-part study assessing the dissipation of these compounds in a biosolids amended soil. Chapter 5 describes the results of the second part of this study, in which dissipation of the compounds was studied under field conditions.

4.2. MATERIALS AND METHODS

4.2.1. Soil and Biosolids

A bulk soil sample was collected from a field site at Mount Compass in South Australia (SA) (35°21‟44.95 S and 138°32‟44.95 E), which is located approximately 70 km south of Adelaide, for use in this study. This soil had a pH of 4.4, which was determined from a soil solution ratio of 1:5 in 0.01M $CaCl_2$, an organic carbon content of 2.5%, and consisted of 96% sand, 2.5% silt and 1.5% clay. The bulk sample was dried at 40°C prior to being homogenized by grinding with a mortar and pestle and sieved to 2 mm. A subsample from this bulk soil was then taken for chemical analysis using the method outlined below to

ensure that there were no background concentrations of the target compounds (i.e. 4tOP, 4NP, TCS and BPA) prior to the commencement of all experimental work.

Two South Australian biosolids were collected and used in this study. Both biosolids had been treated by anaerobic digestion, but thereafter one of the biosolids had been centrifuge dried (CDB) and the other had been solar dried in a lagoon system (LDB). The CDB was collected immediately following centrifugation, whereas the LDB was collected from a stockpile that had completed treatment less than one month prior to collection. The moisture contents of the biosolids were 63% for the CDB and 52% for the LDB and for the experiment the biosolids were used as collected (i.e., wet).

4.2.2. Experimental design and set up

Individual 50 g samples were weighed from the dried bulk soil into glass jars and hydrated to 50% of their maximum water holding capacity (MWHC) with Milli Q (MQ) water (the method used to determine the MWHC is outlined in Jenkinson & Powlson, 1976). All samples were then placed in closed containers in the dark and pre-incubated at 22°C for 14 days to rejuvenate and stabilise soil microbial communities. After the pre-incubation either the CDB or LDB biosolids were added to the hydrated soil, at a rate equivalent to 50 dry t/ha (assuming a soil bulk density of 1.3 g/cm^3 and an incorporation depth of 10 cm). Five replicate samples from each biosolids treatment were then immediately freeze dried and stored in the dark until analysed as the initial sample (t_0). All the remaining sample jars were weighed, then placed on wet paper towel in containers with lids and kept in the dark at a constant temperature of 22°C. The samples were opened to the air on a daily basis and the moisture content in the soil was maintained throughout the experiment by weight at 50% MWHC. At eight additional sampling intervals (3, 7, 14, 28, 56, 112, 168 and 224

days post biosolids addition), triplicate sample jars were removed from each of the biosolids treatments and freeze dried for immediate analysis of the target compounds.

4.2.3. Sample extraction and GCMS analysis

For sample extraction and analysis, 10 g from each freeze dried sample was extracted three times with 1:1 methanol and acetone in an ultrasonic bath. For each sample the extracts were combined then diluted with MQ water and loaded onto Oasis HLB® solid phase extraction (SPE) cartridges. Elution of the samples was conducted using 3 × 2.5 mL methanol, followed by 3 × 2.5 mL acetone and 3 × 2.5 mL ethyl acetate and reconstituted in 4 mL of methanol. Each sample was then derivatized in 400 µL of pyridine and 100 µL of the silylation agent *N,O*-bis-(trimethylsilyl)-trifluorocetamide (BSTFA) + 1% trimethyl-chlorosilane (TMCS) (based on the method of Shareef et al., 2006) and anthracene-d_{10} was added to each sample as an instrument internal standard (IS). Samples were analysed using an Agilent 6890 Series GC system that was interfaced with an Agilent 5973 Network Mass Spectrometer (MS). The concentrations of each of the compounds were determined from relative response factors based on the IS and then adjusted for extraction recoveries based on labelled surrogates (i.e. TCS-$^{13}C_{12}$, BPA-d_{16} and 4nNP-d_8) which were spiked into the samples one day prior to extraction. The limit of detection (LOD) and limit of quantification (LOQ) for each of the compounds were determined as 3- and 10-times the signal to noise ratio and were, 30 and 100 µg/kg respectively for 4NP, 0.6 and 2.0 µg/kg respectively for 4tOP, 0.3 and 1.0 µg/kg respectively for BPA, and 0.8 and 2.7 µg/kg respectively for TCS.

4.2.4. Statistical analysis and interpretation

Prior to any statistical analysis all the concentration data were converted to a ratio of the initial concentration (C_t/C_0). This normalised the data to an initial mean value of 1 and removed any variation at t_0 between the biosolids treatments and the compounds.

4.2.4.1. Analysis of variance

The normalised concentration data were analysed statistically in PASW Statistics® Version 17, using a univariate analysis of variance (ANOVA) with the independent factors of time and biosolids treatment. Time was treated as an independent factor (as opposed to a repeated measures factor), as the samples in the experiment were in individual jars and therefore were not necessarily correlated to each other. An ANOVA was conducted on the dissipation data of each of the compounds individually to produce significance levels for the main effects of time and biosolids treatment on the concentrations of the compounds, as well as the interaction of time and biosolids at a significance level of $\alpha = 0.05$.

4.2.4.2. Nonlinear regression to determine the dissipation rate and pattern of the compounds

Nonlinear regression modelling was conducted on the normalised concentration data for each of the compounds within both biosolids treatments, using SigmaPlot®. There were two models used to predict the dissipation patterns of each of the compounds based on first-order kinetics which are outlined below.

The first model was a standard first-order exponential decay model with two fitting parameters and is represented by equation 4-1,

$$C_t = C_0 e^{-kt} \qquad (4\text{-}1)$$

where C_t is the concentration of the compound at time t, C_0 is the initial concentration of the compound and k is the dissipation rate constant. The significance of the nonlinear regression is produced by SigmaPlot® to show if the degrading model provides a statistically better fit to the data (by comparing the residual sums of squares and total sums of squares) than that of a one parameter model indicating no change (using a significance level of $\alpha = 0.05$). The rate of dissipation, presented as a DT50, was also determined from the first-order regression fit using equation 4-2.

$$DT50 = ln(2) / k \qquad (4\text{-}2)$$

A visual representation of the first-order model, with the calculation of the DT50 value from this model is presented in Figure 4-1.

The term DT50 was used in this study as it refers to the dissipation of the compounds rather than specifically to the degradation, therefore incorporating other factors, for example, leaching, runoff and volatilisation, which may play a role in decreases in concentration. These additional factors are unlikely to play a considerable role in a laboratory experiment such as the one conducted in this study, however they may play a more significant role under field conditions. As this study is part of a larger study, that also involves a field-based study, the term dissipation was used for consistency.

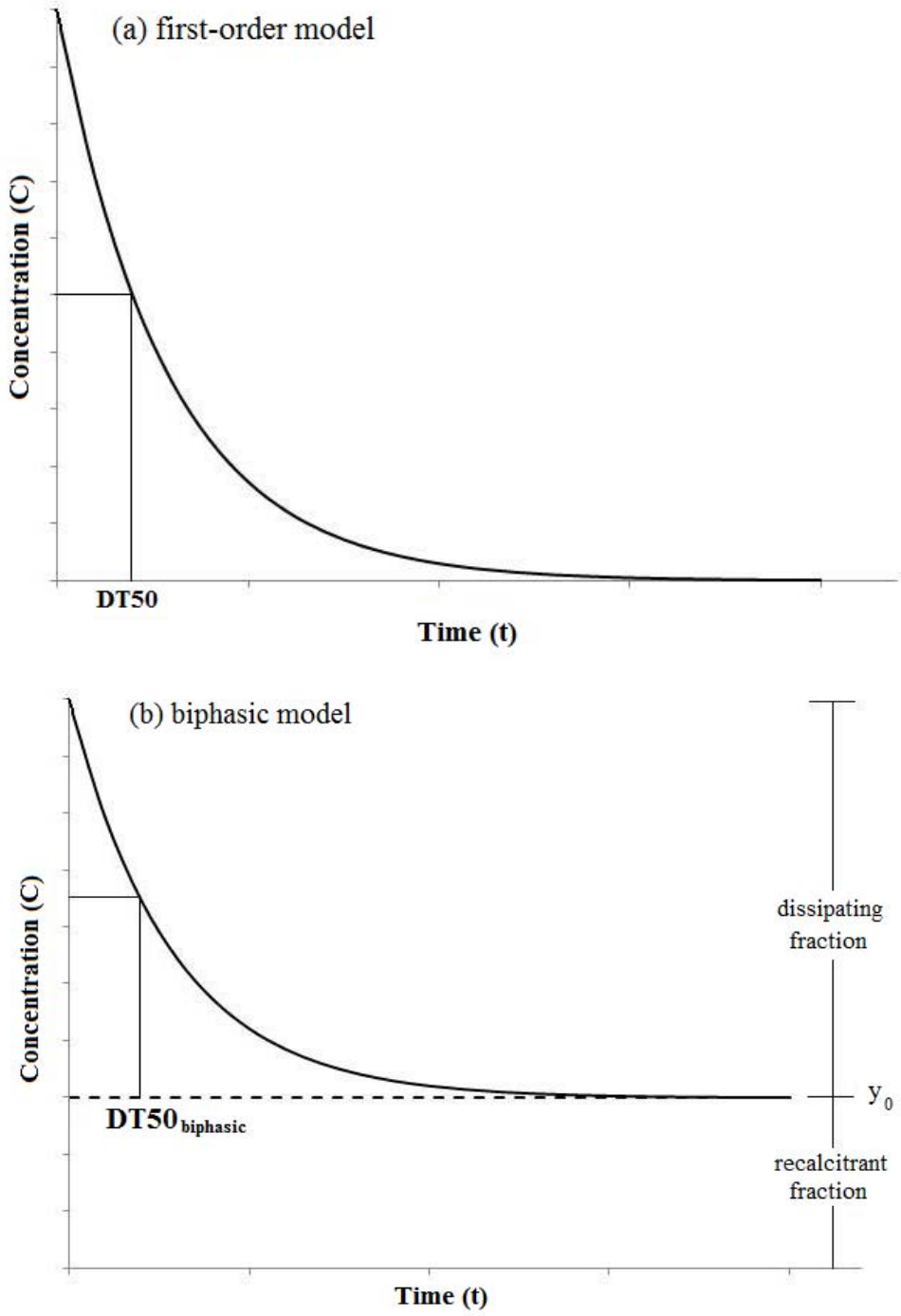


Figure 4-1: Schematic diagram of (a) a first-order model and how the dissipation half-life (DT50) was calculated and (b) a biphasic model and how the biphasic dissipation half-life ($DT50_{biphasic}$) was calculated and indicating the dissipating and recalcitrant fraction. The concentration of the recalcitrant fraction is indicated by the y-intercept (y_0).

A comparison of the fit to the first-order and biphasic models was carried out by calculating the F statistic (F_{stat}) value from the residual sums of squares (RSS) from each model as shown in equation 4-5,

$$F_{stat} = \frac{(RSS_{FO} - RSS_{BI})/(df_{FO} - df_{BI})}{(RSS_{BI} / df_{BI})} \quad (4\text{-}5)$$

where, RSS_{FO} and RSS_{BI} are the RSS for the first-order and biphasic models respectively, and df_{FO} and df_{BI} are the degrees of freedom for the first-order and biphasic models, respectively. In all cases $df_{FO} - df_{BI} = 1$, i.e. the biphasic model has one less degree of freedom than the first-order. The F_{stat} values were converted to probability values (p-values) using the F_{dist} function in Microsoft Excel®. If a p-value was less than 0.05 then the addition of the extra fitting parameter significantly improved the fit of the data and the biphasic model was considered the better fit. Conversely, if a p-value was greater than 0.05 then the extra fitting parameter did not significantly improve the fit of the data and therefore there was considered to be no statistical merit in the additional fitting parameter of the biphasic model.

4.3. RESULTS

The initial average concentrations of the t_0 samples ranged from 1.69 to 11.8 mg/kg for 4NP, 73 to 129 µg/kg for 4tOP, 5.9 to 9.8 µg/kg for BPA and 184 to 361 µg/kg for TCS (Table 4-1). For each compound there were variations in concentration between the biosolids treatments (Table 4-1), with the concentrations of TCS, BPA and 4tOP being approximately two-times greater in the LDB treated soil than the CDB treated soils. For 4NP, the initial concentrations were approximately 7-times higher in the CDB treated soils than the LDB treated soils. Following the initial t_0 sample, the concentrations of all four

compounds remained above their LOQ throughout the 224 day duration of this study. The dissipation of these compounds is shown in Figures 4-2, 4-3, 4-4 and 4-5 for 4NP, 4tOP, BPA and TCS, respectively, along with the fits for the first-order and biphasic models. For both of the models fitted to the data, the coefficient of determination (R^2), DT50, $DT50_{biphasic}$, recalcitrant fraction (y0, as a proportion of the t_0 concentration), the significance of the fits and which of the models best fitted the data are presented in Table 4-2.

Table 4-1: The average and range of concentrations of the compounds 4-nonylphenol (4NP), 4-t-octylphenol (4tOP), bisphenol A (BPA) and triclosan (TCS) in the initial (t_0) sample for the centrifuge dried biosolids (CDB) and lagoon dried biosolids (LDB) treatments.

Biosolids treatment	**Initial compound concentration (µg/kg)**			
	4NP	**4tOP**	**BPA**	**TCS**
CDB	11800 (7780-16600)	73 (40-105)	5.9 (4.1-8.1)	184 (146-236)[a]
LDB	1690 (607-2480)	129 (53-193)	9.8 (5.0-15)	361 (238-503)

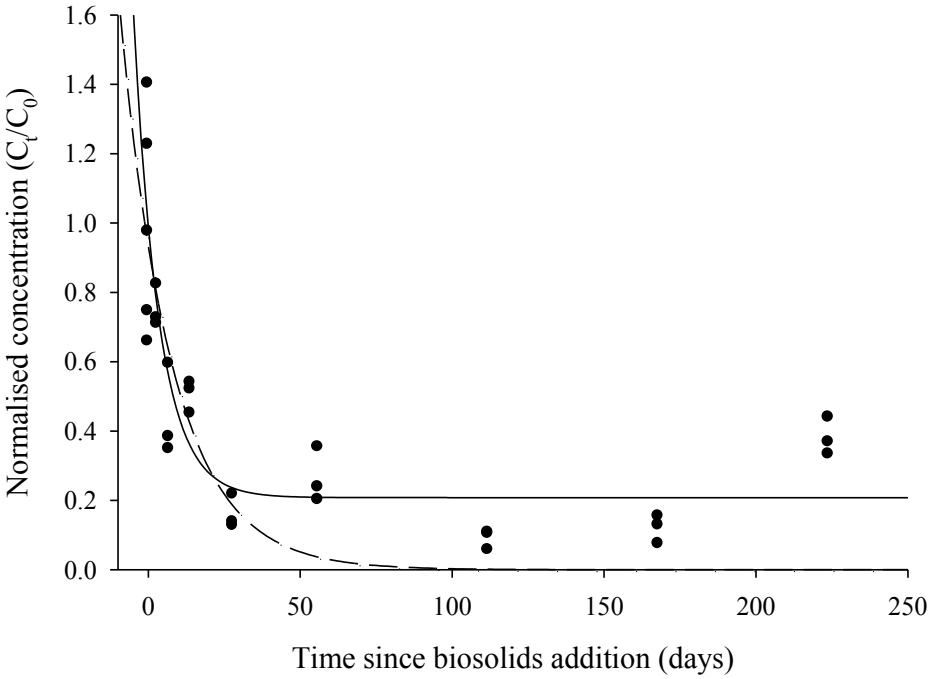
[a] The actual upper limit of this range was 462 µg/kg, however this value was removed as an outlier

Table 4-2: Summary of the degradation information from the first-order and biphasic models for the compounds 4-nonylphenol (4NP), 4-t-octylphenol (4tOP), bisphenol A (BPA) and triclosan (TCS) for the centrifuge dried biosolids (CDB) and lagoon dried biosolids (LDB) treatments. The dissipation half lives determined using the first-order and biphasic models (DT50 and $DT50_{biphasic}$ respectively) are shown in days and the y-intercept (y_0) values correspond to the C_t/C_0 values. The significance values were calculated using equation 4-5.

Model	**Measure**	**4NP**		**4tOP**		**BPA**		**TCS**	
		CDB	**LDB**	**CDB**	**LDB**	**CDB**	**LDB**	**CDB**	**LDB**
first-order	**R^2**	0.62	0.68	0.81	0.79	0.29	0.55	0.17	0.57
	DT50	12	25	14	10	102	18	301	73
	p-value[a]	<0.001	<0.001	<0.001	<0.001	0.003	<0.001	0.03	<0.001
biphasic	**R^2**	0.78	0.73	0.83	0.80	0.53	0.68	0.58	0.76
	$DT50_{biphasic}$	5.8	14	9.9	8.7	8.7	7.7	1.2	6.3
	y_0	0.21	0.17	0.10	0.06	0.42	0.24	0.51	0.30
	p-value[b]	<0.001	0.04	0.07	0.34	0.001	0.003	<0.001	<0.001
	best fit	biphasic	biphasic	first order	first order	biphasic	biphasic	biphasic	biphasic

[a] significance of the first-order model; [b] significance of the biphasic model compared to the first-order model

(a) CDB



(b) LDB

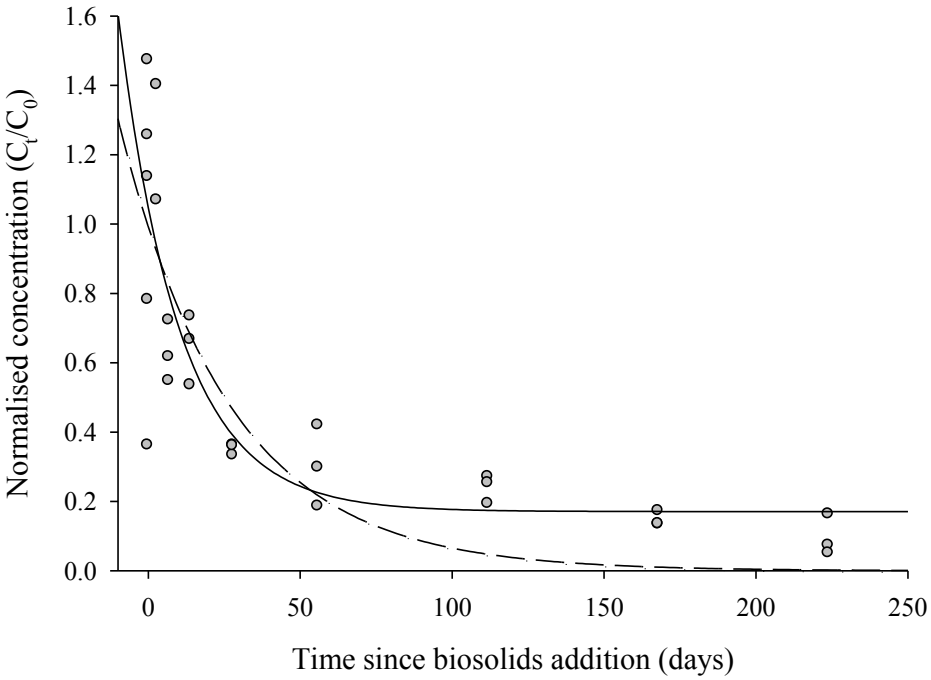
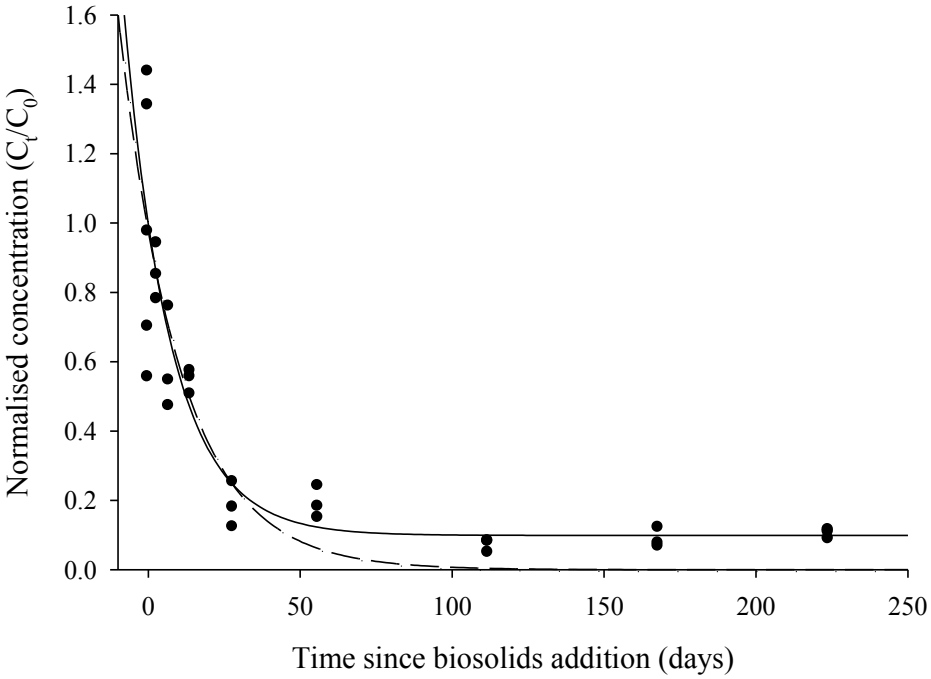


Figure 4-2: Dissipation of 4-nonylphenol following the addition of (a) centrifuge dried biosolids (CDB) and (b) lagoon dried biosolids (LDB) to a soil. All concentration data is normalised as a ratio of the concentration at each sampling interval to the initial concentration (C_t/C_0). The nonlinear regression fits for first-order model and biphasic models are represented by the dashed line and the solid line, respectively.

(a) CDB



(b) LDB

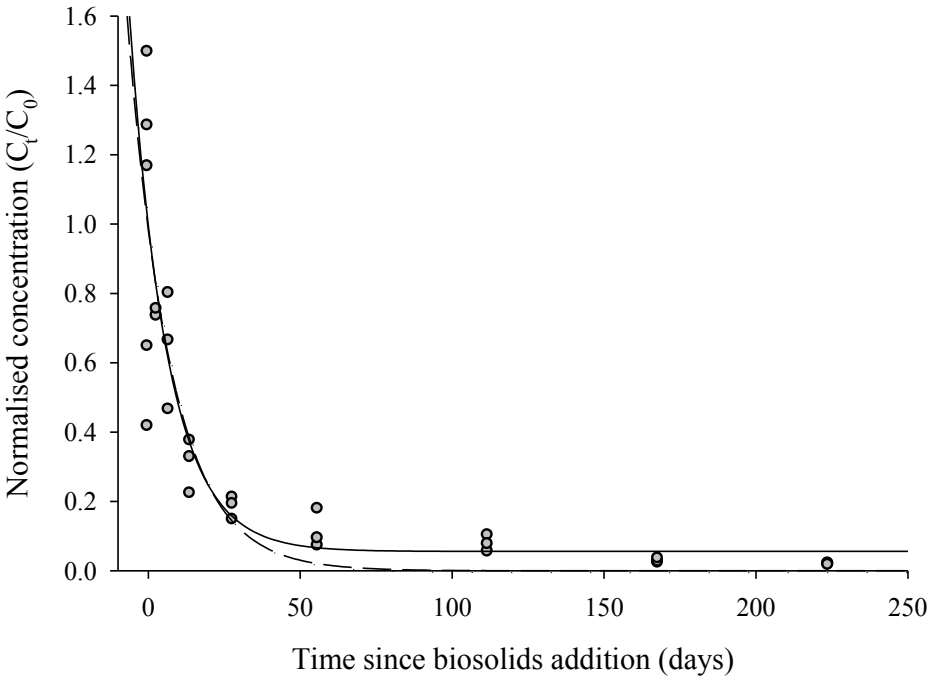


Figure 4-3: Dissipation of 4-t-octylphenol following the addition of (a) centrifuge dried biosolids (CDB) and (b) lagoon dried biosolids (LDB) to a soil. All concentration data is normalised as a ratio of the concentration at each sampling interval to the initial concentration (C_t/C_0). The nonlinear regression fits for first-order model and biphasic models are represented by the dashed line and the solid line, respectively.

4.3.1. Dissipation of 4-nonylphenol (4NP) from biosolids amended soils

There was significant dissipation of 4NP following the addition of both biosolids treatments to the soil over the 224 days of the study (Figure 4-2), indicated by the main effect of time ($p < 0.0005$). For both biosolids treatments, at 7 days post biosolids application, the concentration of 4NP was significantly lower ($p < 0.05$) than the t_0 concentration, however, from 28 days to the completion of the experiment (i.e. 224 days) there was no significant change ($p > 0.05$) in concentration. There was also a main effect of biosolids treatment on the 4NP concentration throughout the experiment ($p = 0.008$), with the overall concentration in the CDB treatment being significantly higher than the LDB treatment. This difference was driven mainly by the differences between the two treatments at 224 days (Figure 4-2). The interaction of time by biosolids for 4NP was non-significant ($p = 0.632$)

The fit of the first-order model for the 4NP dissipation data to both biosolids treatments was significant (both p-values < 0.001) and had R^2 values of 0.62 and 0.68 for the CDB and LDB treatments respectively (Figure 4-2 and Table 4-2). The DT50 values for 4NP obtained from this model were 12 and 25 days for the CDB and LDB treatments, respectively. The statistical comparison of the two models (i.e. first-order and biphasic) to the 4NP dissipation data, showed that the biphasic model explained the data significantly better than the first-order model (both p-values ≤ 0.04, Table 4-2). The effect of adding the third parameter in the biphasic model on the fit to the data was more marked for the CDB treatment ($p < 0.001$) than for the LDB treatment ($p = 0.04$). The $DT50_{biphasic}$ values for 4NP were 5.8 days in the CDB treatment and 14 days in the LDB treatment (Table 4-2). The biphasic model fitted to the data dissipation data produced y_0 values (i.e., C_t/C_0) for the CDB and LDB treatments of 0.21 and 0.17 respectively, indicating 21% of the initial concentration of 4NP in the CDB treatment and 17% of the initial concentration of 4NP in

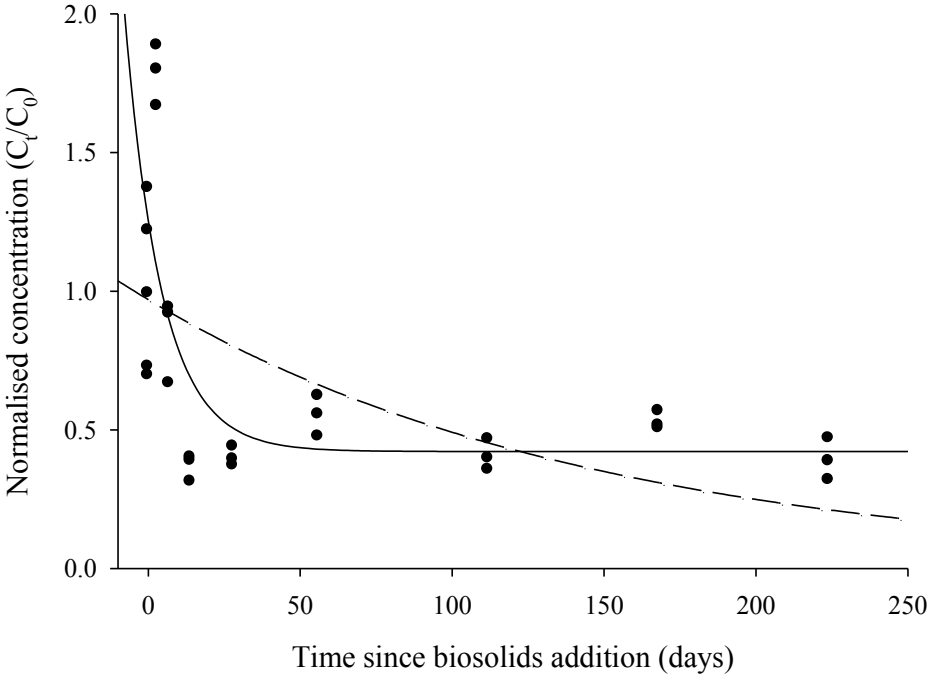
the LDB treatments was recalcitrant. These recalcitrant fractions corresponded to 4NP concentrations of 2500 μg/kg in the CDB treatment and 290 μg/kg in the LDB treatment at the completion of this study.

4.3.2. Dissipation of 4-t-octylphenol (4tOP) from biosolids amended soils

There was significant dissipation of the compound 4tOP over the 224 days of this study (Figure 4-3), indicated by the significant main effect of time ($p < 0.0005$). The first significant difference in concentration observed from the initial 4tOP concentration occurred 7 days post biosolids addition, however, from 28 days post addition through to the completion of the experiment (i.e. 224 days), there was no significant changes in the concentration of 4tOP. The main effect of biosolids and the interaction of time by biosolids were both non-significant (p-values 0.123 and 0.776, respectively).

The fit of the first-order model to the 4tOP normalised dissipation data was significant for both the biosolids treatments (both p-values < 0.001) and also produced high R^2 values (0.81 for the CDB treatment and 0.79 for the LDB treatment) (Figure 4-3 and Table 4-2). The DT50 values obtained for 4tOP from this model were 14 days for the CDB treated soil and 10 days for the LDB treated soil. The fit of the biphasic model to the 4tOP dissipation data produced marginally higher R^2 values than the first-order model, however, it did not significantly improve the fit of the dissipation data for 4tOP (p-values = 0.07 and 0.34 for the CDB and LDB treated soils respectively, Table 4-2). DT50 biphasic were not calculated as the biphasic model did not fit the data significantly better than the first-order model.

(a) CDB



(b) LDB

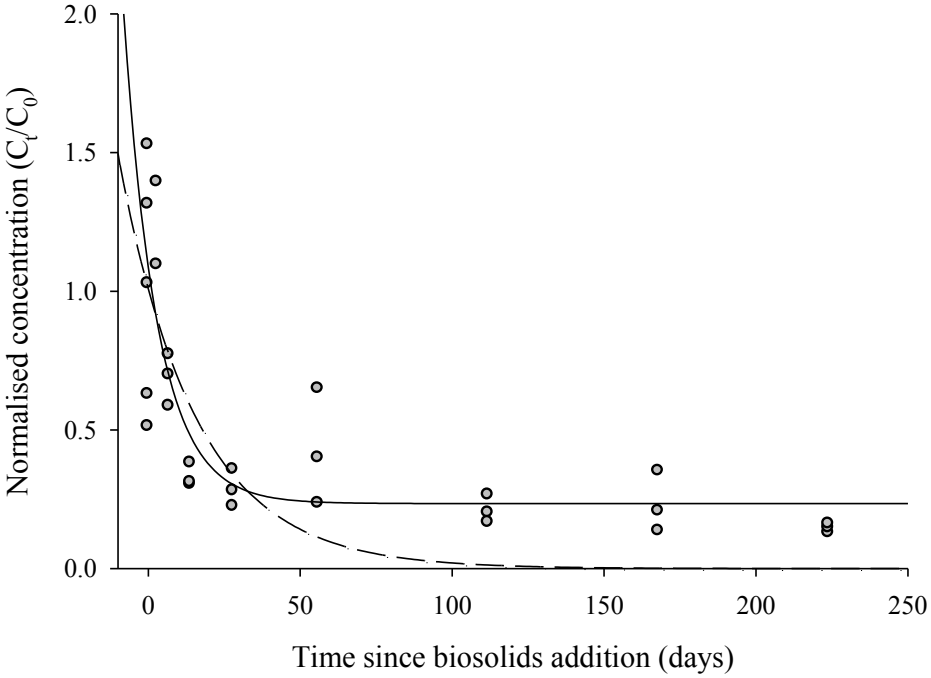


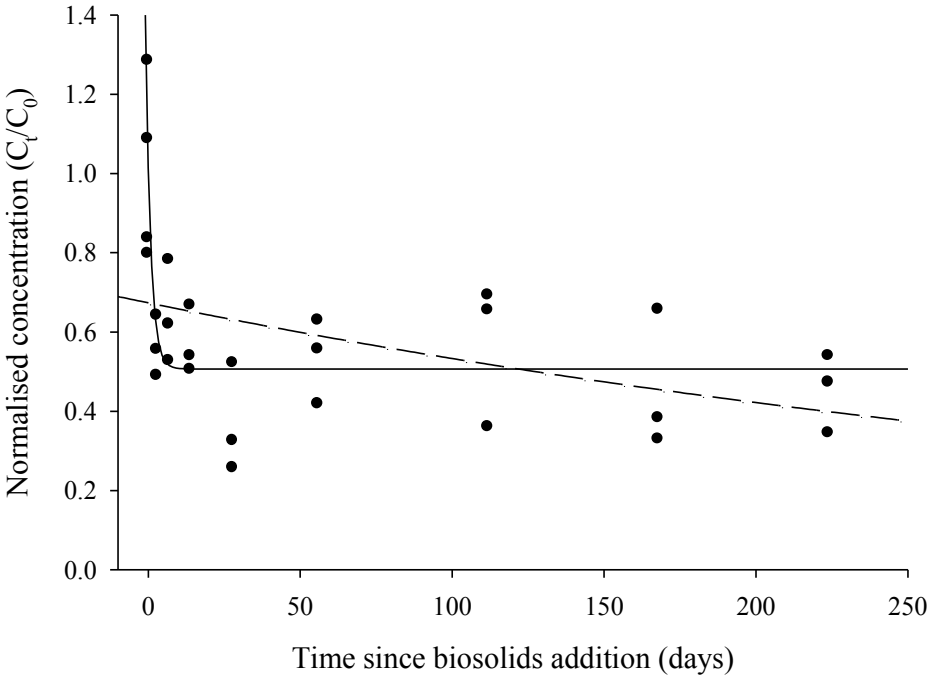
Figure 4-4: Dissipation of bisphenol A following the addition of (a) centrifuge dried biosolids (CDB) and (b) lagoon dried biosolids (LDB) to a soil. All concentration data is normalised as a ratio of the concentration at each sampling interval to the initial concentration (C_t/C_0). The nonlinear regression fits for first-order model and biphasic models are represented by the dashed line and the solid line, respectively.

4.3.3. Dissipation of bisphenol A (BPA) from biosolids amended soils

The dissipation data for BPA over the 224 days of the study showed significant main effects for time ($p < 0.0005$) and biosolids ($p = 0.037$) and a significant interaction of time by biosolids ($p = 0.014$). The significant interaction was likely driven by two factors. First, there was an increase in the concentration of BPA in the CDB treated soils at 3 days post biosolids addition that was not observed in the LDB treated soils (Figure 4-4). Second, the initial significant decrease in BPA concentration was observed at day 14 in the CDB treatment and at day 28 in the LDB treatment. Following these initial decreases, however, there were no significant differences in the concentrations of all subsequent samples.

The fitting of the first-order model to the BPA dissipation data was significant (both p-values ≤ 0.003) and produced R^2 values of 0.29 for the CDB treated and 0.55 for the LDB treated soils (Figure 4-4 and Table 4-2). The DT50 values that were obtained from the first-order model differed considerably between the two biosolids treatments, being 102 days for the CDB treated soils and 18 days for the LDB treated soils. The additional parameter in the biphasic model significantly improved the fit to the BPA dissipation data for both the CDB and LDB treated soils (both p-values ≤ 0.003). The biphasic model accounted for 53% of the variation in the dissipation data from the CDB treated soils and 68% of the variation from the LDB treated soils (Table 4-2). The $DT50_{biphasic}$ values calculated from this model were 8.7 days in the CDB treated soils and 7.7 days in the LDB treated soils (Table 4-2). The proportion of the initial BPA concentration than was predicted by the biphasic model to be recalcitrant at the completion of the experiment, was 42% in the CDB treated soils and 24% in the LDB treated soils (Table 4-2). These recalcitrant fractions corresponded to virtually the same concentration in the two biosolids treatments at the end of the experiment, with values of 2.5 µg/kg and 2.4 µg/kg, respectively.

(a) CDB



(b) LDB

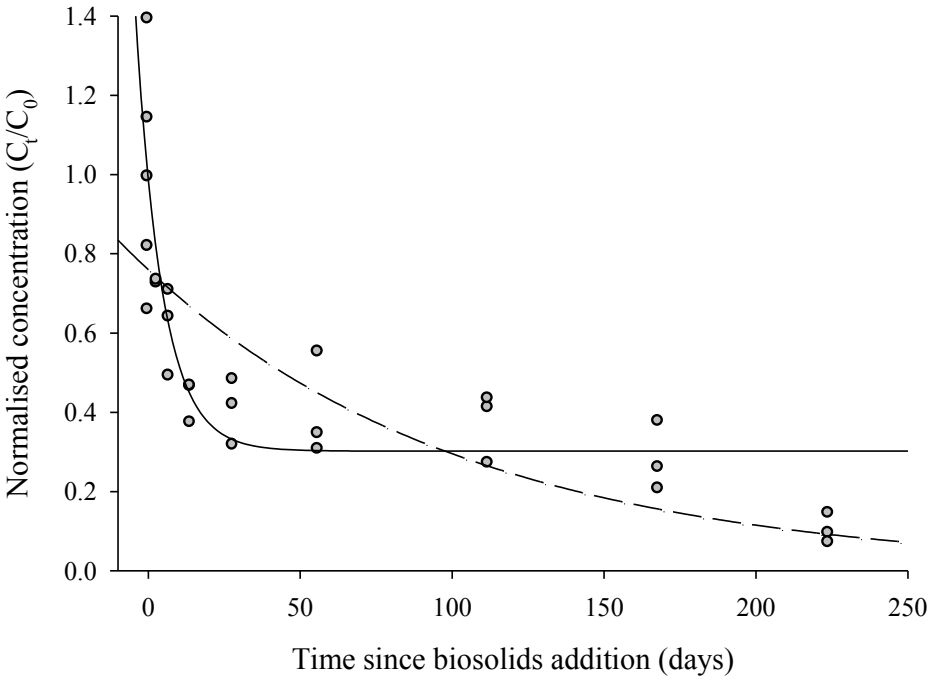


Figure 4-5: Dissipation of triclosan following the addition of (a) centrifuge dried biosolids (CDB) and (b) lagoon dried biosolids (LDB) to a soil. All concentration data is normalised as a ratio of the concentration at each sampling interval to the initial concentration (C_t/C_0). The nonlinear regression fits for first-order model and biphasic models are represented by the dashed line and the solid line, respectively.

4.3.4. Dissipation of triclosan (TCS) from biosolids amended soils

The concentrations of TCS significantly decreased throughout the duration of the experiment (Figure 4-5), as indicated by the significant main effect of time ($p < 0.0005$). There was a rapid decrease in the concentration of TCS at the commencement of the experiment and this resulted in a significant decrease observed 3 days post biosolids addition for both biosolids treatments. The concentrations of TCS did not change significantly, however, from 14 days post biosolids addition until the completion of the experiment (i.e. 224 days). The main effect of biosolids was only marginally significant ($p = 0.044$) and was driven by the overall concentration in the CDB treatment being slightly higher than in the LDB treatment. The interaction of time by biosolids was non-significant ($p = 0.817$).

The fit of the first-order model to the TCS dissipation data was significant for both the CDB and LDB treated soils (both p-values ≤ 0.03), however, this model only accounted for 17% of the variation in the data for the CDB treatment, whereas, for the LDB treatment it accounted for 57% of the variation (Figure 4-5 and Table 4-2). The DT50 values calculated from the first-order model varied considerably between the two biosolids treatments and were 301 days and 73 days for the CDB and LDB treatments, respectively (Table 4-2). The fit of the biphasic model to the dissipation data for TCS from both biosolids treatments showed higher R^2 values of 0.58 and 0.76 for the CDB and LDB treatments, respectively (Table 4-2), when compared to the first-order model. When the fits of the two models were compared statistically, the biphasic model significantly improved the explanation of variation in the data (both p-values < 0.001, Table 4-2). The $DT50_{biphasic}$ values obtained for TCS were 1.2 days in the CDB treatment and 6.3 days in the LDB treatment. The y0 values obtained from the biphasic model for the TCS dissipation data in the CDB and LDB treatments were 0.51 and 0.30, respectively,

indicating that 51% and 30% of the initial TCS remained in the soil. These recalcitrant fractions corresponded to 94 µg/kg and 108 µg/kg of TCS in the CDB and LDB treatments, respectively.

4.4. DISCUSSION

In this study, the concentrations of the four compounds, 4NP, 4tOP, BPA and TCS, added to the soils with the addition of biosolids, decreased significantly over the 224 days of the experiment, with the data showing a significant fit to the first-order dissipation model. Although in all cases the fit of the first-order model was significant ($p < 0.05$), there were large variations observed in the R^2 values obtained from this fit (R^2 values ranged from 0.17 to 0.81). The compound 4tOP showed the best fit to this model with 79 to 81% of the variation in the data explained. In comparison, for BPA and TCS in the CDB treatment, this model explained a low proportion of variation in the data (29% and 17%, respectively).

When the DT50 values that were obtained in this study are compared to those that have been reported in the literature, they are generally similar or only slightly higher when the majority of the variation in the data is explained by the model. For example, Brown et al. (2009) reported half life values for 4NP from a biosolids amended soil of 16 to 26 days, which is in the same range as those reported in this study of 12 to 25 days. For 4tOP, in a study where the compound was spiked into a soil, the average half life was reported to be 5 days (Ying & Kookana, 2005), which is approximately 2- to 3-times smaller than those reported in this study of 10 to 14 days. These small differences may be due to variations in experimental conditions and also from the addition of the compound through spiking rather than in biosolids. For the two compounds BPA and TCS, in the cases where the fit to the first-order model was reasonably good (i.e. ≥ 55%), the DT50 values were only

marginally larger than those reported in literature. In this study the DT50 value for BPA in the LDB treatment was 18 days, whereas others have reported values ranging from 1 to 7 days (Ying & Kookana, 2005; Xu et al., 2009). For TCS also in the LDB treatment, the DT50 value in this study was 73 days, which is only slightly larger than the value reported by Wu et al. (2009a) (i.e. 58 days), however it is approximately 4-times larger than that reported by Ying et al. (2007). However, for BPA and TCS in the CDB treatment, where a low proportion of the variation in the data was explained by the first-order model, the DT50 values are considerably larger than those calculated for the LDB treatment and those in other studies. The DT50 value for BPA in the CDB treatment was approximately 15-times larger than the highest value reported in other studies (Ying & Kookana, 2005) and for TCS in the same biosolids treatment, the value was approximately 5-times longer than the highest reported elsewhere (Wu et al., 2009a). Due to the poor fit of the first-order model to the dissipation data for these two compounds in the CDB treatment, it is likely that the use of a DT50 value is not sufficient in explaining the dissipation rate of the compound and provides an unreliable prediction of the persistence of this compound.

When an additional parameter was used in the biphasic model, the fit to the data was significantly improved for 4NP, BPA and TCS. This was not the case for 4tOP, where the additional fitting parameter in the biphasic model provided no statistically significant improvement in explaining the variation in the data. This is likely to be due, in part, to the fit of the first-order model being quite good for this compound (R^2 = 0.79 and 0.81) and the fact that the C_t/C_0 values decrease more than the other compounds over the duration of the study (Table 4-2 and Figure 4-3). The results observed in this study for the compounds 4NP, BPA and TCS are consistent with other research, for example Sjostrom et al. (2008), which reported recalcitrant fractions of 26 – 35% for NP following the addition of sewage sludge to soil. There are several possible suggestions for the presence of a recalcitrant

fraction of these compounds in a biosolids amended soil. It has been suggested that the presence of a recalcitrant fraction of compounds in biosolids amended soils is due to the distribution of the compound throughout the heterogeneous aggregates of the biosolids (Hesselsoe et al., 2001; Sjostrom et al., 2008). The formation of biosolids aggregates tends to produce aerobic zones in the outer areas and anaerobic zones in the centre of aggregates, which can result in persistent or recalcitrant concentrations of the compounds contained with the biosolids (Hesselsoe et al., 2001). As the compounds assessed in this study degrade predominately under aerobic conditions (e.g. McAvoy et al., 2002; Ying & Kookana, 2005; Press-Kristensen et al., 2008), the presence of anaerobic zones in the biosolids aggregates is likely to have resulted in the degradation slowing considerably or halting. A further hypothesis is that the recalcitrant fraction is due to sorption that is non-reversible which means that there is a sorbed fraction that is not available to microorganisms and hence non-degradable (Wu et al., 2009b). In addition to these above suggestions, it should be noted that generally a biosolids matrix is complex and may involve many components. Various organic compounds may sorb more strongly to the matrix or to different components of the matrix, resulting in the presence of recalcitrant fractions. This may explain the differing proportions of each of the compounds in this study that were recalcitrant in soil amended with different biosolids and the lack of a recalcitrant fraction (statistically) for the compound 4tOP.

Overall, the results from this study raise concerns relating to the potential accumulation of organic compounds in biosolids amended soils particularly if repeat applications are made. This is particularly the case for the compounds BPA and TCS which had the highest recalcitrant fractions (42% and 51% respectively). In addition, this study also showed that the use of a single parameter, for example DT50, is insufficient in explaining the dissipation of the four compounds 4NP, 4tOP, BPA and TCS. Although in most cases a

large proportion of the data was explained by the first-order model, this was significantly improved by the biphasic model. The use of the most appropriate model is crucial when determining the risks associated with these compounds following the addition of biosolids to land, as use of an incorrect model could lead to significant underestimation of the persistence of organic compounds in biosolids amended soils.

4.5. CONCLUSIONS

The four compounds 4-nonylphenol (4NP), 4-t-octylphenol (4tOP), bisphenol A (BPA) and triclosan (TCS) were found to degrade over time when added to a soil via two biosolids. The time taken for 50% of the initial concentrations of the compounds to dissipate (DT50), based on a standard first-order decay model, were 12 to 25 days for 4NP, 10 to 14 days for 4tOP, 18 to 102 days for BPA and 73 to 301 days for TCS. The use of the first-order model produced DT50 values that were consistent with other research only when a considerable portion of the variation was explained by the model. When the first-order model did not explain a considerable portion of the variation, the calculated DT50 values were markedly longer than those reported in the literature. For 4NP, BPA and TCS, a biphasic model, which accounts for a recalcitrant fraction, fitted the dissipation data significantly better than the first-order model. The recalcitrant concentrations for these three compounds as predicted by the biphasic model were 297 – 2480 μg/kg for 4NP, 2.4 – 2.5 μg/kg for BPA and 94 – 108 μg/kg for TCS, which corresponded to 17 to 21%, 24 to 42% and 30 to 51% of the initial concentrations, respectively. In contrast, for 4tOP, the first-order model was sufficient for predicting its dissipation thus indicating that there was no statistical evidence for a recalcitrant fraction of this compound. It appears that different biosolids matrices may influence the degradation of these compounds. The better fit of the biphasic model for some organic contaminants found in biosolids is possibly related to anaerobic conditions within biosolids aggregates and differential non-

reversible sorption of compounds to the biosolids matrix. This study shows that the use of the most appropriate model for dissipation is crucial when assessing the persistence of compounds in soils following the addition of biosolids.

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Chapter 5

Persistence of 4-nonylphenol, 4-t-octylphenol, triclosan and bisphenol A following biosolids addition to land. Part B: Dissipation under field conditions in South Australia

Abstract

The addition of biosolids to land is a route of entry into the environment for contaminants that are not removed through waste treatment processes. The duration of time that these contaminants are likely to persist in the environment following the application of biosolids to agricultural land needs to be determined in order to assess the risks associated with this practice. The following study examined the dissipation of four organic compounds, 4-nonylphenol (4NP), 4-t-octylphenol (4tOP), bisphenol A (BPA) and triclosan (TCS) in biosolids amended soils, under field conditions in South Australia over 336 days. The pattern of dissipation was also assessed to determine if a first-order or a biphasic model better described the data, with comparisons made between laboratory (Chapter 4) and field results. The compounds 4NP, 4tOP and BPA showed a clear decrease in concentration over time. Conversely, the concentration of TCS did not appear to decrease over the 336 days of the trial. The time taken for 50% of the initial concentrations of the compounds to dissipate (DT50) in a soil amended with two different biosolids, based on a standard first-order decay model, were 248 to 257 days for 4NP, 75 to 231 days for 4tOP and 43 to 289 days for BPA. These field DT50 values were approximately 10- to 20-times slower for 4NP and 4tOP and 2.5-times slower for BPA than DT50 values determined in laboratory incubations. The use of the biphasic model significantly improved the fit to the 4tOP data in both biosolids treatments, however, for 4NP and BPA it only improved the fit for one treatment. This study showed that the use of laboratory experiments to predict the persistence of compounds in soils amended with biosolids may overestimate the rates of dissipation and inaccurately predict the patterns of dissipation that occur in the field.

CONTENTS

5.1. INTRODUCTION

Land application of biosolids is a potential route of entry into the environment for numerous organic compounds that may pose a potential risk to organisms and ecosystems. Four specific organic compounds that have received considerable interest recently are the surfactant metabolites 4-nonylphenol (4NP) and 4-t-octylphenol (4tOP), the plasticiser bisphenol A (BPA) and the antimicrobial agent triclosan (TCS). Most of the environmental concern surrounding 4NP, 4tOP and BPA is that they have the ability to mimic natural estrogens by interacting with estrogen receptors (Jobling & Sumpter, 1993; Jobling et al., 1996; Fukuhori et al., 2005). TCS has also been shown to cause endocrine disruption in some organisms (e.g. Veldhoen et al., 2006; Crofton et al., 2007), however, this compound can also exert a high level of toxicity, both in terrestrial and aquatic environments (e.g Orvos et al., 2002; Ishibashi et al., 2004; Waller & Kookana, 2009).

The degradation of 4NP, 4tOP, BPA and TCS in soils has been assessed in several studies and in some cases the results have been used to provide an indication of their expected persistence in the environment following the addition of biosolids to land. In experiments that involved spiking the compounds into soil samples, degradation half lives have been reported of 1 to 17 days for 4NP (Topp & Starratt, 2000; Roberts et al., 2006), approximately 5 days for 4tOP (Ying & Kookana, 2005), 1 to 7 days for BPA (Ying & Kookana, 2005; Xu et al., 2009) and 13 to 58 days for TCS (Ying et al., 2007; Wu et al., 2009a; Xu et al., 2009). Slightly longer half lives of 16 to 23 days have been reported for 4NP in a 45-day glasshouse trial, when the source of the contamination in the soil was solely through the addition of biosolids (i.e. 4NP was contained within the biosolids and not spiked into the samples) (Brown et al., 2009). The degradation of the remaining three compounds from a biosolids source is unknown.

In the previous laboratory-based study (Chapter 4), the dissipation of these four compounds, 4NP, 4tOP, BPA and TCS, was measured when added to a soil as part of the addition of two different biosolids. Dissipation was measured over 224 days in dark conditions with constant temperature and soil moisture. The dissipation rates, expressed as the time taken for 50% of the initial compound to dissipate (DT50), based on a first-order exponential decay model ranged from 12 to 25 days for 4NP, 10 to 14 days for 4tOP, 18 to 102 days of BPA and 73 to 301 days for TCS. These dissipation rates were found to be similar to or slightly longer than those reported in other research when the first-order model provided a good fit to the data. In the case of BPA and TCS, in one of the biosolids treatments, the first-order model was a poor fit to the data and the DT50 values obtained were considerably higher than that in the other treatment, at 102 days and 301 days respectively. In addition, as the experiment was conducted over an extended period of time (i.e. 224 days), the long term pattern of dissipation could also be assessed. It was determined that the dissipation pattern of 4NP, BPA and TCS had a degrading fraction and a recalcitrant fraction in both of the biosolids treatments. This biphasic pattern was not observed, however, for 4tOP, where the entire compound dissipated. For the compounds where the recalcitrant fraction was present, it indicates that there is possibly some influence of the biosolids matrix on the degradation of the compounds. These results were consistent with other research (Hesselsoe et al., 2001; Sjostrom et al., 2008; Wu et al., 2009b), which suggested that this pattern of dissipation is due to limited oxygen within the centre of biosolids aggregates (i.e. anaerobic zones) and/or non-reversible sorption of the compounds to various components of the biosolids matrix.

When biosolids are applied to agricultural land, the dissipation of compounds contained within the biosolids is likely to be influenced by the environmental conditions as well as the biosolids matrix. Variations in temperature and available moisture are likely to play a

role in the dissipation of the compounds. In a laboratory to field comparison study using the ^{14}C-labelled isotope of TCS, the concentration of the compound was found to decrease much more quickly in the laboratory study than in the field (Al-Rajab et al., 2009). As most degradation studies of compounds in biosolids have been conducted under laboratory or glasshouse conditions, field environmental conditions are often not considered. Therefore, it is possible that the persistence of the compounds may have been underestimated.

The aims of this study were to (i) determine the rate of dissipation of 4NP, 4tOP, BPA and TCS, following the addition of biosolids to agricultural land under field conditions in South Australia; (ii) determine if the pattern of dissipation followed a first-order or a biphasic degradation model; and (iii) compare the rate and patterns of dissipation of the compounds between the field and the laboratory (Chapter 4).

5.2. MATERIALS AND METHODS

5.2.1. Field trial design and set up

The field site was located at Mount Compass, South Australia (SA), which is approximately 70 km south of Adelaide (35°21‟44.95 S and 138°32‟44.95 E). This soil had a pH of 4.4, which was determined from a soil:solution ratio of 1:5 in 0.01M $CaCl_2$, an organic carbon content of 2.5%, and consisted of 96% sand, 2.5% silt and 1.5% clay. The climate at this location is Mediterranean consisting of wet cold winters and dry hot summers. Weather conditions were monitored throughout the duration of this study using a weather station at the field site that measured ambient temperature, rainfall, humidity and soil temperature and moisture.

The field trial was established in May 2008, which is the start of the cereal cropping season in southern Australia. The trial consisted of three treatments, two locally produced biosolids and a control each conducted in triplicate. The overall plot design consisted of nine plots, each 2 m × 2 m, that were randomised in a latin square design. The two types of biosolids that were used in the field trial were collected from different locations in SA. Both of the biosolids had been anaerobically digested. One had then been centrifuge dried (CDB) while the other had been solar dried in a lagoon system (LDB). The moisture content of the biosolids was 39% and 48%, respectively. The biosolids used in this field trial corresponded to those that were used in the previous laboratory study (Chapter 4), however, they were collected at different times. The biosolids were transported to the field site immediately following collection, for addition to the field plots. The biosolids were then applied to the surface of the required plots at a rate equivalent to 2 times the nitrogen limiting biosolids application rate (NLBAR). This rate is twice the permissible amount that can be added to agricultural soils under South Australian guidelines (SA EPA, 1997). This rate was equivalent to approximately 25 dry t/ha for the CDB treatment and 45 dry t/ha for the LDB treatment. There was no addition made to the 3 control plots. All of the plots (including the controls) were then rotary hoed to a depth of 10 cm to incorporate the biosolids or in the case of the control plots to simulate the biosolids treatments. Immediately following incorporation, duplicate composite samples were taken from each of the plots and returned to the laboratory for freeze drying and homogenisation for analysis to represent the initial (t_0) concentration of the four selected contaminants. Duplicate composite samples were then taken from each of the plots at intervals throughout a 336 day trial (i.e., 28, 56, 112, 168, 224, 280, 336 days post biosolids addition) and prepared for chemical analysis as described earlier.

5.2.2. Sample extraction and GCMS analysis

For sample extraction and analysis, 10 g from each freeze dried sample was extracted three times with 10 mL of 1:1 methanol and acetone in an ultrasonic bath. For each sample, the extracts were combined then diluted with MQ water and loaded onto Oasis HLB® solid phase extraction (SPE) cartridges. Elution of the samples was conducted using 3 × 2.5 mL methanol, followed by 3 × 2.5 mL acetone and 3 × 2.5 mL ethyl acetate and reconstituted in 4 mL of methanol. Each sample was then derivatized in 400 µL of pyridine and 100 µL of the silylation agent *N,O*-bis-(trimethylsilyl)-trifluorocetamide (BSTFA) + 1% trimethyl-chlorosilane (TMCS) (based on the method of Shareef et al., 2006) and anthracene-d_{10} was added to each sample as an instrument internal standard (IS). Samples were analysed using an Agilent 6890 Series GC system that was interfaced with an Agilent 5973 Network Mass Spectrometer (MS). The concentrations of each of the compounds were determined from relative response factors based on the IS and then adjusted for extraction recoveries. The extraction recoveries were determined by extracting and analysing a duplicate set of samples that had been spiked with known concentrations of the compounds 4tOP, BPA and TCS. The recoveries were then determined by difference from the two sets of samples. For the compound 4NP, the extraction recoveries were determined from the labelled surrogate 4-n-nonylphenol-d_8 (4nNP-d_8), which was spiked into the duplicate samples. The reason for the different method for recovery determination for 4NP was that the concentrations of this compound were relatively high within the samples, therefore by using a labelled surrogate the spiked and unspiked compounds could be easily distinguished. The limit of detection (LOD) and limit of quantification (LOQ) for each of the compounds were determined as 3- and 10-times the signal to noise ratio and were, 30 and 100 µg/kg respectively for 4NP, 0.6 and 2.0 µg/kg respectively for 4tOP, 0.3 and 1.0 µg/kg respectively for BPA, and 0.8 and 2.7 µg/kg respectively for TCS.

5.2.3. Statistical analysis and data interpretation

Prior to all statistical analyses all the concentration data at each sampling time were converted to a ratio of the initial concentration (C_t/C_0). This procedure normalised all the data to an initial mean value of 1 and removed any variation at t_0 between the biosolids treatments and the compounds.

5.2.3.1. Analysis of variance

The normalised concentration data were analysed statistically in PASW Statistics® Version 17, using a repeated measured general linear model (GLM). The repeated measures factor was time and the independent variable was biosolids treatment. A GLM was conducted on the dissipation data of each of the compounds individually to produce significance levels for the repeated measure main effect of time and the interaction of time by biosolids, as well as the between subjects main effect of biosolids treatment at a significance level of $\alpha = 0.05$.

5.2.3.2. Nonlinear regression to determine the dissipation rates and patterns

Two nonlinear regression models were fitted to the normalised concentration data. These consisted of (i) a standard first-order exponential decay model with two fitting parameters (equation 5-1) which assumes the concentration decreases to zero; and (ii) a first-order exponential decay model with three fitting parameters that represents a biphasic pattern of degradation (equation 5-2). The biphasic model used in this study assumes that there is a fraction of the compound that is dissipating and a fraction that is recalcitrant (see figure 1 in Chapter 4):

$$C_t = C_0 e^{-kt} \quad (5\text{-}1)$$

$$C_t = C_0 e^{-kt} + y_0 \quad (5\text{-}2)$$

where C_t is the concentration of the compound at time t, C_0 (or $C_0 + y_0$ from eqn 5-2) is the initial concentration of the compound, k is the rate constant and y_0 in eqn 5-2 is the recalcitrant fraction of the compound. The rate constant, k, was then used to determine the DT50 and the $DT50_{biphasic}$ values using equation 5-3, where the $DT50_{biphasic}$ indicates the time taken for the dissipating fraction to decrease by 50% and is independent of y_0.

$$DT50 \text{ or } DT50_{biphasic} = \ln 2 / k \qquad (5\text{-}3)$$

The significance of the first-order model was produced by SigmaPlot® (i.e. if the first-order decay model was significantly better than that of no change). The significance of the biphasic model against the first-order model was determined by a comparison of the residual sums of squares (RSS) for each of the first-order and biphasic models (procedure outlined in detail in Chapter 4).

5.3. RESULTS

5.3.1. Weather station data

The weather station data for the average weekly ambient temperature and total weekly rainfall for the duration of the field trial is shown in Figure 5-1, along with the timing of each soil sampling event. The majority of rainfall took place in the initial 15-weeks of the trial during the winter months, which is typical of Mediterranean climates. The average weekly rainfall in this 15 week period was 22 mm with a weekly maximum of 67 mm in week 11. The overall average weekly temperature in this period was 9.8°C. In comparison, in the summer months (i.e. from approximately week 30), the total weekly rainfalls were close to zero and average weekly temperature for this period was 18.5°C, which peaked at week 36 of the trial with an average weekly temperature of 29°C.

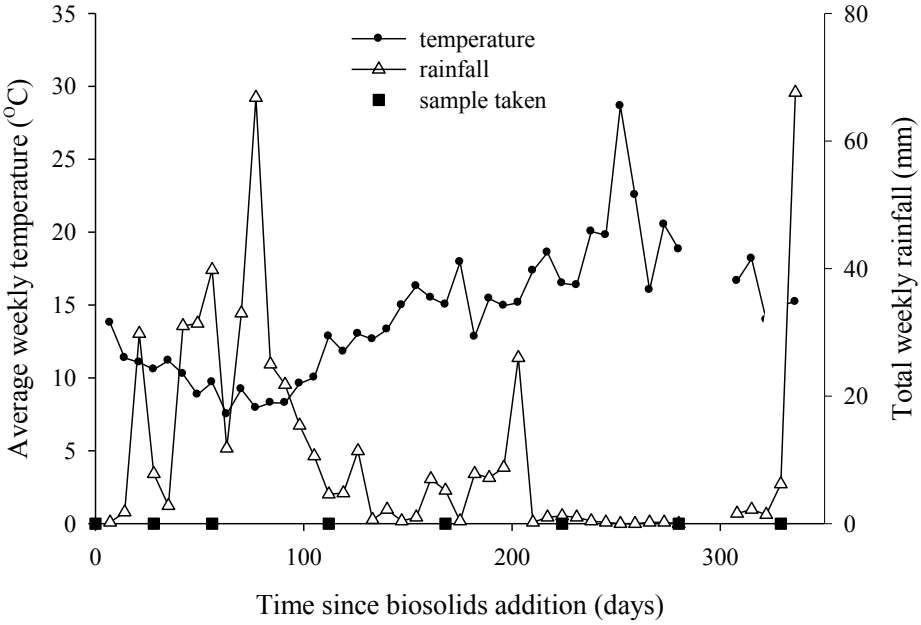


Figure 5-1: Average weekly temperatures and total weekly rainfall for the 336 day duration of the field trial. Times at which samples were taken for chemical analysis are also shown. The initial sample (t_0) was collected 26th May 2008.

5.3.2. Dissipation of the compounds following application of biosolids

The initial concentrations of the four compounds, 4NP, 4tOP, BPA and TCS, in the CDB and LDB treated soils, are shown in Table 5-1. The concentrations of BPA were the lowest and ranged from 2.1 to 6.2 µg/kg while the concentrations of 4NP were the highest and ranged from 475 to 887 µg/kg. For the LDB treated soil the concentrations of 4tOP, BPA and TCS were higher than in the CDB treated soils, whereas this trend was reverse for 4NP, where the concentration was higher in the CDB treated soils. The initial concentrations in the field trial were, for all compounds, lower than the initial concentrations in the laboratory study (Figure 5-2). The differences were between approximately 3- to 13-times in the CDB treatment and 2- to 5-times in the LDB treatment and were due to the higher application rate that was used in the laboratory (i.e. 50 dry t/ha) and the biosolids being collected at different times. At all samplings intervals, the concentrations of all four compounds were below their LOD in the control soils.

Table 5-1: The average and range of concentrations of the compounds 4-nonylphenol (4NP), 4-t-octylphenol (4tOP), bisphenol A (BPA) and triclosan (TCS) at the initial t_0 sample in the centrifuge dried biosolids (CDB) and lagoon dried biosolids (LDB) treated soil.

Biosolids treatment	**Initial compound concentration (µg/kg)**			
	4NP	**4tOP**	**BPA**	**TCS**
CDB	887 (519-1640)	7.0 (4.2-14)	2.1 (1.0-3.7)	39 (25-69)
LDB	475 (189-934)	27 (12-49)	6.2 (3.2-11)	106 (47-207)

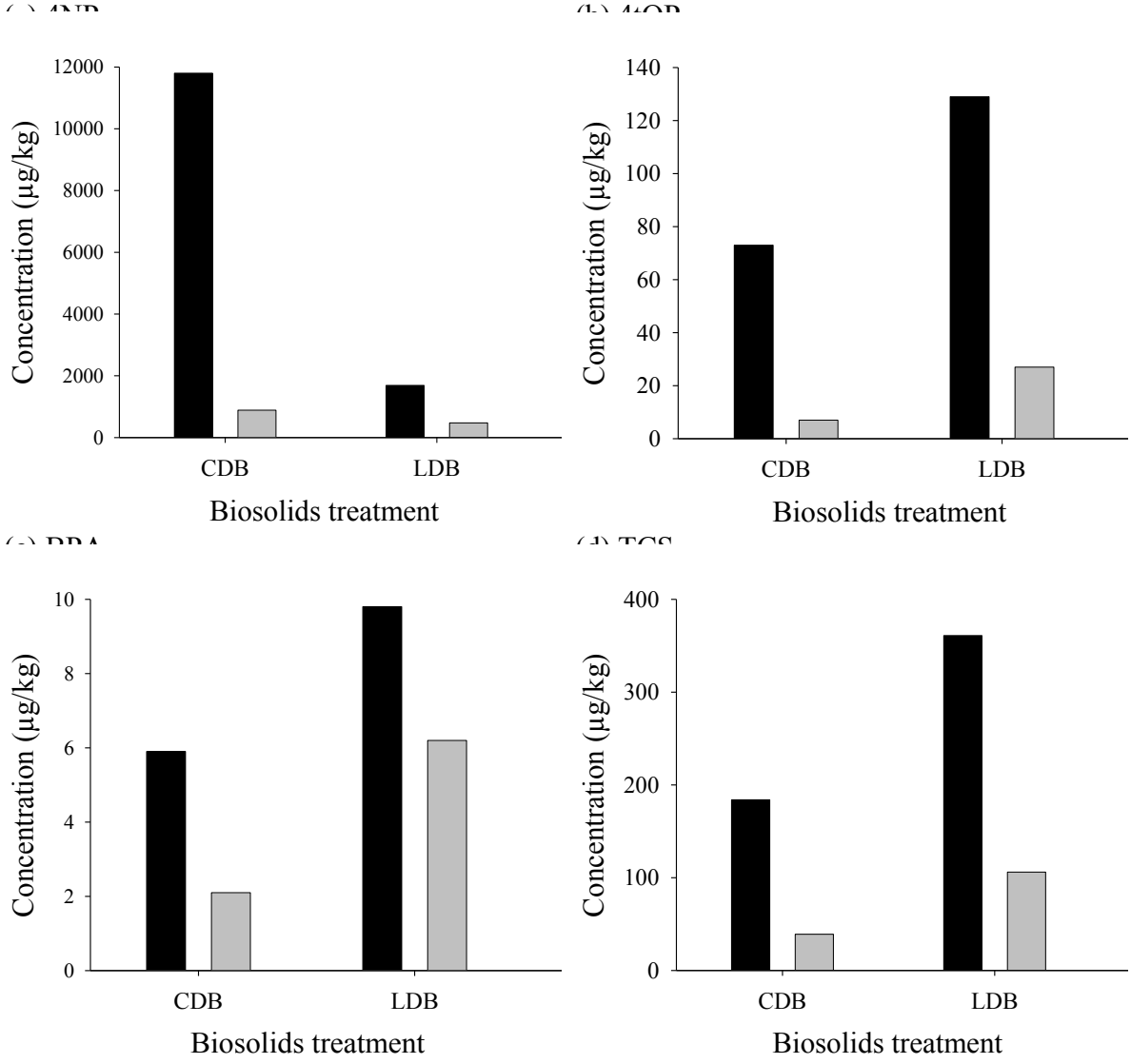


Figure 5-2: Comparison of laboratory (black bars) and field (grey bars) initial concentrations of (a) 4-nonylphenol (4NP), (b) 4-t-octylphenol (4tOP), (c) bisphenol A (BPA) and (d) triclosan (TCS) in the centrifuge dried biosolids (CDB) and lagoon dried biosolids (LDB).

The normalised concentrations for each compound within each biosolids treatment are shown for the duration of the experiment in Figures 5-3, 5-4, 5-5 and 5-6, for 4NP, 4tOP, BPA and TCS, respectively. The nonlinear regression fit for the first-order and biphasic models to the normalised concentration data are also shown in Figures 5-3, 5-4, 5-5 and 5-6. The R^2 values obtained from both of the models are shown in Table 5-2, along with the DT50 values determined from the first-order model, and the y-intercept (y_0) and the $DT50_{biphasic}$ values obtained from the biphasic model. Table 5-2 also shows the significance (p-value) of the first order model and of the additional parameter when the first-order and biphasic models were compared statistically and the "best fit" based on this comparison.

5.3.2.1. Dissipation of 4-nonylphenol (4NP) from biosolids amended soils

There was significant dissipation of 4NP following the addition of both biosolids treatments to the soil over the 336 days of the study (Figure 5-3), indicated by the significant main effect of time ($p < 0.0005$). In the two biosolids treatments, there was a significant decrease in the concentration of 4NP 112 days post biosolids addition to the soils. There was no significant main effect of biosolids ($p = 0.708$) and the interaction of time by biosolids was also non-significant ($p = 0.258$).

The fit of the first-order model to the 4NP dissipation data in this study was significant, however the R^2 values derived were low at 0.20 and 0.21 in the CDB and LDB treated soils respectively (Table 5-2 and Figure 5-3). The DT50 of 4NP determined from the first-order model was 257 days in the CDB treatment and 248 days in the LDB treatment. The additional parameter in the biphasic model significantly improved the fit to the 4NP dissipation data in the CDB biosolids treatment ($p = 0.014$), however was non-significant for the LDB biosolids treatment ($p = 0.092$) (Table 5-2). The $DT50_{biphasic}$ values for 4NP

in the CDB and the LDB biosolids treatments were 41 days and 58 days, respectively, and the y-intercepts from both treatments were approximately 50% (Table 5-2). The remaining concentration therefore at the completion of the 336 days was 417 µg/kg for the CDB treatment and 228 µg/kg for the LDB treatment.

5.3.2.2. Dissipation of 4-t-octylphenol (4tOP) from biosolids amended soils

There was a significant main effect of time ($p < 0.0005$) on the dissipation of 4tOP following the addition of the biosolids to the soil (Figure 5-4). In the two biosolids treatments, the 4tOP concentration significantly decreased from the initial concentration 56 days post biosolids addition. From 224 days through till the end of the field trial at 336 days there was no significant change in the concentration of 4tOP. There was also a significant main effect of biosolids ($p = 0.005$) on the concentrations of 4tOP, where overall the concentrations in the LDB treatment were lower than the CDB treatment. The interaction of time by biosolids was non-significant ($p = 0.684$).

The fit of the first-order model to the dissipation data for 4tOP was significant (both p-values ≤ 0.001) and the R^2 values were 0.23 for the CDB treatment and 0.56 for the LDB treatment (Table 5-2). The DT50 values calculated from the first-order model were 231 days in the CDB treatment and 75 days in the LDB treatment (Table 5-2). The additional fitting parameter in the biphasic model significantly improved the fit to the 4tOP dissipation data (p-values of 0.002 and 0.033 in the CDB and LDB treatments respectively) (Table 5-2). The $DT50_{biphasic}$ values obtained from this model were 33 days for the CDB treatment and 41 days for the LDB treatment with the y-intercept values indicating that 42% and 16% of the initial concentrations remained at the completion of the experiment. These percentages corresponded to concentrations of 2.94 µg/kg in the CDB treatment and 4.32 µg/kg in the LDB treatment.

Table 5-2: Summary of the degradation information from the first-order and biphasic models for the compounds 4-nonylphenol (4NP), 4-t-octylphenol (4tOP), bisphenol A (BPA) and triclosan (TCS) for the centrifuge dried biosolids (CDB) and lagoon dried biosolids (LDB) treated soils. The dissipation half-lives estimated from first-order and biphasic models (DT50 and $DT50_{biphasic}$ respectively) are shown in days and the y-intercept (y_0) values correspond to the C_t/C_0 values.

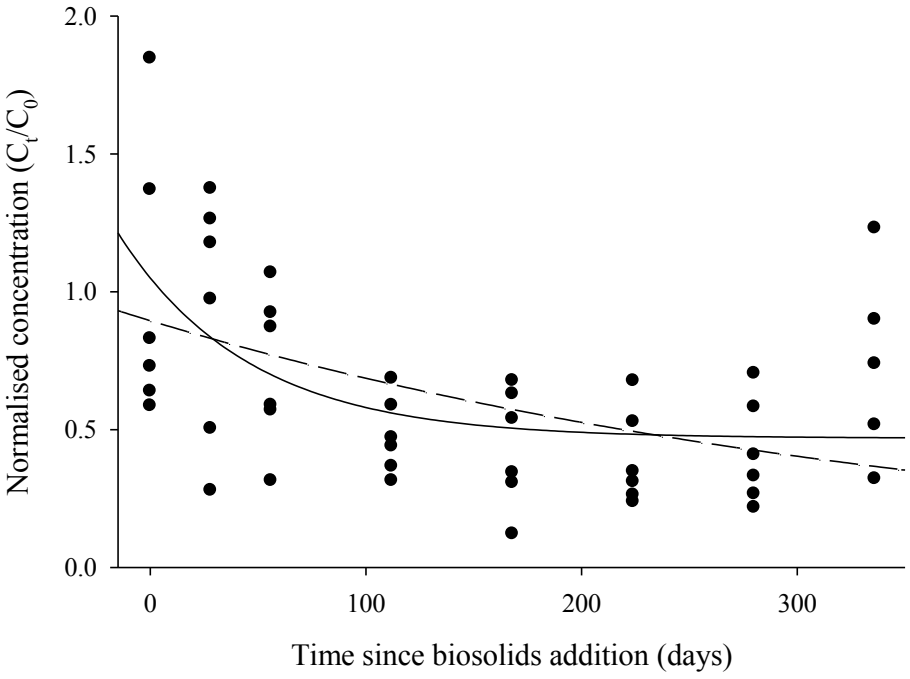
Model	**Measure**	**4NP**		**4tOP**		**BPA**		**TCS**	
		CDB	**LDB**	**CDB**	**LDB**	**CDB**	**LDB**	**CDB**	**LDB**
first order	**R^2**	0.20	0.21	0.23	0.56	0.09	0.37	-	-
	DT50	257	248	231	75	289	43	-	-
	p-value[a]	0.002	0.001	0.001	<0.001	0.046	<0.001	1.00	1.00
biphasic	**R^2**	0.30	0.26	0.39	0.60	0.17	0.63	0.06[c]	0.05[d]
	$DT50_{biphasic}$	41	58	33	41	17	16	-	-
	y_0	0.47	0.48	0.42	0.16	0.45	0.23	0.74	0.73
	p-value[b]	0.014	0.092	0.002	0.033	0.056	<0.001	-	-
	best fit	biphasic	first order	biphasic	biphasic	first order	biphasic	-	-

[a] significance of the first-order model; [b] significance of the biphasic model compared to the first-order model (explained in detail Chapter 4);

[c] the significance of the biphasic model against that of no change was $p = 0.26$

[d] the significance of the biphasic model against that of no change was $p = 0.30$

(a) CDB



(b) LDB

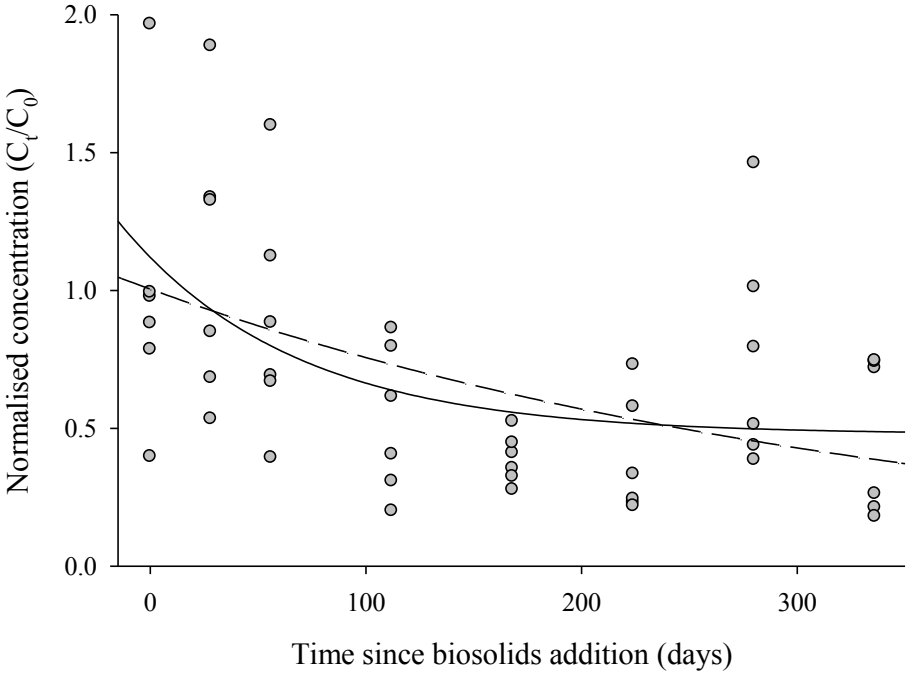
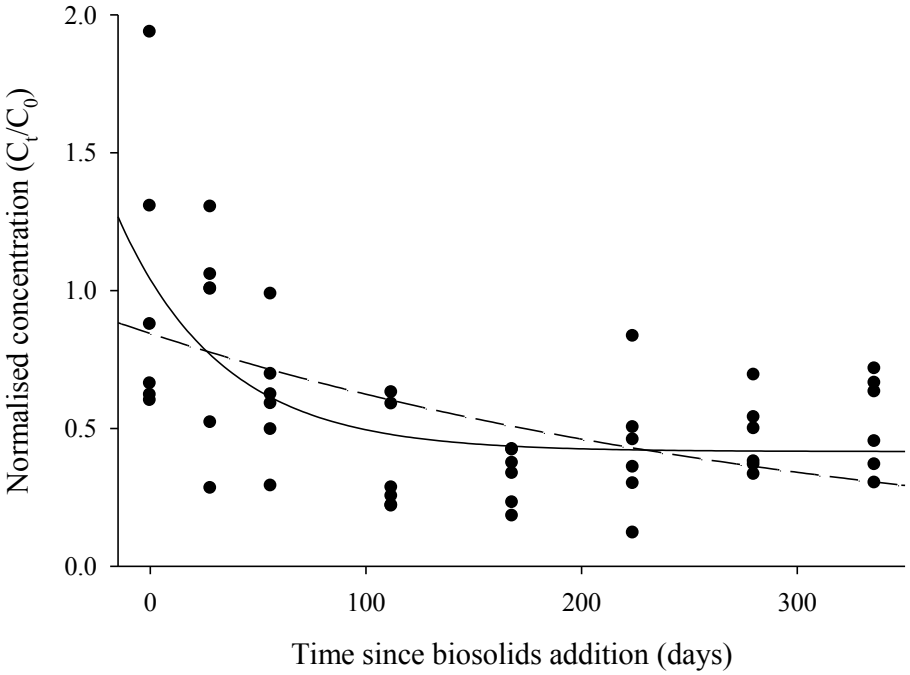


Figure 5-3: Dissipation of 4-nonylphenol following the addition of (a) centrifuge dried biosolids (CDB) and (b) lagoon dried biosolids (LDB) to a soil. All concentration data is normalised as a ratio of the concentration at each sampling interval to the initial concentration (C_t/C_0). The nonlinear regression fits for the first-order model and biphasic model are represented by the dashed line and the solid line, respectively.

(a) CDB



(b) LDB

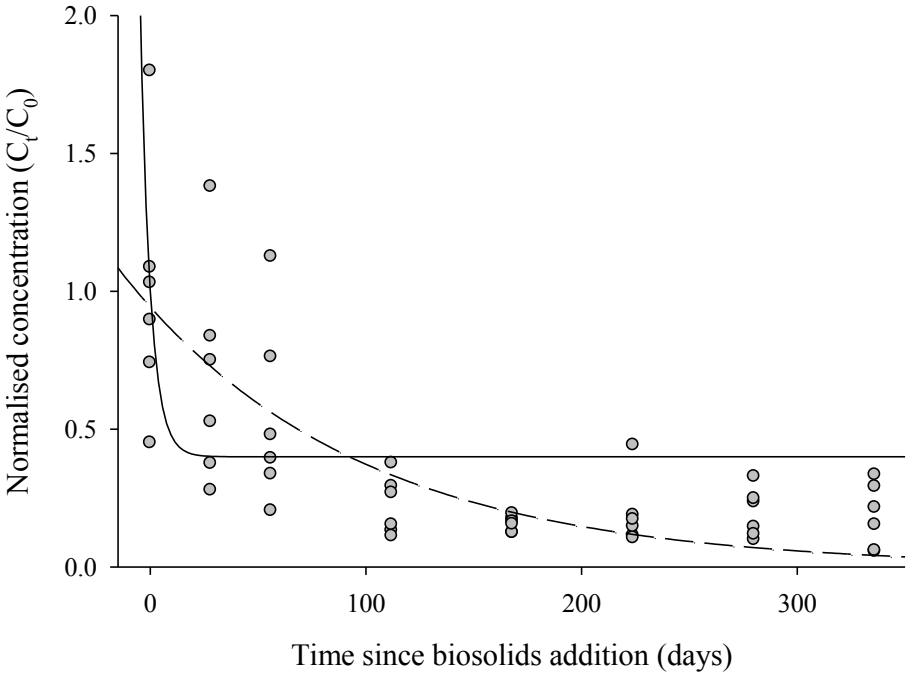


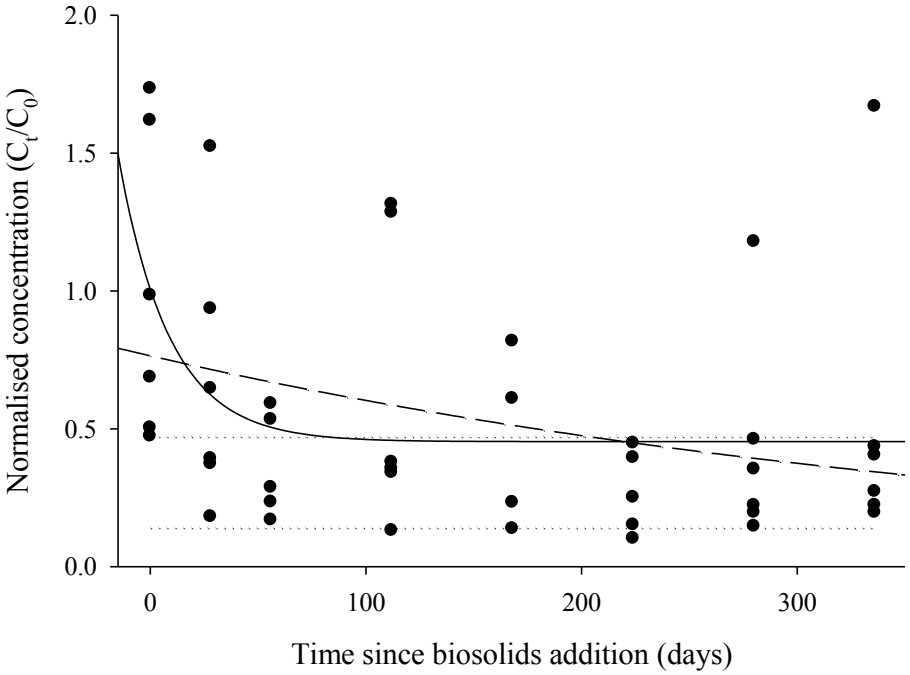
Figure 5-4: Dissipation of 4-t-octylphenol following the addition of (a) centrifuge dried biosolids (CDB) and (b) lagoon dried biosolids (LDB) to a soil. All concentration data is normalised as a ratio of the concentration at each sampling interval to the initial concentration (C_t/C_0). The nonlinear regression fits for the first-order model and biphasic model are represented by the dashed line and the solid line, respectively.

5.3.2.3. Dissipation of bisphenol A (BPA) from biosolids amended soils

Throughout the 336 days of the trial, the concentrations of BPA in both of the biosolids treatments decreased to concentrations below the LOQ of 1.0 µg/kg and in a small number of cases below the LOD of 0.3 µg/kg. For this reason, the normalised concentrations (C_t/C_0) that correspond to the LOD and LOQ for this compound are shown on Figure 5-5. For the repeated measures GLM analysis for this compound, all of the values were, however, used to avoid missing values. There was a significant main effect of time ($p = 0.011$) on the concentration of BPA across the two biosolids treatments. The concentrations of BPA were significantly lower than the initial concentration at 56 days post biosolids addition. After this there were no further significant changes in the BPA concentration through to the completion of the trial at 336 days. There was also a significant main effect of biosolids observed ($p = 0.040$), where, overall, the normalised concentrations of BPA in the LDB biosolids treatment were significantly lower than those in the CDB biosolids treatment. The interaction of time by biosolids was non-significant ($p = 0.457$)

The fit of the first-order model to the dissipation data was only marginally significant for the CDB treatment ($p = 0.046$), however, it was highly significant for the LDB treatment ($p < 0.001$) and explained 9% and 37% of the variation in the data respectively (Table 5-2). The DT50 values calculated from this model for BPA were 289 days for the CDB treatment and 43 days for the LDB treatment (Table 5-2). The additional fitting parameter in the biphasic model did not significantly improve the fit for the BPA dissipation data in the CDB ($p = 0.056$) (Table 5-2). The fit was significantly improved for the LDB treatment ($p < 0.001$), however, and explained 63% of the variation in the data. The $DT50_{biphasic}$ value for BPA in the LDB treatment was 16 days and 23% of the initial concentration was identified as the recalcitrant fraction (i.e. 1.4 µg/kg).

(a) CDB



(b) LDB

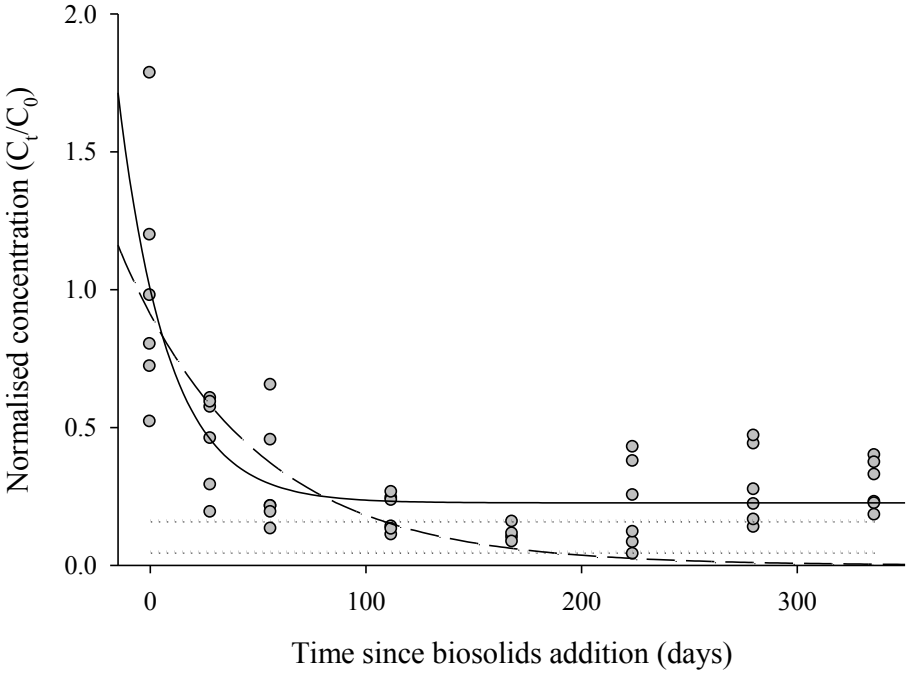
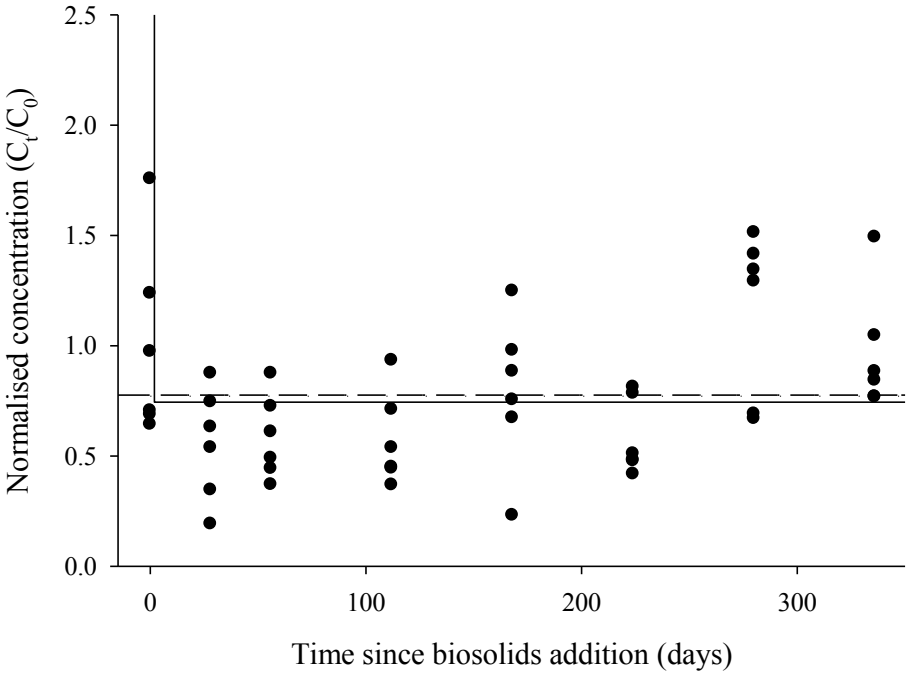


Figure 5-5: Dissipation of bisphenol A following the addition of (a) centrifuge dried biosolids (CDB) and (b) lagoon dried biosolids (LDB) to a soil. All concentration data is normalised as a ratio of the concentration at each sampling interval to the initial concentration (C_t/C_0). The nonlinear regression fits for the first-order model and biphasic model are represented by the dashed line and the solid line, respectively. The upper dotted line in the plots indicates the LOQ and the lower dotted line indicates the LOD for BPA converted to the normalised concentration (C_t/C_0).

(a) CDB



(b) LDB

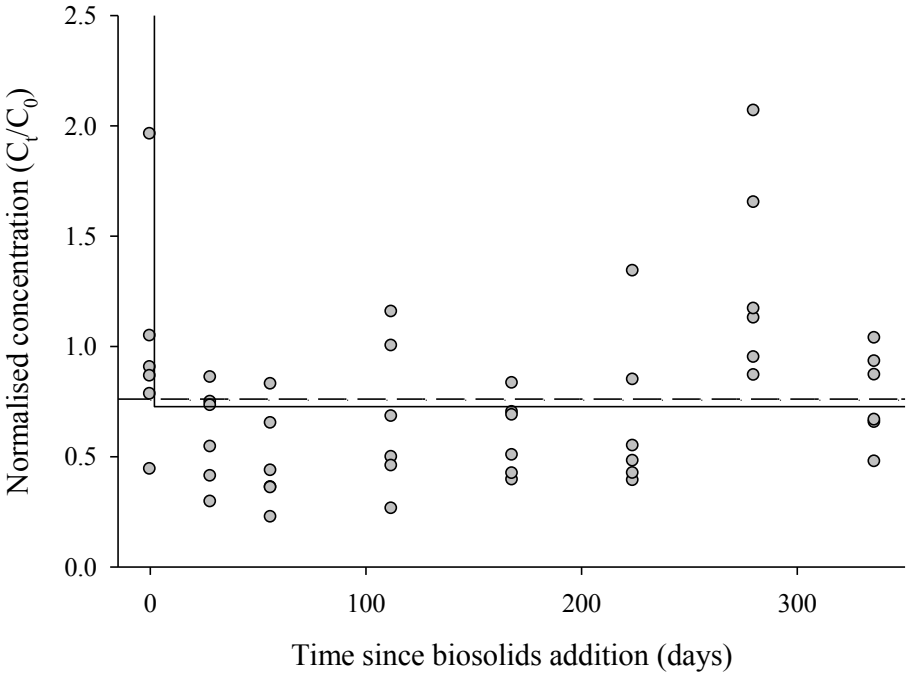


Figure 5-6: Dissipation of triclosan following the addition of (a) centrifuge dried biosolids (CDB) and (b) lagoon dried biosolids (LDB) to a soil. All concentration data is normalised as a ratio of the concentration at each sampling interval to the initial concentration (C_t/C_0). The nonlinear regression fits for the first-order model and biphasic model are represented by the dashed line and the solid line, respectively.

5.3.2.4. Dissipation of triclosan (TCS) from biosolids amended soils

The dissipation data for TCS throughout the experiment showed a significant main effect of time ($p < 0.0005$). This significance is due to the concentrations within both of the biosolids treatments being lower than the initial concentration at 28, 56 and 112 days post biosolids addition. However, at the final four sampling intervals (i.e. 168, 224, 280 and 336) the concentrations were not significantly different from the initial concentration. The main effect of biosolids and the interaction of biosolids by time were both non-significant (both p-values ≤ 0.784).

The fit of the first-order model to the dissipation data for TCS in both of the biosolids treatments was non-significant (both p-values = 1.00). In addition, the fit of the biphasic model was also non-significant (both p-values ≥ 0.26). Therefore DT50 and $DT50_{biphasic}$ values were not calculated.

5.4. DISCUSSION

For three of the compounds assessed in this study, 4NP, 4tOP and BPA, there was a clear effect of time on their concentrations following the addition of two different biosolids to a soil. This effect was evident from both the repeated measures GLM and the significance of the first-order regression model to the dissipation data. For the remaining compound, TCS, this was not the case and minimal or no dissipation was detected throughout the field trial. Although there were some significant differences observed for the concentrations of TCS compared to the initial concentrations, these differences did not persist through to the completion of the experiment. In addition to this, the first-order model did not provide a significant fit to the dissipation data for this compound in soils treated with both biosolids.

When the DT50 values were calculated from the first-order model, 4NP showed little difference in the dissipation rate between the two biosolids treatment. The DT50 values for this compound were 257 days in the CDB treatment and 248 days in the LDB treatment. For the compounds 4tOP and BPA there was considerable difference in the DT50 values between the two biosolids treatments. In both cases, the CDB treatment showed higher DT50 values of 231 and 289 days, respectively, and the LDB treatment which showed the lower values of 75 and 43 days, respectively. This showed that for these two compounds the dissipation rate following the addition of the biosolids was faster in the LDB treatment. This variation in dissipation rates may be due to differences in the biosolids matrices, however, the values are also likely to be affected by the poor fit of the first-order model to the 4tOP and BPA data in the CDB treatment (R^2 values of 0.23 and 0.09 respectively, Table 5-2). This poor fit of the model to the data indicates that the single value DT50 is not a good representation of the dissipation.

The dissipation rates compared between the field to the laboratory (presented in Chapter 4) showed that the compounds 4NP and 4tOP presented a similar trend. For both these compounds the rate in the field was approximately 20-times slower in the CDB treatment and 10-times slower in the LDB treatment than the corresponding laboratory-based values. For the compound BPA, the difference in dissipation rate between the laboratory and the field was not as extreme with the field rates being approximately 2.5-times longer for both of the biosolids treatments. For the compound TCS, DT50 values could not be calculated due to the non-significant fit of the data to the first-order model, which produced a horizontal fit to the data and a p-value of 1.0, indicating no dissipation of this compound. These differences in dissipation rates between the field and the laboratory are likely to be due to less than optimal environmental conditions for degradation of the compounds in the

field and indicate that the use of laboratory experiments to predict dissipation rates in the field should be used with caution.

The additional parameter in the biphasic model significantly improved the fit for the dissipation data of 4NP in the CDB treatment, 4tOP in both treatments and BPA in the LDB treatment. For TCS in both of the biosolids treatments, the improvement of the fit for the biphasic model was again non-significant. The comparison between the laboratory and field dissipations patterns, as shown by the biphasic model, will be discussed for each compound separately below.

Based on the previous laboratory results (Chapter 4), it was expected that 4NP would have a degrading fraction and a recalcitrant fraction in both of the biosolids treatments. This was not the case for the LDB treatment, where the improvement in fit for the biphasic model was not statistically significant (Table 5-2). There are several suggestions for these differences between the laboratory- and field-based dissipation results. The variation in the dissipation data at each of the sampling intervals for this compound was large, which is common for field trials of this nature due to difficulties in obtaining a homogeneous sample. It is possible that this variation resulted in a non-significant improvement in the fit of the biphasic model. A further suggestion is that the portion of the dissipation curve that was obtained from this field trial does not cover all of the initial exponential degrading phase, and therefore the duration of the trial was not sufficient to clearly show the recalcitrant fraction. This is due to the considerably slower dissipation rate of this in the field compared to the laboratory study.

For 4tOP, in both biosolids treatments there was a recalcitrant fraction that remained after nearly one year of this field trial with 42% remaining in the soil of the CDB treatment and

16% in the LDB treatment. The dissipation of this compound under laboratory conditions (Chapter 4) showed the most complete dissipation of all four compounds assessed and for neither treatment indicated a recalcitrant fraction was present. Therefore based on these results, it is possible that this compound will show an additional exponential phase of degradation under more favourable climatic conditions. As the weeks leading up to the completion of this trial showed little or no rainfall, the soil, which consisted of 96% sand, contained little moisture. This lack of moisture may have resulted in the processes required for the degradation of this compound halting. Therefore, an additional rain event may lead to continued degradation of this compound.

For BPA in the CDB treatment, the results observed from both dissipation model curve fits did not produce conclusive results due to the variation in the data (Figure 5-5) and the fact that the majority of concentrations measured were below the LOQ of 1.0 µg/kg. The initial concentration of the compound in this treatment was possibly too low to adequately determine the rate and pattern of dissipation. For BPA in the LDB treatment, however, this was not the case and the pattern of dissipation was more evident. Although both the DT50 and the $DT50_{biphasic}$ were higher in the field compared to the laboratory (by 2.5-times and 2-times respectively), the recalcitrant fraction under both experimental conditions was similar at approximately 23% (Chapter 4). These results for BPA indicate that this compound showed the least variation between the laboratory and the field of all four compounds measured. This may be due to the degradation of this compound not being affected to the same degree by temperature and moisture as the other compounds.

The fit of the biphasic model to the TCS data in both biosolids treatments was non-significant indicating that this model was not sufficient in explaining the variation in the data for this compound throughout the experiment. This result, in combination with the

non-significant first-order model indicates that under the environmental conditions in this field trial, there was no significant dissipation of this compound. This result is considerably different from what was found in the laboratory, where dissipation was observed with a recalcitrant fraction of 30 to 51% (Chapter 4). The persistence of this compound beyond the completion of this trial is likely to be extensive as essentially a full annual cycle was covered. This result therefore creates concern about accumulation of TCS in soils where biosolids have been applied to land in similar environmental and soil conditions. Accumulation of this compound may lead to adverse effects on organisms in the surrounding soil environment or aquatic organisms due to runoff or leaching.

Overall, this study showed that the dissipation of the compounds under the field conditions assessed in the trial significantly slowed, or, in the case of TCS, completely halted dissipation compared to comparable laboratory-based studies (Chapter 4). These differences can be attributed to the specific environmental conditions that are found in the area where this field trial was conducted. In general, the climate in South Australia is Mediterranean, with the majority of rainfall occurring in the winter months and little or no rainfall in the summer months (Figure 5-1). These combinations of environmental conditions in this study resulted in a marked delay in the dissipation of the compounds assessed and also in some cases on the pattern of dissipation observed. It should be noted that the results reported in the study are site specific, in terms of both climate as well as soil type, however, they do show that the dissipation rates of the compounds observed in the laboratory can grossly over-estimate of dissipation rates observed in the field following biosolids addition.

5.5. CONCLUSIONS

Following the addition of biosolids to soil under field conditions in South Australia with a Mediterranean climate, the compounds 4NP, 4tOP and BPA were found to dissipate over a 336 day period, however, the compound TCS did not show any dissipation. The time taken for 50% of the initial concentrations of the compounds to dissipate (DT50) were 248 to 257 days for 4NP, 75 to 231 days for 4tOP and 43 to 289 days for BPA. Dissipation in the field took place approximately 10- to 20-times slower than in the laboratory for 4NP and 4tOP and approximately 2.5-times slower for BPA. The use of a biphasic model significantly improved the fit to the 4NP data in the CDB treatment but not in the LDB treatment. For 4tOP, the biphasic model improved the fit in both biosolids treatments and for BPA only in the LDB treatment. It is expected that with additional time, seasonal variation, and an additional rain event, the dissipation of 4NP and 4tOP would continue. The main factor that resulted in differences between the laboratory and the field was likely to be the unfavourable environmental conditions for degradation at the location of this field trial. The results reported in this study are site-specific, however they show that the use of laboratory experiments to predict the persistence of compounds contained within biosolids, may overestimate dissipation rates and inaccurately predict dissipation patterns.

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Chapter 6

Comparison of dissipation rates and patterns between indigenous and spiked bisphenol A and triclosan in a biosolids amended soil

Abstract

Degradation experiments following spiking of compounds into soils are often used to provide a measure of the persistence of organic compounds following land application of biosolids. In this study, the rate and pattern of dissipation of bisphenol A (BPA) and triclosan (TCS) indigenous to two different biosolids samples were compared to those of labelled surrogates, i.e. bisphenol A-d_{16} (BPA-d_{16}) and triclosan-$^{13}C_{12}$ (TCS-$^{13}C_{12}$), that were spiked into a biosolids amended soil. The biosolids used were a centrifuge dried biosolids (CDB) and a lagoon dried biosolids (LDB). The duration of the experiment was 224 days, and the dissipation data were compared by fitting a first-order decay model and a biphasic model to the data. The DT50 (time taken for the initial concentration of the compound to decrease by 50%) determined from the first-order decay model was 10- to 100-times higher for the indigenous BPA compared to the spiked BPA-d_{16}. A biphasic model better explained the pattern of dissipation for the indigenous BPA in both biosolids treatments, however, the first-order model was sufficient for the spiked BPA-d_{16}, which dissipated to below the detection limit in both treatments. When the DT50 values obtained from the model that was the best fit to the data were compared, (i.e. DT50 for BPA-d_{16} and $DT50_{biphasic}$ for BPA), the rate of dissipation of the spiked BPA-d_{16} was approximately 5-times faster than the indigenous BPA. The indigenous TCS did not appear to show any dissipation in CDB treatment, however in the LDB treatment a DT50 value of 89 days was obtained. This was approximately 1.6-times higher than the DT50 for the spiked TCS-$^{13}C_{12}$ in the two biosolids treatments. The biphasic model was a significant improvement to the dissipation data of the spiked TCS in the CDB treatment, however, not in the LDB treatment. This study showed that the dissipation of spiked compounds did not occur at the same rate or follow the same pattern as that of indigenous forms of the same compounds in biosolids. Therefore spiking experiments may not be suitable to predict the persistence of organic compounds following land application of biosolids.

CONTENTS

6.1. INTRODUCTION

The organic compounds bisphenol A (BPA) and triclosan (TCS) are commonly detected in wastewater streams and due to their hydrophobic nature (i.e. log K_{OW} values of 3.13 and 4.76, respectively), they are often detected in the solid waste by-product, biosolids (e.g. McAvoy et al., 2002; Kinney et al., 2006; Chu & Metcalfe, 2007). The release of BPA and TCS into the environment through the application of biosolids to agricultural land has received increasing interest recently due to their high level of toxicity and/or their potential to cause endocrine disruption effects (e.g. Orvos et al., 2002; Fukuhori et al., 2005; Veldhoen et al., 2006; Crofton et al., 2007; Waller & Kookana, 2009). To assist in determining the potential risks that these compounds may pose to the environment following the application of biosolids to land, an understanding of their persistence in the environment is required.

The duration of time required for BPA and TCS to degrade when added to soils has been assessed in several laboratory-based studies following spiking the compounds into soil samples. In general, BPA and TCS have been shown to degrade under aerobic conditions, with little or no degradation occurring under anaerobic conditions (McAvoy et al., 2002; Ying & Kookana, 2005; Press-Kristensen et al., 2008). Half-lives or DT50s (time taken for 50% of the initial concentration of the compound to dissipate) of these compounds in spike degradation experiments in soils have been reported for BPA ranging from 1 to 7 days (Ying & Kookana, 2005; Xu et al., 2009) and for TCS from 13 to 58 days (Ying et al., 2007; Wu et al., 2009a; Xu et al., 2009).

In a previous study, (presented in Chapter 4), the dissipation of BPA and TCS in a South Australian agricultural soil was assessed, when the addition of biosolids to the soil was the source of the contamination (i.e. the compounds were indigenous to the biosolids at the

time of addition). The DT50 values found were longer than those reported in previous research where the compounds had been spiked into soil samples, and varied considerably between the two different biosolids that were tested. The DT50 values obtained ranged from 18 to 102 days for BPA and 73 to 301 days for TCS. In addition, it was also determined that there was a non-degrading or recalcitrant fraction for both compounds, which persisted in the biosolids amended soils for the 224 days of the experiment. The marked differences in the DT50 values of spiked and indigenous BPA and TCS raises the question of whether it is possible to use spiked degradation experiments to simulate the degradation of compounds that are indigenous to biosolids. This concern has only been addressed in a small number of studies. Some work using the surfactant metabolite compound 4-nonylphenol, however, does suggest that the degradation of a compound spiked into a soil is more complete than that of a compound contained within organic waste products (Mortensen & Kure, 2003). If the degradation observed for a compound which is spiked into a soil differs from that of one which is added to a soil with biosolids, it may not be suitable to use experiments involving spiking to predict degradation of compounds following land application of biosolids.

The aim of this study was to compare the dissipation rates and patterns of BPA and TCS that are indigenous to biosolids at the time of addition with those of the same compounds that had been spiked into the same samples. The spiking was conducted using the isotopically labelled surrogate compounds, BPA-d_{16} and TCS-$^{13}C_{12}$.

6.2. MATERIALS AND METHODS

6.2.1. Soil and Biosolids

A bulk soil was collected from a field site at Mount Compass in South Australia (SA) (35°21‟44.95 S and 138°32‟44.95 E), which is located approximately 70 km south of

Adelaide, for use in this study. The soil had a pH of 4.4 (determined using a soil:solution ratio of 1:5 in 0.01M $CaCl_2$), an organic carbon content of 2.5%, and consisted of 96% sand, 2.5% silt and 1.5% clay. The bulk sample was dried at 40°C prior to being homogenized by grinding with a mortar and pestle and sieved to 2 mm.

Two locally produced biosolids were also collected for use in this study. Both biosolids had been treated by anaerobic digestion, but thereafter one of the biosolids had been centrifuge dried (CDB) and the other had been solar dried in a lagoon system (LDB). The moisture contents of the biosolids were 63% for the CDB and 52% for the LDB and for the experiment the biosolids were used as collected (i.e. wet).

6.2.2. Experimental design and set up

Individual 50 g samples were weighed from the dried bulk soil into glass jars and hydrated to 50% of their maximum water holding capacity (MWHC) with Milli Q (MQ) water (the method used to determine the MWHC is outlined in Jenkinson & Powlson, 1976). All samples were then placed in closed containers in the dark and pre-incubated at 22°C for 14 days to rejuvenate and stabilise soil microbial communities. After the pre-incubation, either the CDB or LDB treatments were added to the hydrated soil, at a rate equivalent to 50 dry t/ha (assuming a soil bulk density of 1.3 g/cm^3 and an incorporation depth of 10 cm). All of the samples were then spiked with 200 µL of a stock solution containing the compounds BPA-d_{16} and TCS-$^{13}C_{12}$ in methanol (at a concentration of 25 mg/L). This spiking solution was added to the surface of the samples so the expected soil concentration in each test container was 100 µg/kg for each compound, BPA-d_{16} and TCS-$^{13}C_{12}$. Five replicate samples from each of the biosolids treatments were then freeze dried immediately and stored in the dark until analysed as the initial sample (t_0). All the remaining sample jars were weighed, then placed on wet paper towel in containers with

lids and kept in the dark at a constant temperature of 22°C. The samples were opened to the air daily and the moisture content in the soil was maintained throughout the experiment by weight at 50% MWHC. At eight additional sampling intervals (3, 7, 14, 28, 56, 112, 168 and 224 days post biosolids addition and sample spiking), triplicate sample jars were removed from each of the biosolids treatments and freeze dried for immediate analysis of the target compounds.

6.2.3. Sample extraction and GCMS analysis

For sample extraction and analysis, 10 g from each freeze dried sample was extracted three times with 1:1 methanol and acetone in an ultrasonic bath. For each sample the extracts were combined then diluted with MQ water and loaded onto Oasis HLB® solid phase extraction (SPE) cartridges. Elution of the samples was conducted using 3 × 2.5 mL methanol, followed by 3 × 2.5 mL acetone and 3 × 2.5 mL ethyl acetate and reconstituted in 4 mL of methanol. Each sample was then derivatized in 400 µL of pyridine and 100 µL of the silylation agent *N,O*-bis-(trimethylsilyl)-trifluorocetamide (BSTFA) + 1% trimethyl-chlorosilane (TMCS) (based on the method of Shareef et al., 2006) and anthracene-d_{10} was added to each sample as an instrument internal standard (IS). Samples were analysed using an Agilent 6890 Series GC system that was interfaced with an Agilent 5973 Network Mass Spectrometer (MS). The concentrations of each of the compounds were determined from relative response factors based on the IS and then adjusted for extraction recoveries based on BPA-d_{16} and TCS-$^{13}C_{12}$ that had been spiked into an additional set of samples one day prior to extraction. The limit of detection (LOD) and limit of quantification (LOQ) for each of the compounds were determined as 3- and 10-times the signal to noise ratio and were 0.3 and 1.0 µg/kg, respectively, for BPA/BPA-d_{16}, and 0.8 and 2.7 µg/kg, respectively, for TCS/TCS-$^{13}C_{12}$.

6.2.4. Statistical analysis and interpretation

Prior to any statistical analysis, all the concentration data at each sampling time were converted to a ratio of the initial concentration (C_t/C_0). This normalised the data to an initial mean concentration of 1 and removed any variation at t_0 between the biosolids treatments and the compounds.

6.2.4.1. Nonlinear regression modelling

Two nonlinear regression models were fitted to the normalised concentration data of all compounds. These consisted of (i) a standard first-order exponential decay model with two fitting parameters (equation 6-1) which assumes the concentration decreases to zero; and (ii) a first-order exponential decay model with three fitting parameters that represents a biphasic pattern of dissipation (equation 6-2). The biphasic model used in this study assumes that there is a fraction of the compound that is dissipating and a fraction that is recalcitrant:

$$C_t = C_0 e^{-kt} \quad (6\text{-}1)$$

$$C_t = C_0 e^{-kt} + y_0 \quad (6\text{-}2)$$

where C_t is the concentration of the compound at time t, C_0 (or $C_0 + y_0$ from eqn 6-2) is the initial concentration of the compound, k is the rate constant and y_0 in eqn 6-2 is the recalcitrant fraction of the compound. The rate constant, k, was then used to determine the DT50 and the $DT50_{biphasic}$ values using equation 6-3.

$$DT50 \text{ or } DT50_{biphasic} = ln2 / k \quad (6\text{-}3)$$

The significance of the first-order model was produced by SigmaPlot® (i.e. if the first-order decay model was significantly better than that of no change), and the significance of the biphasic model against the first-order model was determined by a comparison of the residual sums of squares (RSS) for each of the first-order and biphasic models (procedure outlined in detail in Chapter 4).

6.3. RESULTS

The initial average concentrations of the t_0 samples were found to range from 6.4 to 11 µg/kg for BPA, 81 to 97 µg/kg for BPA-d_{16}, 213 to 361 µg/kg for TCS and 80 to 88 µg/kg for TCS-$^{13}C_{12}$ (Table 6-1). The normalised dissipation data of the four compounds is shown in Figures 6-1, 6-2, 6-3 and 6-4, respectively, along with the fits for the first-order and biphasic models. Table 6-2 presents the coefficient of determination (R^2) for both of the models fitted to the dissipation data, the DT50 from the first order model and the $DT50_{biphasic}$ and recalcitrant fraction (i.e. y_0) from the biphasic model. In addition, Table 6-2 indicates which model is the best fit to the data.

Table 6-1: The average and range of concentrations of bisphenol A (BPA), bisphenol A-d_{16} (BPA-d_{16}), triclosan (TCS) and triclosan-$^{13}C_{12}$ (TCS-$^{13}C_{12}$) for the initial (t0) sample for the centrifuge dried biosolids (CDB) and lagoon dried biosolids (LDB) treatments.

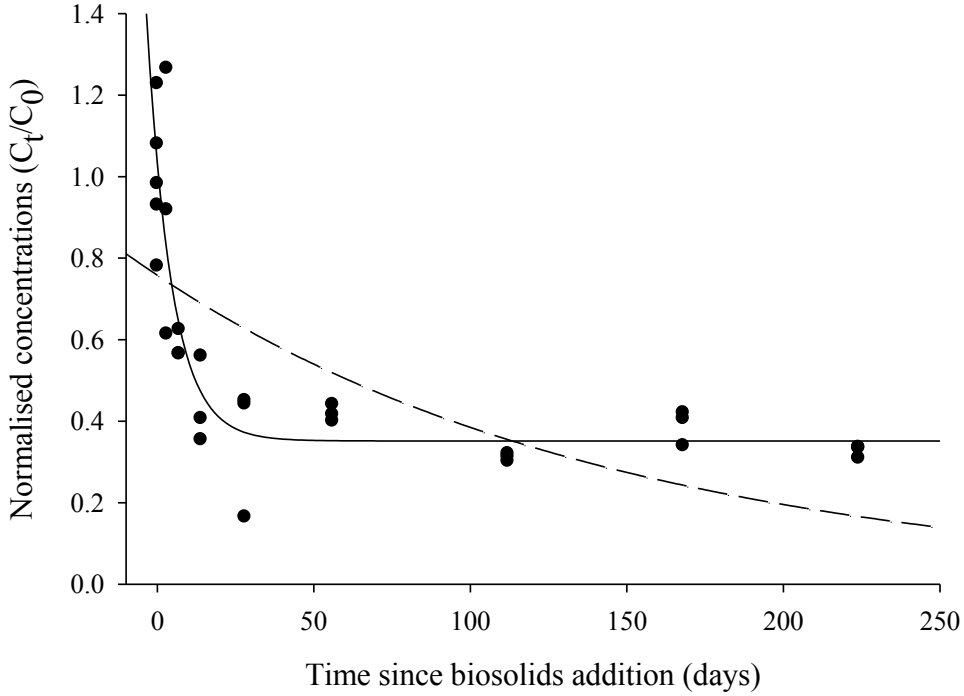
Biosolids treatment	**Initial compound concentration (µg/kg)**			
	BPA	**BPA-d_{16}**	**TCS**	**TCS-$^{13}C_{12}$**
CDB	6.4 (5.0-7.8)	81 (72-92)	213 (166-263)	88 (77-101)
LDB	11 (4.1-21)	97 (78-123)	361 (149-572)	80 (52-108)

Table 6-2: Summary of the degradation information from the first-order and biphasic models for the compounds bisphenol A (BPA), bisphenol A-d_{16} (BPA-d_{16}), triclosan (TCS) and triclosan-$^{13}C_{12}$ (TCS-$^{13}C_{12}$) for the centrifuge dried biosolids (CDB) and lagoon dried biosolids (LDB) treatments. The dissipation half lives determined using the first-order and biphasic models (DT50 and $DT50_{biphasic}$, respectively) are shown in days and the y-intercept (y_0) values correspond to the C_t/C_0 values.

Model	**Measure**	**BPA**		**BPA-d_{16}**		**TCS**		**TCS-$^{13}C_{12}$**	
		CDB	**LDB**	**CDB**	**LDB**	**CDB**	**LDB**	**CDB**	**LDB**
first-order	**R^2**	0.41	0.45	0.98	0.95	0.06	0.41	0.79	0.79
	DT50	102	9.2	1.1	1.5	462	89	55	57
	p-value[a]	<0.001	<0.001	<0.001	<0.001	0.22	<0.001	<0.001	<0.001
biphasic	**R^2**	0.80	0.60	0.98	0.95	0.74	0.42	0.83	0.81
	$DT50_{biphasic}$	5.6	5.0	1.0	1.4	< 1	49	27	36
	y_0	0.35	0.23	0.02	0.01	0.45	0.16	0.18	0.15
	p-value[b]	<0.001	<0.001	0.289	0.624	<0.001	0.691	0.015	0.165
	best fit	Biphasic	biphasic	first-order	first-order	biphasic	first-order	biphasic	first-order

[a] significance of the first-order model; b significance of the biphasic model compared to the first-order model (explained in detail Chapter 4)

(a) CDB



(b) LDB

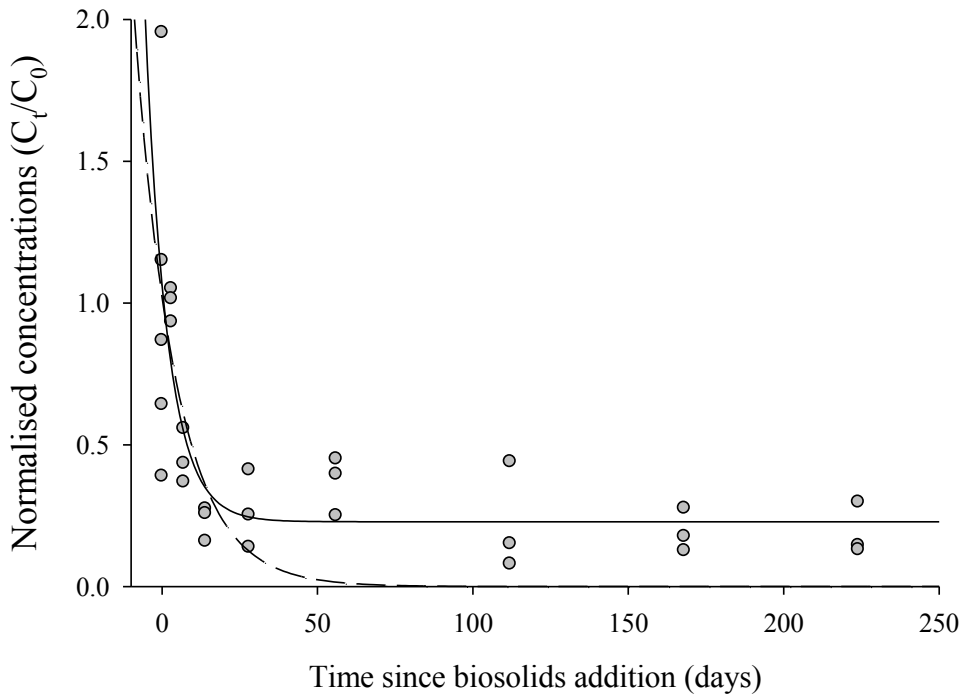
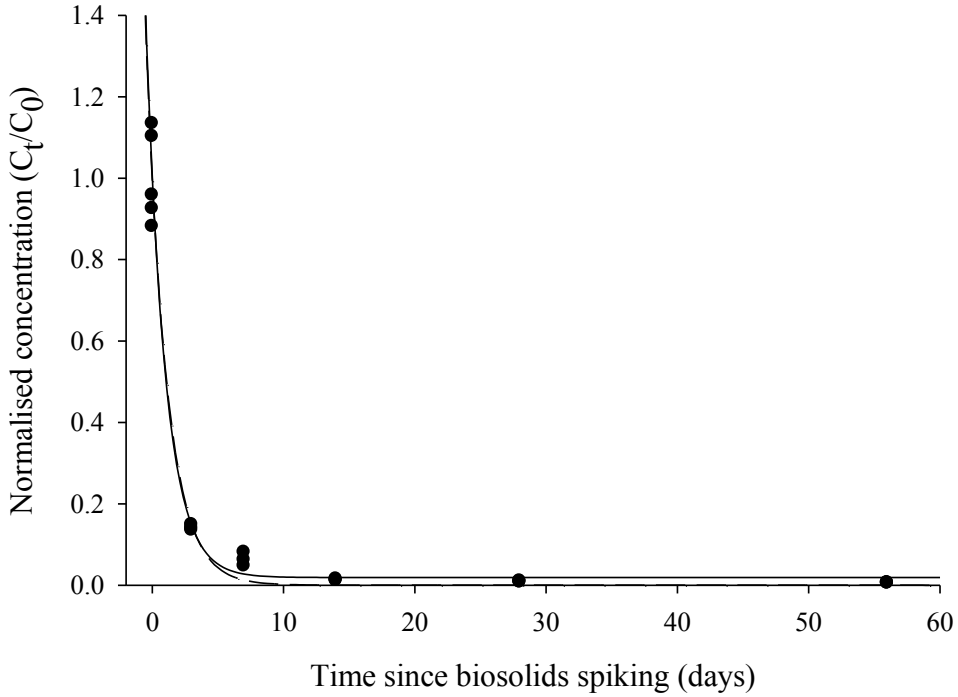


Figure 6-1: Dissipation of indigenous bisphenol A following the addition of (a) centrifuge dried biosolids (CDB) and (b) lagoon dried biosolids (LDB) to a soil. All concentration data is normalised as a ratio of the concentration at each sampling interval to the initial concentration (C_t/C_0). The nonlinear regression fits for first-order model and biphasic models are represented by the dashed line and the solid line, respectively.

(a) CDB



(b) LDB

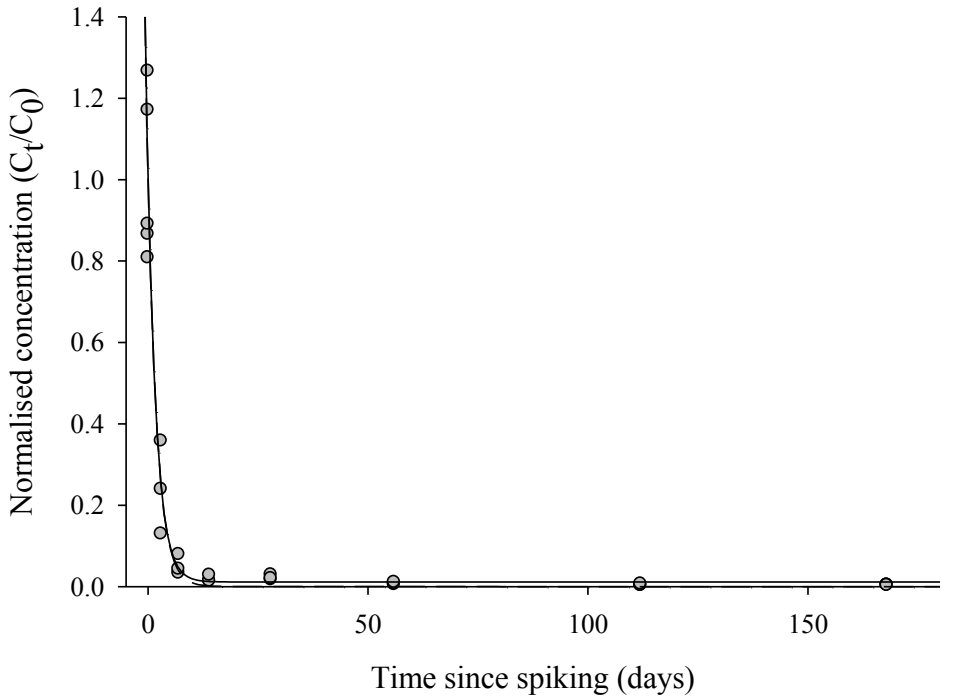


Figure 6-2: Dissipation of spiked bisphenol A-d_{16} following the addition of (a) centrifuge dried biosolids (CDB) and (b) lagoon dried biosolids (LDB) to a soil. All concentration data is normalised as a ratio of the concentration at each sampling interval to the initial concentration (C_t/C_0). The nonlinear regression fits for first-order model and biphasic models are represented by the dashed line and the solid line, respectively.

6.3.1. Dissipation of indigenous bisphenol A and spiked bisphenol A-d_{16}

Over the 224 day duration of the experiment there was dissipation of both spiked and indigenous BPA and BPA-d_{16} in both biosolids treatments (Figure 6-1 and 6-2). The indigenous BPA remained above the LOD of 0.3 µg/kg for the entire duration of the experiment (Figure 6-1), whereas, the concentration of the BPA-d_{16} decreased to below the LOD after 56 days in the CDB treatment and after 168 days in the LDB treatment (Figure 6-2). The plots in Figure 6-2 for BPA-d_{16} only present the data for the duration of the experiment when the compound was above the LOD.

The fit of the first-order model to the BPA and BPA-d_{16} data in both biosolids treatments was significant (all p-values < 0.001) and had R^2 values of 0.41 and 0.98, respectively, for the CDB treatment and 0.45 and 0.95, respectively for the LDB treatment (Figures 6-1 and 6-2; Table 6-2). The DT50 values for BPA ranged from 9.2 to 102 days and for BPA-d_{16}, while the DT50 values ranged from 1.1 to 1.5 days (Table 6-2).

The statistical comparison of the biphasic and first-order models showed that the fit for the indigenous BPA was significantly improved (both p-values < 0.001) by the biphasic model, however, for the spiked BPA-d_{16}, the biphasic model did not significantly improve the fit (both p-values ≥ 0.289) (Table 6-2). The $DT50_{biphasic}$ values from the significant fit of the BPA data were 5.6 and 5.0 days in the CDB and LDB treatments, respectively and the y_0 values (i.e. C_t/C_0) representing the recalcitrant fraction were 0.35 (35% of the initial concentration) and 0.23 (23% of the initial concentration), respectively (Table 6-2). These recalcitrant fractions of BPA corresponded to 2.24 µg/kg in the CDB treatment and 2.53 µg/kg in the LDB treatment.

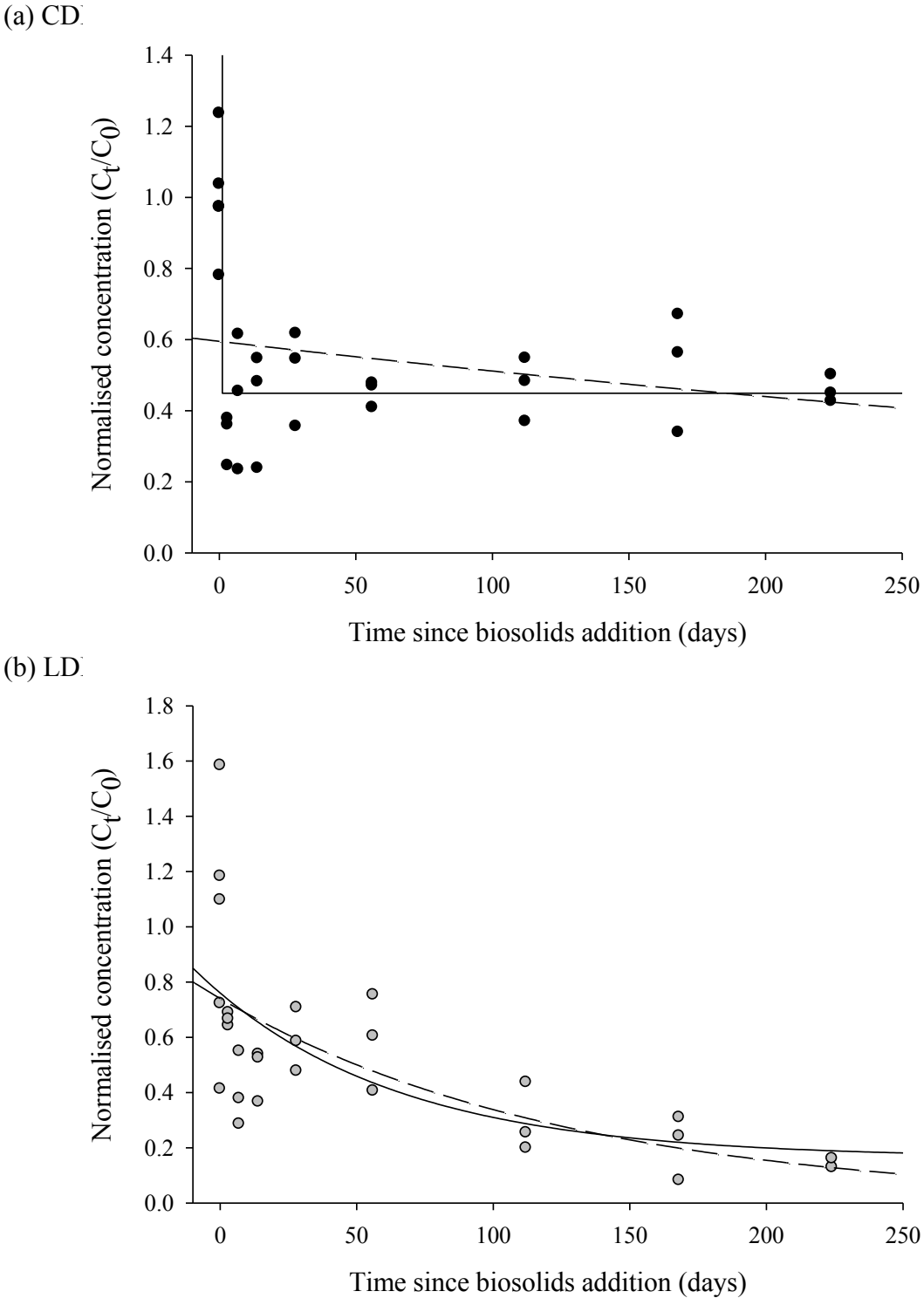
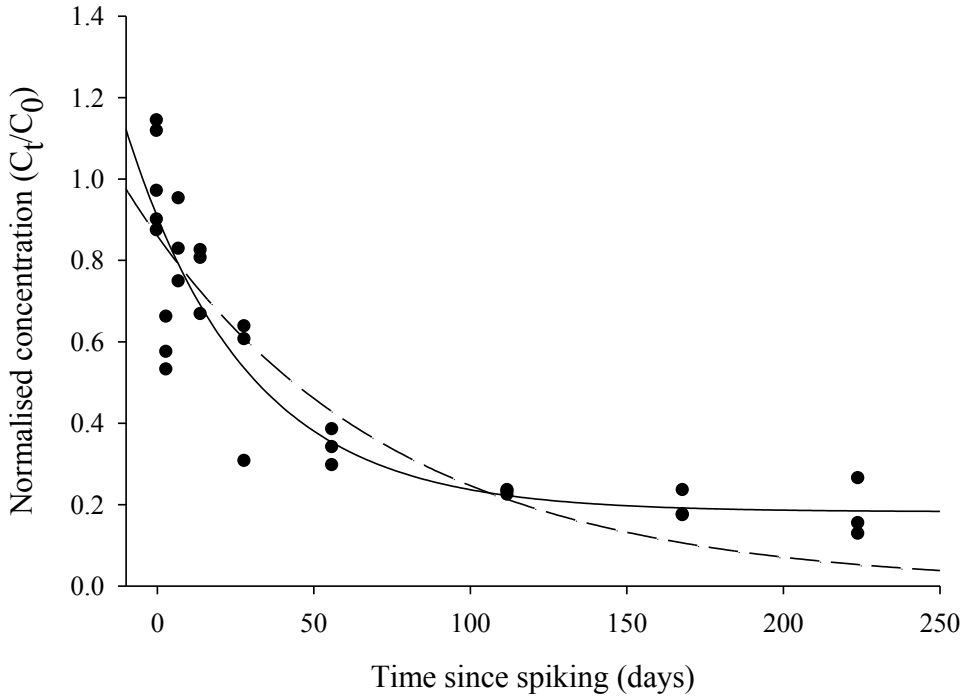


Figure 6-3: Dissipation of indigenous triclosan following the addition of (a) centrifuge dried biosolids (CDB) and (b) lagoon dried biosolids (LDB) to a soil. All concentration data is normalised as a ratio of the concentration at each sampling interval to the initial concentration (C_t/C_0). The nonlinear regression fits for first-order model and biphasic models are represented by the dashed line and the solid line, respectively.

(a) CDB



(b) LDB

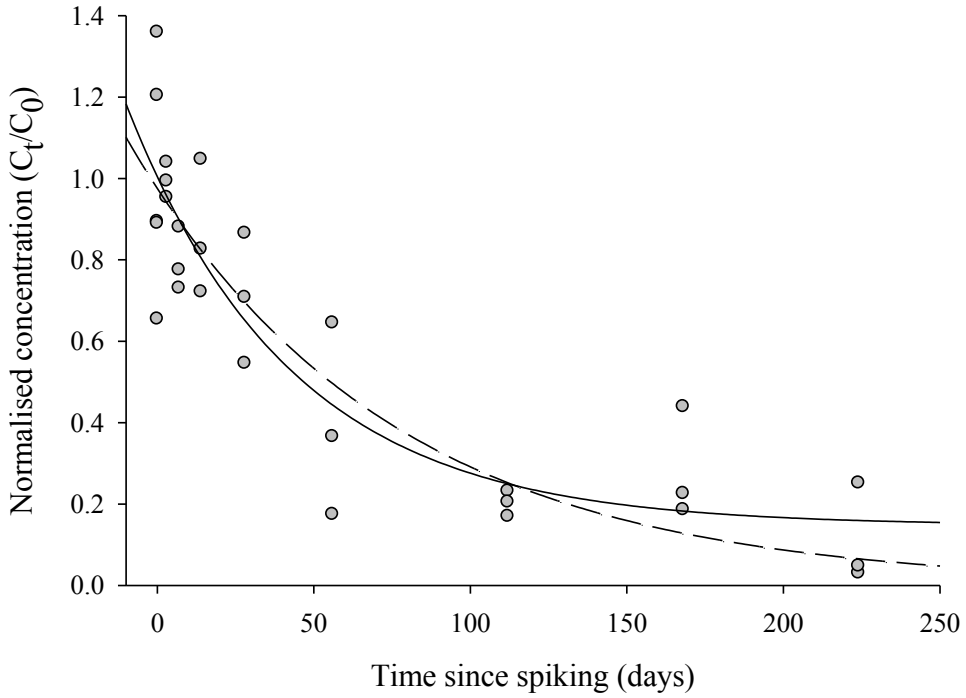


Figure 6-4: Dissipation of spiked triclosan-$^{13}C_{12}$ following the addition of (a) centrifuge dried biosolids (CDB) and (b) lagoon dried biosolids (LDB) to a soil. All concentration data is normalised as a ratio of the concentration at each sampling interval to the initial concentration (C_t/C_0). The nonlinear regression fits for first-order model and biphasic models are represented by the dashed line and the solid line, respectively.

6.3.2. Dissipation of indigenous triclosan and spiked triclosan-$^{13}C_{12}$

Over the 224 day duration of the experiment there was some dissipation of both spiked and indigenous TCS and TCS-$^{13}C_{12}$ in both biosolids treatments (Figure 6-3 and 6-4). The fit of the first-order model was significant for the indigenous TCS in the LDB treatment ($p < 0.001$) and for the spiked TCS-$^{13}C_{12}$ in both of the biosolids treatments (both p-values < 0.001) (Table 6-2). The R^2 values for this fit were 0.41 for the TCS in the LDB treatment and 0.79 for the TCS-$^{13}C_{12}$ in both of the biosolids treatments (Table 6-2). For the indigenous TCS in the CDB, the fit of the first-order model was non-significant ($p = 0.22$). The DT50 values calculated for the statistically significant fit of the first-order model were 89 days for the indigenous TCS in the LDB and 55 to 57 days for the spiked TCS-$^{13}C_{12}$.

The statistical comparison of the biphasic and first-order models to the TCS and TCS-$^{13}C_{12}$ data only showed a significant improvement in the fit for both the indigenous and spiked compounds in the CDB treatment (both p-values ≤ 0.015) (Table 6-2). The $DT50_{biphasic}$ values from this fit in the CDB treatment were < 1 day for the indigenous TCS and 27 days for the spiked TCS-$^{13}C_{12}$. For TCS and TCS-$^{13}C_{12}$ in the CDB treatment there was a recalcitrant fraction with y_0 values of 0.45 and 0.18, respectively (Table 6-2) which represent 45% and 18% of the initial concentrations. These y_0 values corresponded to recalcitrant concentrations of 96 µg/kg for TCS and 16 µg/kg for TCS-$^{13}C_{12}$ at the completion of the experiment.

6.4. DISCUSSION

In this study, the concentrations of the indigenous compounds, BPA and TCS, and the spiked compounds, BPA-d_{16} and TCS-$^{13}C_{12}$, decreased in the biosolids amended soils over time. The spiked BPA-d_{16} in both of the biosolids treatments however was the only compound to decrease to below the LOD for that compound of 0.3 µg/kg during the

experiment. For all other compounds (i.e. BPA, TCS and TCS-$^{13}C_{12}$), concentrations were above the LOQ in all samples collected over the 224 days of the experiment.

By comparison of the data for the indigenous BPA and the spiked BPA-d_{16}, it can be seen that the different method of addition of the compounds produced both different rates and patterns of dissipation. The DT50 values produced were between 10- and 100-times higher for the indigenous BPA compared to the spiked BPA-d_{16}. The DT50 values obtained in this study for the spiked BPA-d_{16} are within the range reported in other research in spike degradation experiments (i.e. 1 to 7 days) (Ying & Kookana, 2005; Xu et al., 2009). The DT50 values for the indigenous BPA are considerably higher than those reported in previous spike degradation studies, however, they are similar to those that were reported in the previous study (presented in Chapter 4) when the compound was indigenous to biosolids (i.e. 102 and 18 days in the CDB and LDB treatments, respectively).

When the biphasic model was fitted to the dissipation data for the indigenous BPA and the spiked BPA-d_{16} there were very clear differences observed in both biosolids treatments. The biphasic model provided a significantly better fit to the data for the indigenous BPA in both treatments, indicating that this compound had a recalcitrant fraction. The presence of a recalcitrant fraction of the indigenous BPA is consistent with that of the previous laboratory-based study (presented in Chapter 4), where the dissipation of BPA following the addition of biosolids to a soil was shown to be biphasic with recalcitrant fractions of 53 to 68%. In contrast, for the spiked BPA-d_{16}, the biphasic model showed no significant improvement to the fit of the data, indicating that there was no recalcitrant fraction present. This is also evident as the concentration of the BPA-d_{16} in both of the biosolids treatments decreased to below the LOD of 0.3 µg/kg prior to the completion of the experiment. A comparison of these results between the indigenous and spiked compound

indicate that the mechanism responsible for the presence of a recalcitrant fraction does not play a role when the compound is spiked into the sample.

Due to the variations in the pattern of dissipation between the indigenous BPA and the spiked BPA-d_{16}, the DT50 calculated from the first-order model cannot be used to compare the dissipation rates of the compounds. If the DT50 values, as determined from the first-order, are compared between the indigenous and spiked compounds, it implies that the dissipation rate is 100-times slower for the indigenous BPA in the CDB treatment and 10-times slower in the LDB treatment. This comparison may be misleading as the dissipation of the indigenous BPA is explained better by the biphasic model. Instead, if the dissipation rate obtained from the model that provided the best fit to the data is used (i.e. $DT50_{biphasic}$ for the indigenous BPA and DT50 for the spiked BPA-d_{16}), a more meaningful comparison can be made. By using this method of comparison, the dissipation rate is approximately 5-time slower for the indigenous BPA compared to the spiked BPA-d_{16}. Although this value is considerably less than the 10- to 100- times observed if only the DT50 values are compared, it does still show that the dissipation rate varies depending on the method used for the addition of the compounds to the soils. These results for BPA clearly show that both the rate and pattern of dissipation varies for the indigenous and spiked compounds, therefore indicating that the use of spiking to predict dissipation of this compound following biosolids application to land may not be suitable.

The fit of the first-order model to the indigenous TCS in the CDB treatment was non-significant, indicating that this first-order decay model did not significantly improve the fit to the data compared to a horizontal line indicating no change (i.e. no dissipation). In comparison, the spiked TCS-$^{13}C_{12}$ in the same CDB treatment did show a significant fit to this model indicating that there was dissipation, with a DT50 value of 55 days. For the

LDB treatment, for both the indigenous and spiked compounds, the first-order model was significant and the difference in DT50 values was approximately 1.6-times. This showed that for TCS, in this biosolids treatment, the dissipation was only slightly slower for the indigenous compound compared to the spiked compound. Similar to what was found for BPA, the DT50 values obtained in this study for the spiked TCS-$^{13}C_{12}$ are within the range of those reported in other research using spike degradation experiments (i.e. 18 to 58 days) (Ying et al., 2007; Wu et al., 2009a; Xu et al., 2009). For the indigenous TCS in the LDB treatment, the DT50 value was only slightly longer at 89 days, however, in the CDB treatment there appeared to be no dissipation of the indigenous TCS.

The differences in the dissipation pattern of indigenous TCS and spiked TCS-$^{13}C_{12}$ as shown by the biphasic model were not as clear as those observed for BPA, however, some differences were seen. The fit of the data for both the indigenous and the spiked form of this compound in the CDB biosolids was significantly improved by the additional parameter in the biphasic model. Although this was the case, by observing the fit to the indigenous data presented in Figure 6-3a, it can be seen that this model does not explain the data particularly well and that the significant improvement may not be meaningful. The $DT50_{biphasic}$ that was calculated for this dataset was less than one day (Table 6-2) as a result of the rapid initial decrease of the concentration. Following this there does not appear to be any change in the concentration through to the completion of the experiment at 224 days. It should be noted however, that a similar pattern was observed for TCS in this biosolids treatment in the previous laboratory study (Chapter 4). The dissipation data for the spiked TCS-$^{13}C_{12}$, shows a better fit to the biphasic model and produced a $DT50_{biphasic}$ value of 27 days. It is likely that the indigenous TCS in the CDB treatment showed no considerable dissipation throughout the 224 days of this experiment, whereas the spiked TCS-$^{13}C_{12}$ did show dissipation and followed a biphasic pattern.

For the indigenous and spiked TCS in the LDB treatment, the biphasic model did not improve the fit of the data, indicating that in this treatment, neither compound showed a recalcitrant fraction. These differing results between the two biosolids treatments show that the dissipation patterns of this compound vary with differing biosolids treatments. This indicates that there is some influence of the biosolids matrix on the pattern of dissipation of TCS, and therefore this needs to be considered when assessing its persistence following land application of biosolids. These results for TCS, although not as clear as those discussed previously for BPA, do indicate that it may not be suitable to use spike degradation experiments to predict the persistence of this compound following the addition of biosolids to a soil.

The differences observed in this study between the dissipation rate of the indigenous and spiked compounds indicate that the use of spike degradation experiments may not provide an accurate assessment of the persistence of organic compounds in biosolids following land application. This finding may also apply to other organic soil amendments that are applied to agricultural land such as animal manures. In all cases, the rates were faster for the compounds that had been spiked into the samples compared to the indigenous compounds. In addition to this, the BPA clearly showed that when it was added to a soil as an indigenous component of biosolids, there was a fraction that was recalcitrant whereas for the spiked compound, the concentration continued to decrease to a concentration that was below detection. This indicates that the use of spiked degradation experiments may not account for the recalcitrant fraction that may be present for some compounds in biosolids amended soils. In other studies, recalcitrant fractions of compounds in biosolids or biosolids amended soils have also been observed (e.g. Hesselsoe et al., 2001; Sjostrom et al., 2008; Wu et al., 2009b). It is unclear what the mechanism that is responsible for this

is, however, there are several suggestions. The presence of anaerobic zones within the aggregates of biosolids may result in delayed degradation due to limited oxygen availability (Hesselsoe et al., 2001). Both of the compounds assessed in this study degrade solely under aerobic conditions (McAvoy et al., 2002; Ying & Kookana, 2005; Press-Kristensen et al., 2008), therefore, limited oxygen availability is likely to influence the degradation rates of the compounds. A further suggestion relates to non-reversible sorption of the compounds to the biosolids matrix (Wu et al., 2009b). As biosolids matrices can be particularly complex and contain various components, sorption may vary from one biosolids to another. This could explain the differences observed in this study between the biosolids treatments in terms of the dissipation rates for both compounds and the presence of the recalcitrant fraction for TCS in the CDB treatment however not in the LDB treatment. Compounds that are spiked into biosolids or biosolids amended soils are unlikely to sorb to the matrix in the same manner as that of compounds that are present within biosolids throughout the treatment process. In addition, the distribution of a spiked compound within a biosolids aggregate is likely to differ to that of a compound that is indigenous to a biosolids sample. The spiked compound is likely to sorb to the outer portions of a biosolids aggregate and therefore be more available to microbes in the presence of oxygen which will result in faster rates of degradation. Although it is unclear what mechanism or combination of mechanisms are responsible for the differing dissipation of the compounds assessed in this study, it is clear that the use of spike degradation experiments to predict the degradation of a compound following the addition of biosolids to land may provide inaccurate estimates of persistence.

6.5. CONCLUSIONS

In this study, the indigenous BPA and spiked BPA-d_{16} both showed dissipation over the 224 days. The time taken for the initial concentration to decrease by 50% (DT50)

calculated from the first-order model was 10- to 100-times longer for the indigenous compound than the spiked compound. The indigenous BPA showed a biphasic pattern of dissipation with a recalcitrant fraction, whereas the spiked BPA-d_{16} degraded to a concentration that was below detection. Based on a biphasic dissipation model, the rate of dissipation for the indigenous BPA was approximately 5-times slower than that of the spiked BPA-d_{16}. The indigenous TCS did not appear to show any dissipation in the centrifuge dried biosolids (CDB) treatments, but did so in the lagoon dried biosolids (LDB) treatment, with a DT50 value of 89 days. This DT50 value was approximately 1.6-times slower than the spiked TCS-$^{13}C_{12}$ in the two biosolids treatments. The dissipation data for the spiked TCS-$^{13}C_{12}$ in the CDB treatment followed a biphasic pattern whereas in the LDB the dissipation data for both the indigenous and spiked TCS followed a first-order decay. This study shows that spiking compounds into soils can produce differences in dissipation, in terms of both the rates and the patterns, to those of compounds indigenous to biosolids. Therefore the use of spiking experiments to predict the persistence of organic compounds following land application of biosolids may provide misleading results.

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Chapter 7

Comparison of dissipation rates between indigenous and spiked 4-nonylphenol and 4-t-octylphenol in a biosolids amended soil

Abstract

The potential for organic contaminants to accumulate in soils following the land application of biosolids needs to be understood in order to assess the risks associated with this practice. The persistence of organic compounds in soils and, by inference, in biosolids amended soils is often determined by using spiked degradation experiments, in which a compound is added to soil. This study measured the dissipation rates of 4-nonylphenol (4NP) and 4-t-octylphenol (4tOP) in soils following the addition of two biosolids to determine if samples that contained additional spiked concentrations of the compounds displayed different dissipation rates to the compounds indigenous to the biosolids. For both compounds, the spiked fractions were distinguishable from the indigenous fractions in the early stages of the experiment, however were not distinguishable towards the completion of the experiment. The time required for the initial concentration of indigenous 4NP to decrease by 50% (DT50) was 22 to 52 days which was longer than the 16 to 30 days for the samples that contained the additional spike of 4NP. The DT50 values for indigenous 4tOP ranged from 13 to 18 days, which were also longer than the 6.4 to 7.1 days for the samples that contained the additional spike of 4tOP. This study showed that the rates of dissipation of compounds spiked into biosolids amended soil differed from those of the indigenous compounds. Therefore spiked degradation experiments may not be suitable for estimating the persistence of organic compounds following land application of biosolids and their potential to accumulate in soils.

CONTENTS

7.1. INTRODUCTION

The surfactant metabolites 4-nonylphenol (4NP) and 4-t-octylphenol (4tOP) are produced from their parent alkylphenol ethoxylate compounds during wastewater treatment processes (Ying & Kookana, 2005). These shorter chained metabolite compounds tend to be more persistent than their parent compounds, with degradation only occurring under aerobic conditions (Ying & Kookana, 2005; Press-Kristensen et al., 2008). Due to the hydrophobic nature of 4NP and 4tOP they tend to concentrate in the solid waste phase of waste water treatment plants, i.e. biosolids. As a result, these compounds have been detected in biosolids at concentrations ranging from 0.6 to 440 mg/kg for 4NP and 0.2 to 2.4 mg/kg for 4tOP (Kinney et al., 2006). The application of biosolids to land is a route of entry for 4NP and 4tOP into the environment which may result in accumulation in soils and exposure of terrestrial organisms and aquatic organisms if off-site migration occurs (Langdon et al., in press). The main concern for organisms from these compounds is that they have been shown to interfere with estrogen receptors and thereby causing endocrine disruption (e.g. Jobling & Sumpter, 1993). To determine the potential risks that these compounds may pose to the environment, following the application of biosolids to land, an understanding of their persistence is required in addition to an understanding of their toxic effects.

The degradation of 4NP in soils has been assessed quite extensively in the literature due to the high level of concern surrounding this compound internationally. Laboratory-based experiments measuring the mineralisation of ^{14}C labelled 4NP added to soil have been conducted with reported half-life values for the compound ranging from 1 to 17 days (Topp & Starratt, 2000; Roberts et al., 2006). Less research has been conducted on the degradation of 4tOP in soils, however, in a study conducted using a range of Australian

soils, spike degradation experiments found an average half life for this compound of 5 days (Ying & Kookana, 2005).

The persistence of 4NP and 4tOP in soils when the addition of biosolids to the soil is the source of contamination (i.e. the compounds are indigenous to the biosolids at the time of addition) appears to be longer than that observed in spike degradation experiments. For example, Brown et al. (2009) assessed the persistence of 4NP following the addition of biosolids to a soil and reported half-life values ranging from 16 to 23 days. In addition to this, in a previous study (presented in Chapter 4) assessing the persistence of indigenous 4NP and 4tOP following the addition of two different biosolids treatments to a soil reported the time taken for the initial concentration of the compound to decrease by 50% (i.e. dissipation half-life, DT50) ranged from 12 to 25 days for 4NP and 10 to 14 days for 4tOP (presented in Chapter 4). It is possible that the form in which the compounds are added to the soils (i.e. spiked or indigenous to biosolids) may influence the rate of degradation observed and, in turn, results from spike degradation experiments may not be comparable with rates observed for compounds that are indigenous to biosolids. In order to permit direct comparison, however, studies need to be conducted in the same soils and under the same experimental conditions.

A glasshouse study on the degradation of 4NP in soils, which is partially consistent with the above theory, found that degradation was more complete when the compound was spiked into a soil rather than added through the addition of organic wastes (Mortensen & Kure, 2003). The study reported that after 30 days, 16% of the initial indigenous 4NP remained in the soils that had been amended with sludge, whereas, only 0.9% of 4-n-nonylphenol (4nNP, an unbranched isomer of 4NP) that had been spiked into the soils remained. In that study, however, as the isomers of the indigenous and spiked compounds

were different this may have influenced the results, as unbranched compounds tend to degrade more readily than branched compounds (Mortensen & Kure, 2003). It is also well established that the branched chain 4NP isomers are more prevalent in biosolids when compared to the unbranched 4nNP isomer (Mortensen & Kure, 2003). Therefore, although the Mortensen & Kure (2003) study suggests the degradation of the spiked compound differs from that of compounds indigenous to organic waste products, the use of slightly different compounds may result in inconsistencies.

In the present study, the dissipation of 4NP and 4tOP will be examined in biosolids amended soils. The dissipation rates of the compounds that are indigenous to the biosolids will be assessed and compared with the rates when elevated levels of the same compounds have been spiked into the biosolids amended soil samples. This study did not involve the use of isotopically labelled surrogate compounds, as was done previously for the compounds bisphenol (BPA) and triclosan (TCS) (Chapter 6). As a result, in the current study, only comparisons in terms of dissipation rates can be made. Comparisons of dissipation pattern cannot be made (as was done previously in Chapter 6) as the spiked and indigenous fractions of the compounds cannot be distinguished from each other. The two studies will also enable a comparison of the effectiveness of the two different methods of spiking (i.e. isotope labelled and non-isotope labelled) to be made.

7.2. MATERIALS AND METHODS

7.2.1. Soil and Biosolids

A bulk soil was collected from Mount Compass in South Australia (SA) (35°21‟44.95 S and 138°32‟44.95 E), which is located approximately 70 km south of Adelaide, for use in this study. The soil had a pH 4.4 (determined using a soil:solution ratio of 1:5 in 0.01M $CaCl_2$), an organic carbon content of 2.5%, and consisted of 96% sand, 2.5% silt and 1.5%

clay. The bulk sample was dried at 40°C prior to being homogenized by grinding with a mortar and pestle and sieved to 2 mm.

Two locally produced biosolids were collected for use in this study. Both biosolids had been treated by anaerobic digestion, but thereafter one of the biosolids had been centrifuge dried (CDB) and the other had been solar dried in a lagoon system (LDB). The moisture contents of the biosolids were 63% for the CDB and 52% for the LDB and for the experiment the biosolids were used as collected (i.e. wet).

7.2.2. Experimental design and set up

Individual 50 g samples were weighed from the dried bulk soil into glass jars and hydrated to 50% of their maximum water holding capacity (MWHC) with Milli Q (MQ) water (the MWHC was determined using the method outlined in Jenkinson & Powlson, 1976). All samples were then placed in closed containers in the dark and pre-incubated at 22°C for 14 days to rejuvenate and stabilise soil microbial communities. After the pre-incubation either the CDB or LDB biosolids were added to the hydrated soil, at a rate equivalent to 50 dry t/ha (assuming a soil bulk density of 1.3 g/cm^3 and an incorporation depth of 10 cm). Within each of the biosolids treatments, half the samples were spiked with 200 µL of a stock solution in methanol, which contained 4NP and 4tOP at concentrations of 500 mg/L and 25 mg/L respectively. This spike added an additional concentration of 2 mg/kg and 100 µg/kg for 4NP and 4tOP, respectively, to the indigenous concentrations of the compounds, which was already in the samples from the biosolids addition. The higher spiking concentration of 4NP was used due to the higher predicted initial concentration of this compound in the biosolids (as seen in Chapters 4 and 5). This therefore ensured that the spiked fraction could be statistically differentiated from the indigenous fraction already contained within the biosolids. The remaining half of the samples had 200 µL of methanol,

that contained no addition compounds (methanol control), added to them. Therefore these samples contained only the compounds that were indigenous to the biosolids. Five replicate samples from each of the spiking treatments within each of the biosolids treatments were then freeze dried immediately and stored in the dark until analysis as the initial sample (t_0). All the remaining sample jars were weighed, then placed on wet paper towel in containers with lids and kept in the dark at a constant temperature of 22°C. The samples were opened to the air on a daily basis and the moisture content in the soil was maintained throughout the experiment by weight at 50% MWHC. At eight additional sampling intervals (3, 7, 14, 28, 56, 112, 168 and 224 days post biosolids addition), triplicate sample jars were removed from each of the biosolids treatments and freeze dried for immediate analysis of the target compounds.

7.2.3. Sample extraction and GCMS analysis

For sample extraction and analysis, 10 g from each freeze dried sample was extracted three times with 10 mL of 1:1 methanol and acetone in an ultrasonic bath. For each sample the extracts were combined then diluted with MQ water and loaded onto Oasis HLB® solid phase extraction (SPE) cartridges. Elution of the samples was conducted using 3 × 2.5 mL methanol, followed by 3 × 2.5 mL acetone and 3 × 2.5 mL ethyl acetate and reconstituted in 4 mL of methanol. Each sample was then derivatized in 400 µL of pyridine and 100 µL of the silylation agent *N,O*-bis-(trimethylsilyl)-trifluorocetamide (BSTFA) + 1% trimethyl-chlorosilane (TMCS) (based on the method of Shareef et al., 2006) and anthracene-d_{10} was added to each sample as an instrument internal standard (IS). Samples were analysed using an Agilent 6890 Series GC system that was interfaced with an Agilent 5973 Network Mass Spectrometer (MS). The concentrations of each of the compounds were determined from relative response factors based on the IS and then adjusted for extraction recoveries based on the labelled surrogate, 4nNP-d_8, which had

been spiked into the samples one day prior to extraction. The limit of detection (LOD) and limit of quantification (LOQ) for each of the compounds were determined as 3- and 10-times the signal to noise ratio and were 30 and 100 µg/kg respectively for 4NP, 0.6 and 2.0 µg/kg respectively for 4tOP.

7.2.4. Statistical analysis and data interpretation

7.2.4.1. Comparison of concentrations of indigenous and spiked compounds

The concentration data for each of the compounds were compared separately within the biosolids treatments using individual t-tests at each sampling time in PASW Statistics® Version 17. These t-tests determined if the concentrations in the spiked samples were significantly higher than in the indigenous samples at the commencement of the experiment and if this difference continued throughout the 224 days of the experiment. If the compounds in the spiked and indigenous samples were dissipating at the same rate, it was expected that differences would be observed throughout the entire duration of the experiment. The significance level used for all statistical tests was $\alpha = 0.05$.

7.2.4.2. Dissipation rates of indigenous and spiked compounds

The dissipation rates of the compounds were determined by fitting a nonlinear regression to the concentration data of each compound using SigmaPlot® Version 10. Prior to the nonlinear regression modelling, all concentration data across the duration of the experiment were normalised to a ratio of the initial concentration (C_t/C_0). This normalised the data to an initial mean value of 1 and removed any variation at t_0 between the biosolids treatments and the compounds. The dissipation rates of 4NP and 4tOP in the indigenous samples and the spiked samples were determined using a first-order exponential decay regression model, shown in equation 7-1,

$$C_t = C_0 e^{-kt} \quad (7\text{-}1)$$

where, C_t is the concentration of the compound at time, t, C_0 is the initial concentration of the compound and k is the rate constant. The rate constant, k, was then used to determine the DT50 for each of the compounds in the indigenous and spiked samples by using equation 7-2.

$$DT50 = ln(2) / k. \quad (7\text{-}2)$$

In the current study only the first order model was used to assess the dissipation of the compounds (as opposed to the first-order and biphasic models in Chapters 4, 5 and 6) as this enabled the relative rates of dissipation to be compared. As the spiked fractions and indigenous fractions of each of the compounds were not independent from each other (i.e. they were the same compound), the more complex pattern determined by the biphasic model could not be compared between the two fractions (as was done in previous Chapters). In addition to this, in the previous laboratory study (Chapter 4), the fit of the dissipation data for 4tOP was not significantly improved by the biphasic model.

7.3. RESULTS

7.3.1. Comparison of concentrations of indigenous and spiked compounds

The initial concentrations for 4NP in the indigenous and spiked samples ranged from 9843 to 13720 µg/kg respectively in the CDB treatment and from 1500 to 3540 µg/kg respectively in the LDB treatment. For 4tOP the initial concentrations ranged from 67 to 174 µg/kg respectively in the CDB treatment and from 120 to 246 µg/kg respectively in the LDB treatment (Table 7-1). For 4NP in both of the biosolids treatments and for 4tOP in the CDB treatment, the initial concentrations in the spiked samples were significantly

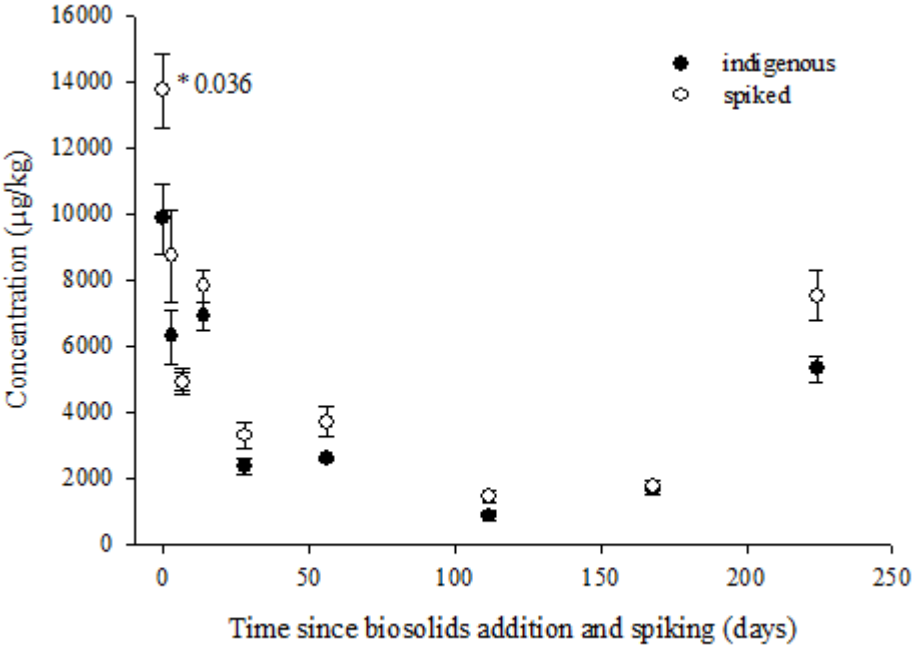
higher than in the indigenous samples (all p-values ≤ 0.036) (Figures 7-1 and 7-2). For 4tOP in the LDB treatment the difference in initial concentrations between the indigenous and spiked samples was non-significant ($p > 0.05$).

Table 7-1: The average and range of concentrations of the compounds 4-nonylphenol and 4-t-octylphenol in the initial (t_0) spiked and indigenous samples for the centrifuge dried biosolids (CDB) and lagoon dried biosolids (LDB) treatments. All concentrations are shown in μg/kg.

Biosolids treatment	**4-nonylphenol**		**4-t-octylphenol**	
	indigenous	**spiked**	**indigenous**	**spiked**
CDB	9843 (6480-11050)	13720 (9280-15590)	67 (41-95)	174 (153-186)
LDB	1500 (938-2125)	3540 (2380-4750)	120 (92-152)	246 (115-409)

For the compound 4NP, in the CDB treatment, there were no significant differences ($p > 0.05$) between the concentrations in the indigenous and spiked samples following that observed in the t_0 samples (Figure 7-1a). For 4NP in the LDB treatment, concentrations in the spiked samples were only significantly higher (all p-values ≤ 0.034) than the indigenous samples on days 14 and 28 after the commencement of the experiment (Figure 7-1b). For the compound 4tOP, in the CDB treatment, the concentrations were only significantly higher (both p-values ≤ 0.015) in the spiked samples on days 3 and 112 after the commencement of the experiment (Figure 7-2a). For 4tOP in the LDB treatment, although the concentrations were not significantly different in the initial t_0 samples, the concentrations in the spiked samples were significantly higher (both p-values ≤ 0.048) than the indigenous samples at days 14 and 28 after the commencement of the experiment (Figure 7-2b).

(a) CDB



(b) LDB

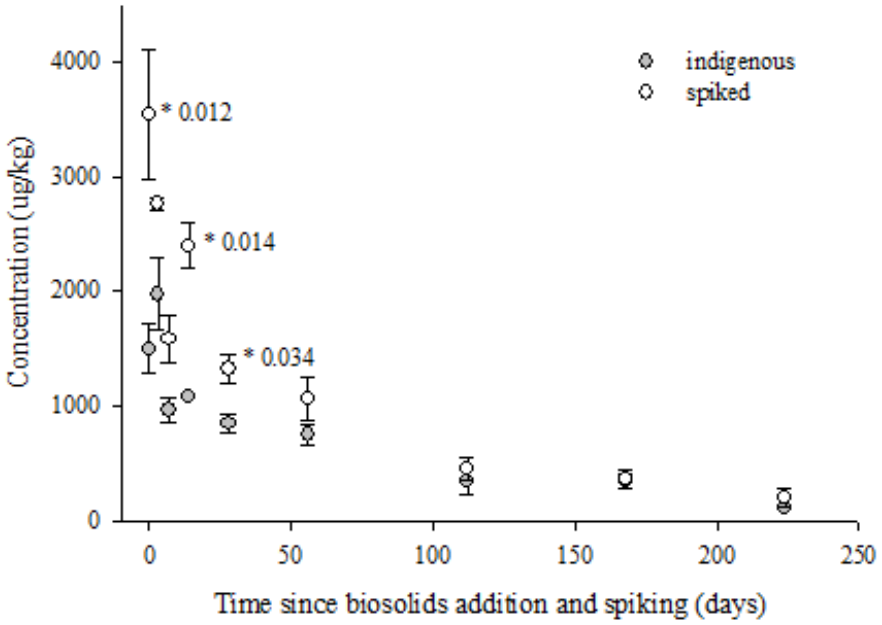
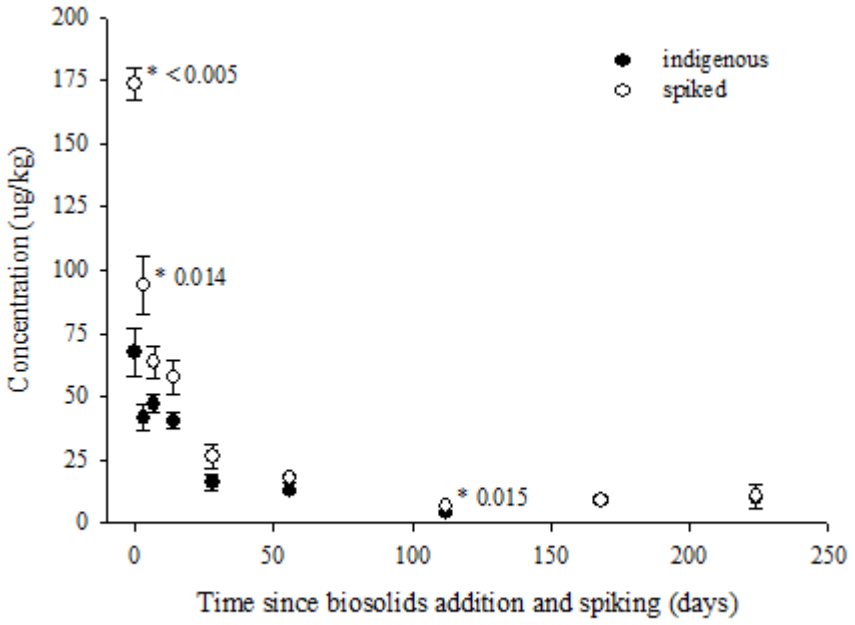


Figure 7-1: Dissipation of indigenous and spiked (being the sum of indigenous added) 4-nonylphenol following the addition of (a) centrifuge dried biosolids (CDB) and (b) lagoon dried biosolids to a soil. Error bars indicate standard errors and pairs of values that are significantly different from each other are indicated by an asterisk (*) with p-values shown.

(a) CDB



(b) LDB

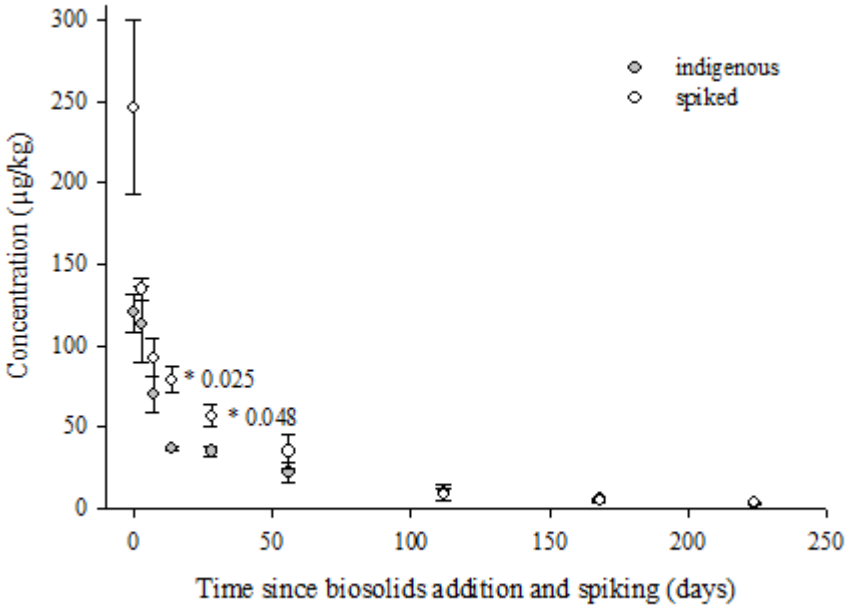


Figure 7-2: Dissipation of indigenous and spiked (being the sum of indigenous added) 4-t-octylphenol following the addition of (a) centrifuge dried biosolids (CDB) and (b) lagoon dried biosolids to a soil. Error bars indicate standard errors and pairs of values that are significantly different from each other are indicated by an asterisk (*) with p-values shown.

7.3.2. Dissipation rates of indigenous and spiked compounds

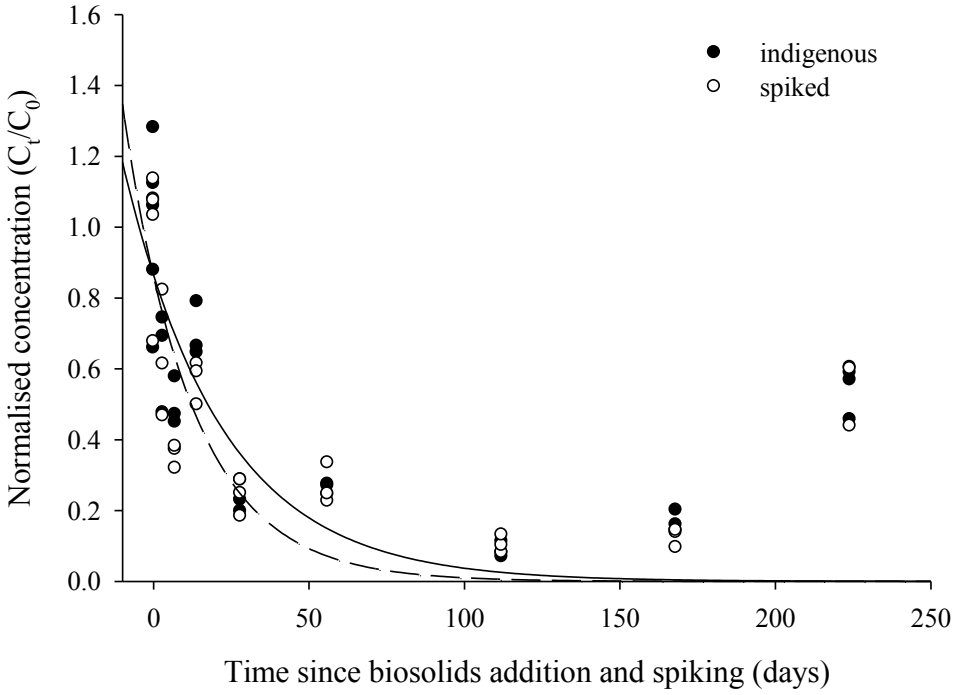
The plots of the normalised concentrations of 4NP and 4tOP for the duration of the experiment are shown in Figures 7-3 and 7-4, respectively, along with the fit of the first-order exponential model, which in all cases was highly significant ($p < 0.001$). The fit of the first-order model to the dissipation data for 4NP explained 39 to 43% of the variation in the data for the CDB treatments and 69 to 75% of the variation in the data for the LDB treatment (Table 7-2). The fit to the 4tOP data by this model was better than that for the 4NP data and explained 79 to 91% of the variation in the dissipation data for the CDB treatment and 71 to 84% of the variation in the data for the LDB treatment (Table 7-2).

The DT50 values that were calculated from the first-order model ranged from 22 to 52 days for 4NP in the indigenous samples and 16 to 30 days in the spiked samples (Table 7-2). The DT50 values for 4tOP were lower and ranged from 13 to 18 days in the indigenous samples and from 6.4 to 7.1 days in the spiked samples.

Table 7-2: Summary information from the first-order regression fit to the dissipation data for 4-nonylphenol and 4-t-octylphenol in the centrifuge dried biosolids (CDB) and the lagoon dried biosolids (LDB) treatments. The time taken for the initial concentrations to dissipate by 50% (DT50) values are shown in days.

Biosolids treatment	**Measure**	**4-nonylphenol**		**4-t-octylphenol**	
		indigenous	**spiked**	**indigenous**	**spiked**
CDB	R^2	0.43	0.39	0.79	0.91
	DT50	22	16	18	6.4
LDB	R^2	0.69	0.75	0.84	0.71
	DT50	52	30	13	7.1

(a) CDB



(b) LDB

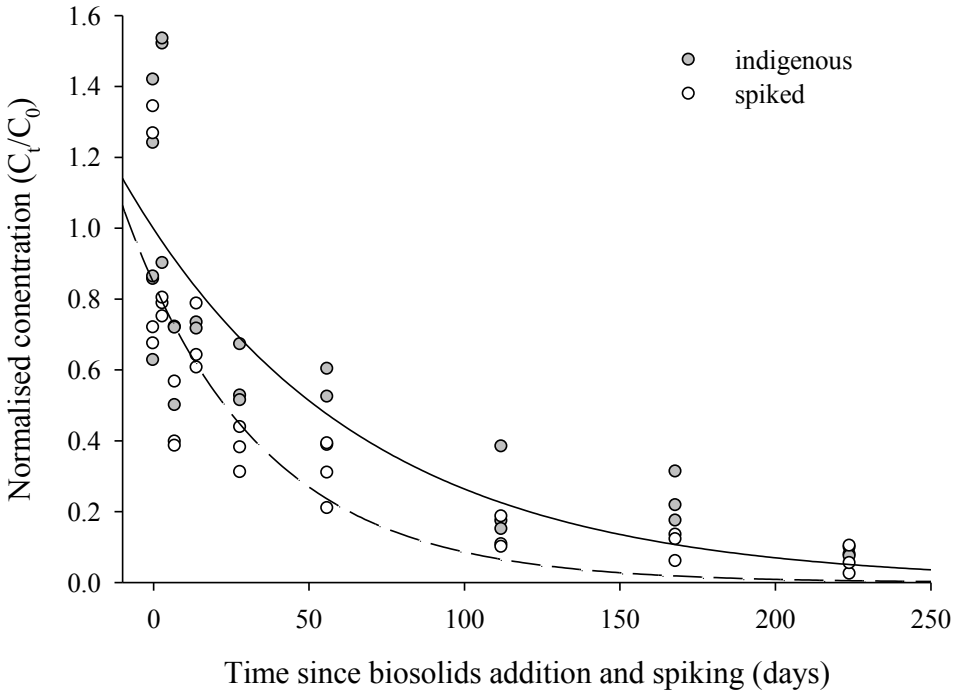
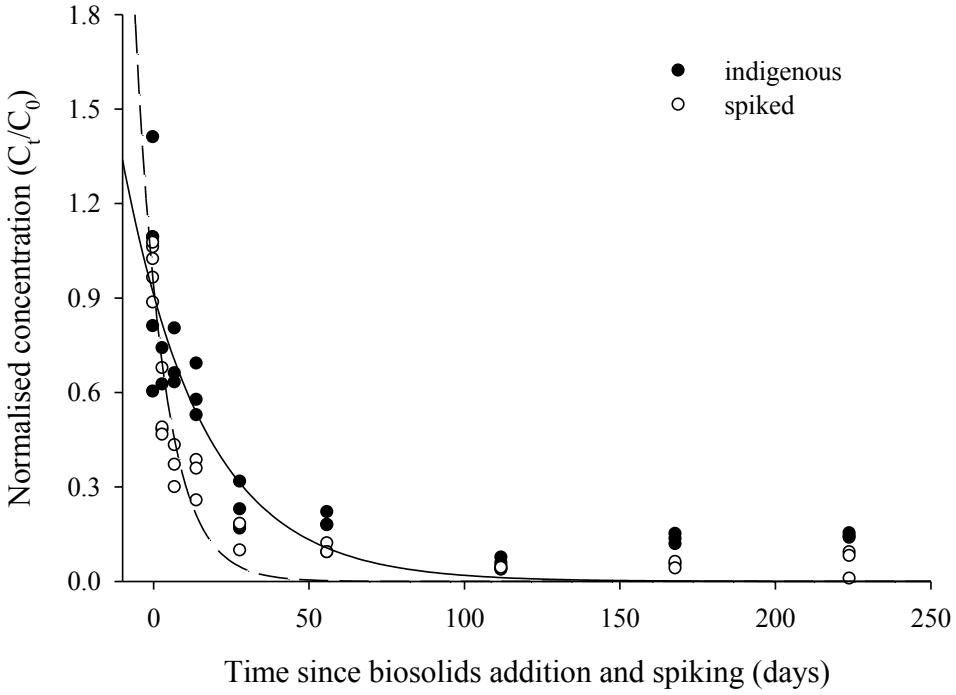


Figure 7-3: Dissipation of indigenous and spiked (being the sum of indigenous added) 4-nonylphenol following the addition of (a) centrifuge dried biosolids (CDB) and (b) lagoon dried biosolids to a soil. All concentration data is normalised as a ratio of the concentration at each sampling interval to the initial concentration (C_t/C_0). The first-order regression fits are shown for the indigenous and spiked samples solid and dashed lines, respectively.

(a) CDB



(b) LDB

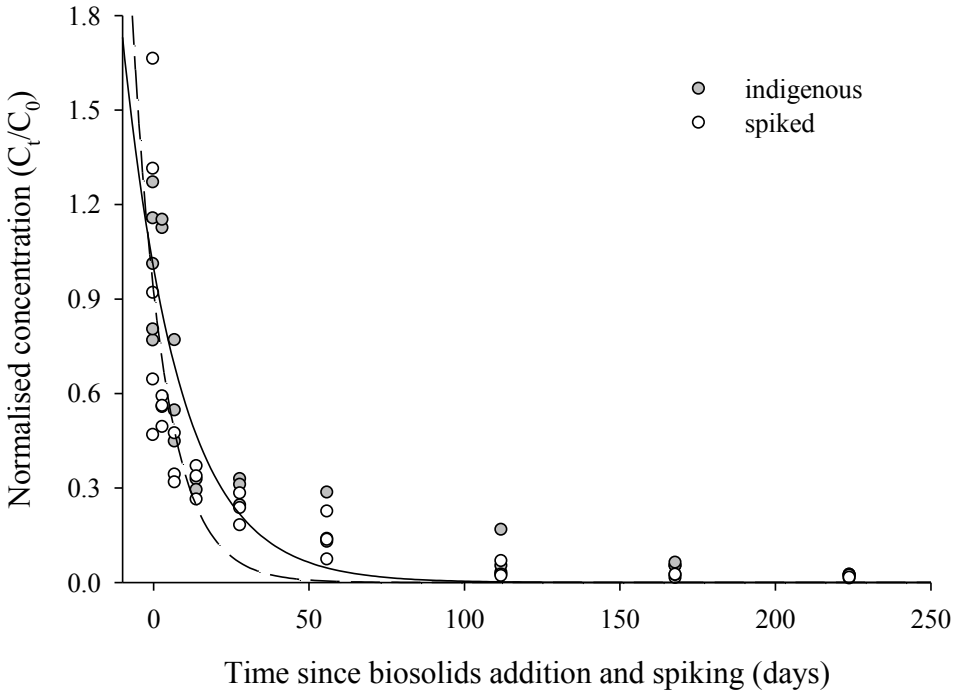


Figure 7-4: Dissipation of indigenous and spiked (being the sum of indigenous added) 4-t-octylphenol following the addition of (a) centrifuge dried biosolids (CDB) and (b) lagoon dried biosolids to a soil. All concentration data is normalised as a ratio of the concentration at each sampling interval to the initial concentration (C_t/C_0). The first-order regression fits are shown for the indigenous and spiked samples solid and dashed lines, respectively.

7.4. DISCUSSION

At the commencement of the experiment, the concentrations in the spiked samples were all significantly higher than the indigenous samples in all cases except for the 4tOP in the LDB treatment. This lack of a clear distinction between the spiked and indigenous fraction for 4tOP in the LDB treatment was due to the large variation that was seen in the replicates from the spiked samples (Table 7-1 Figure 7-2b). This resulted in a large error for this group of samples (Figure 7-2b). Although the concentration of this compound at the initial sampling time was not significantly higher than that of the indigenous sample, Figure 7-2b clearly shows that the mean concentration is considerably higher in the spiked sample than the indigenous sample.

In all treatments there were some statistically significant differences between the concentration in the indigenous and spiked samples in the early stages of the experiment, however, by the completion at day 224 there were no statistical differences (Figures 7-1 and 7-2). These results suggest that the spiked fraction, which was initially distinguishable from the indigenous fraction (indicated by the significantly higher concentrations at the early stages of the experiment), is dissipating faster than the indigenous fraction. By the completion of the experiment, there were no statistically significant differences in concentrations between the indigenous and spiked samples of each compound, indicating that the concentrations in all samples were likely due to the indigenous fractions of 4NP and 4tOP. However, this lack of significance may also be due to higher variability being observed between replicates towards the end of the experiment, making statistically significant differences less likely. In addition to this, it should also be noted that there were five replicate samples for the initial t_0 sample and three replicates at all subsequent sampling times, which would result in a greater likelihood of statistically significant differences observed at t_0 compared to all other sampling times.

The results from the fits of the exponential degradation curves to the normalised concentration data for both compounds support the results obtained from the individual t-tests. The DT50 value for 4NP in the indigenous samples was 22 days in the CDB treatment and 52 days in the LDB treatment. The dissipation of 4NP in the spiked CDB treatment occurred 1.4-times faster than in the corresponding indigenous samples, with a DT50 value of 16 days and dissipation occurred 1.7-times faster in the LDB treatment with a DT50 value of 30 days. The DT50 values for 4tOP calculated in the indigenous samples were 18 days in the CDB treatment and 13 days in the LDB treatment. The DT50 values for the spiked 4tOP were 2.8-times faster than for the indigenous 4tOP in the CDB treatment with a DT50 of 6.4 days and were 1.8-times faster in the LDB treatment with a DT50 of 7.1 days.

The results obtained in this study are consistent with those that were reported by Mortensen & Kure (2003), that indicated that the degradation of spiked 4NP was more complete in a soil than that of a similar compound (4nNP) added through the addition of organic waste products. The mechanism responsible for these differences in dissipation of the compounds that are indigenous to biosolids and that of compounds spiked into the matrix is not clear, however several suggestions can be made. The ageing of an organic compound that takes place within the biosolids matrix throughout waste treatment processes may result in irreversible sorption of organic compounds to the matrix or components of the matrix (Wu et al., 2009). The same degree of ageing is unlikely to be achieved when a compound is spiked directly into a sample. The different degree or type of sorption in the early stages of the experiment would therefore result in the compound being more available to microorganisms, which would in turn promote biodegradation. However, as the dissipation experiment continued the ageing of the spiked compound

would increase and may eventually be similar to that of the indigenous compounds. In addition to this there is also likely to be some influence of the distribution of the compounds throughout a biosolids aggregate that may affect degradation rates. Hesselsoe et al. (2001) found that the ability of oxygen to penetrate a biosolids aggregate was a limiting factor in the degradation of organic compounds. When a compound is spiked into a biosolids or biosolids amended soil sample, the compound is likely to be bound to the outer portions of a biosolids aggregate. In comparison, a compound that is indigenous to the biosolids is likely to be distributed throughout an aggregate. Although future research is needed to determine the exact mechanism(s) responsible for the differences in the dissipation of the spiked and indigenous 4NP and 4tOP, this study does show that the use of spiking experiments to predict the degradation and/or dissipation of a compound following the addition of biosolids to land may provide underestimates of their persistence.

The results from this study are consistent with those obtained for BPA and TCS (Chapter 6), indicating that spiked compounds in a biosolids amended soil dissipate at a faster rate than those that are indigenous to biosolids. As the spiked and indigenous fractions were not independent of each other in the present study (i.e. the compound was not isotopically labelled), the data analysis and comparisons of dissipation were limited. For example, the dissipation pattern (i.e. first-order or biphasic) could not be compared between the spiked and indigenous compounds. As a result, the data obtained in this study are not as conclusive of those obtained using isotopically labelled spikes (Chapter 6). Therefore, although this current method yielded consistent results, the use of isotopically labelled spikes is the favoured method for this type of study.

7.5. CONCLUSIONS

In this study, 4-nonylphenol (4NP) and 4-t-octylphenol (4tOP) were found to dissipate at a faster rate in the samples containing an additional spike of the compounds when compared to that of the indigenous compounds to biosolids. For both compounds, the spiked fractions were distinguishable from the indigenous fractions in the early stages of the experiment, however were not distinguishable towards the completion of the experiment, indicating that the spiked fraction may have degraded. For 4NP the time taken for the initial concentration to decrease by 50% (DT50) in the indigenous samples was 22 days and 52 days in the centrifuge dried biosolids (CDB) and the lagoon dried biosolids (LDB) treatments respectively, whereas in the spiked samples the DT50 values were 16 days and 30 days respectively. For 4tOP the DT50 values in the indigenous samples were 18 days and 13 days for the CDB and LDB treatments respectively, whereas in the spiked samples the DT50 values were 6.4 and 7.1 days respectively. In the spiked samples the decrease in concentration occurred 1.4 to 3 times faster than in the indigenous samples for both compounds. These results show that the use of spike degradation experiments to predict the dissipation of organic compounds applied to soil through the land application of biosolids or other soil amendments are likely to underestimate the persistence of these compounds and are therefore not recommended.

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Chapter 8

Estrogenic activity in soils following addition of biosolids using the recombinant yeast estrogen screen (YES) bioassay

Abstract

Land application of biosolids is a potential route of entry into the environment for many estrogenic compounds. In this study, soil samples were collected at four sampling times over a 112 day period from a field trial following addition of either centrifuge dried biosolids (CDB) or lagoon dried biosolids (LDB). The recombinant yeast estrogen screen (YES) bioassay was then used to determine if estrogenic activity was present in the samples. Estrogenic activity was detected at all sampling times. In the CDB treated soils, the estrogenic activity ranged from below detection to 2.9 µg 17β-estradiol equivalency (EEq)/kg with an overall average of 1.7 µg EEq/kg. The activity in the LDB treated soils was higher and ranged from 0.2 to 3.3 µg EEq/kg with an overall average of 1.1 µg EEq/kg. Further research would need to be conducted to quantify the potential risks posed to the environment from these estrogenic levels measured in the soils.

CONTENTS

8.1. INTRODUCTION

Estrogen compounds, for example, the naturally occurring 17β-estradiol (E2), its metabolites, estrone (E1) and estriol (E3) and the synthetic estrogen, 17α-ethinylestradiol (EE2) are known to occur in biosolids (Ternes et al., 2002; Braga et al., 2005; USEPA, 2009). The naturally occurring compounds, E2, E1 and E3, were found at concentrations ranging from 22 to 355 µg/kg, 27 to 965 µg/kg and 8 to 232 µg/kg respectively, in the recent Targeted National Sewage Sludge Survey (TNSSS) conducted in the United States (USEPA, 2009). For the synthetic compound, EE2, concentrations tend to be lower than for the naturally occurring compounds with concentrations up to 17 µg/kg reported (Ternes et al., 2002). The specific concern surrounding estrogen compounds in the environment is their ability to produce adverse effects on exposed organisms at concentrations in the ng/L range (e.g. Mills & Chichester, 2005). These effect concentrations are often also below the limits of detection of most chemical analyses, therefore making the risks difficult to quantify. For this reason bioassays are often used as an initial screening tool to highlight the presence of estrogenic compounds.

Several *in vitro* bioassays are available to detect the presence of estrogenic activity in waters or in extracts from sediments or soils. In this study, the recombinant yeast estrogen screen (YES) bioassay was used. This bioassay uses yeast strains (*Saccharomyces cerevisiae*) that have had the human estrogen receptor (hERα) stably integrated into the main chromosome, along with an expression plasmid carrying estrogen receptor elements (EREs) and the reporter gene, *Lac-Z* (encoding the enzyme β-galactosidase) (Routledge & Sumpter, 1996). Upon binding an active ligand, the E2-receptor interacts with the EREs to modulate the expression of *Lac-Z* which in turn synthesizes β-galactosidase which is secreted into the medium, where it metabolises the chromogenic substrate, chlorophenol red-β-D-galactopyranoside (CPRG) producing a colourmetric response (Routledge &

Sumpter, 1996). This colourmetric response is then used to indicate the presence of estrogenic activity in the sample. The response in the sample can then be expressed as an E2 equivalent (EEq) value, but represents the total estrogenic activity of all compounds present. The relative potencies of the estrogen compounds, as well as some other non-estrogen compounds, that are known to show estrogenic properties in the environment (xenoestrogens) (Jobling & Sumpter, 1993; Jobling et al., 1996; Fukuhori et al., 2005) are presented in Table 8-1.

Table 8-1: Potency of compounds relative to 17β-estradiol (E2) based on the yeast estrogen screen (YES) (as reported in Rutishauser et al., 2004)

Compound	**Relative potency to E2**
17β-estradiol (E2)	1.0
Estrone (E1)	0.38
Estriol (E3)	2.4×10^{-3}
17α-ethinylestradiol (EE2)	1.19
4-nonylphenol (4NP)	2.5×10^{-5}
4-t-octylphenol (4tOP)	7.8×10^{-6}
Bisphenol A (BPA)	1.1 10^{-4}

The YES bioassay has been used as a tool in some studies to provide information on the estrogenic activity of biosolids products with values ranging up to 35 µg EEq/kg (Holbrook et al., 2002). Holbrook et al. (2002) also used this bioassay in a mass balance study and determined that between 5 and 10% of the estrogenic activity from influent wastewater was found in the biosolids product produced in a municipal wastewater treatment plant. Of the remainder it was found that 25 to 43% was contained in the treated effluent water, whereas, 51 to 67% was degraded through the wastewater or biosolids

treatment processes (Holbrook et al., 2002). In other work it has been found that the treatment processes used on the biosolids product has an influence on the estrogenic activity. In general, biosolids that had undergone aerobic digestion have been shown to contain minimal or no estrogen-receptor gene transcription activity, whereas biosolids that had undergone anaerobic digestion had comparatively higher activity (Lorenzen et al. 2004). The presence of estrogenic activity in soils following the addition of biosolids is unknown. Therefore, the aim of this study was to use the YES bioassay to determine if estrogenic activity could be detected in field soils for 112 days following biosolids addition.

8.2. MATERIALS AND METHODS

8.2.1. Sample collection and preparation

The biosolids amended soil samples used for this study were the same as those collected from the field trial presented in Chapter 5. In brief, the field trial consisted of two biosolids treatments, a centrifuge dried biosolids (CDB) and a lagoon dried biosolids (LDB) treatment, plus a control treatment, all conducted in triplicate. The biosolids were added to the surface of the plots and then all plots, including the control plots were rotary hoed to a depth of 10 cm. Duplicate samples were collected from each plot immediately after biosolids addition (t_0) and returned to the laboratory to be freeze dried and sieved to 2 mm for extraction. Duplicate samples were then collected from each plot at 28, 56 and 112 days post biosolids addition.

The sample extraction and preparation procedure is outlined in detail in Chapter 5 and involved the samples being extracted ultrasonically with methanol and acetone, followed by solid phase extraction (SPE) using Oasis HLB® cartridges (500 mg 6 cc) and elution

with methanol, acetone and ethyl acetate. The extracts from the samples were then reconstituted in 4 mL of methanol.

8.2.2. Recombinant yeast estrogen screen bioassay

Prior to commencing the YES bioassay, each sample extract was serially diluted in methanol by a factor 2 to produce 5 subsequent 200 µL samples (i.e. there were six concentration levels in total, with the highest being 100%). The detailed method for the YES bioassay is described by Routledge & Sumpter (1996). In brief, 10 µL from each dilution was transferred into sterile 96-well microtitre plates and evaporated to dryness. Following this, 200 µL of the yeast medium was added to each well. Each plate contained one row of E2 control standards at concentrations ranging from 1.3 to 2.7 ng/L. The plates were sealed and placed in an incubator for three days at 32°C then read at 540 nm for CPRG. EEq values were then determined for each of the samples by relating the response of the sample relative to the E2 standard curve. A limit of detection (LOD) and limit of quantification (LOQ) were calculated for each E2 standard curve using a signal-to-noise ratio of three and ten respectively for each set of samples.

8.3. RESULTS AND DISCUSSION

In the initial sample taken on the day of biosolids application, there was no estrogenic activity detected in the soil extracts from the control plots, whereas activity was detected in all of the samples that had been treated with biosolids (Table 8-2). Following this in the CDB treated soils, activity was detected in 50% of the replicates at each of the three subsequent sampling times (28, 56 and 112 days). In comparison, activity was detected in all replicates from each sampling time in the LDB treated soils. The average and range of EEq values are shown in Table 8-2. Overall, the estrogenic activity in the samples was low, with EEq values in the CDB treated soils ranging from < LOD to 2.9 µg EEq/kg and

in the LDB treated soils from 0.2 to 3.3 (Table 8-2) with averages over the entire study of 0.7 and 1.1 μg EEq/kg. Although the activity levels do seem to increase in the samples collected at 112 days post biosolids application, there is some variation expected between runs of samples. Therefore as they are all within the same order of magnitude, this increase is not considered to be substansial.

Table 8-2: Summary of the estradiol equivalency values (EEqs) calculated from the yeast estogen screen for soil samples following the addition of biosolids. EEq values are shown in μg/kg.

Biosolids treatment	**Sampling time (days)**			
	0	**28**	**56**	**112**
CDB	0.5 (0.3 – 1.1)	0.2 (< LOD – 0.2)	0.3 (< LOD – 0.3)	1.8 (< LOD – 2.9)
LDB	1.3 (0.2 – 2.5)	0.2 (0.2 – 0.3)	0.5 (0.3 – 1.2)	2.3 (1.7 – 3.3)

< LOD indicates that at least one replicate sample was below the limit of detection.

The YES bioassay cannot be used to identify the specific compounds responsible for the estrogenicity in the soil extracts, however, it is likely that the majority of activity is due to the presence of estrogen compounds (E2, E1, E3 and EE2) based on findings in the literature. The majority of estrogenic activity in treated effluent waters from wastewater treatment plants (WWTPs) has been found to be due to the presence of natural and synthetic estrogens (i.e. E2, E1, E3 and EE2) (Aerni et al., 2004). Tan et al. (2007) reported that the natural estrogens, E2 and E1, contributed to 60% or more of the EEq values measured on influent and effluent samples from a WWTP. Korner et al. (2001) reported that E2 and EE2 contributed 90% or more of the EEq values measured in effluent samples from WWTPs. It is also expected that the estrogen compounds, in particular, E2, E1 and EE2, are the main contributors to estrogenic activity in environmental samples due

to the relative potencies of these compounds compared to other estrogenic compounds (Table 8-1). When the relative potencies of the compounds, 4NP, 4tOP and BPA (as presented in Table 8-1) are compared with the initial known concentrations of these compounds (presented in Chapter 5), the contribution from these compounds ranges from 0.2 to 0.4 µg EEq/kg.

Langdon et al. (in press) conducted an aquatic hazard assessment for estrogenic compounds following the addition of biosolids to an agricultural soil. That study involved predicting the maximum concentrations of compounds that may be present in runoff water from biosolids amended land based on the partitioning of each of the compounds. Maximum runoff water concentrations were then compared to the most sensitive toxicity values available to estimate the hazard they posed using the hazard quotient (HQ) method (Urban & Cook, 1986). If a HQ value was greater than 1.0 then compounds were classed as posing a high hazard. The assessment used a series of conservative assumptions and therefore if the HQ for a compound was less than 1.0 it was considered very unlikely for toxicity to occur and compounds were considered a low (or moderate) hazard. From that study, based on a maximum biosolids amended soil concentration of 1.5 µg/kg, the compound E2 was considered to pose a high hazard with a HQ value of 62. If the average EEq values in the biosolids amended soils from this study are used (0.7 and 1.1 µg EEq/kg) as the concentration of E2 in the soils, then HQ values (using the same method as that outlined in Langdon et al., in press) would range from 29 to 45. This study therefore indicates that although the estrogenic activity is low in these soils, they still pose a high hazard that may lead to adverse environmental effects. It should be noted that Langdon et al. (in press) did use a series of conservative assumptions and as a result the HQ values obtained are over-estimates of the hazard posed and should be used as part of an initial screening level assessment.

8.4. CONCLUSION

Estrogenic activity was detected using the yeast estrogen screen (YES) bioassay in all soils that had been treated with either a centrifuge dried biosolids (CDB) or a lagoon dried biosolids (LDB). In the CDB treated soils, the estrogenic activity in the soils over the 112 days ranged from below detection to 2.9 µg 17β-estradiol equivalency (EEq)/kg. with an overall average of 1.7. The activity in the LDB treated soils was higher and ranged from 0.2 to 3.3 µg EEq/kg with an overall average of 1.1. The estrogenic activity was considered to be at a level that resulted in a potentially high hazard being posed to aquatic ecosystems, however, further research would be required to quantify this more accurately.

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Chapter 9

General discussion and recommendations for future research

CONTENTS

9.1. SUMMARY AND IMPLICATIONS OF FINDINGS

It is well documented that pharmaceuticals and personal care products (PPCPs) and endocrine disrupting compounds (EDCs) are present in wastewater and biosolids. The use of biosolids for land application, although beneficial for the growth of plants and crops, can be a route of entry for these compounds into both the terrestrial and aquatic environments. It is difficult at this stage to gain a full understanding of the potential risk posed to the environment by PPCPs and EDCs following the application of biosolids to land due to the many knowledge gaps. Much of the work that has been conducted to date is compound and site specific which makes broad conclusions difficult to make. There is also the added complexity caused by difficulties in developing reliable analytical methods for many of the compounds within these groups of contaminants.

The work presented in this thesis was conducted to gain a better understanding of the environmental behaviour of PPCPs and EDCs. This was done by conducting a series of related studies. First, a screening level hazard assessment was conducted for all PPCPs and EDCs that have been reported in biosolids to determine if there are likely to be any adverse effects to aquatic ecosystems adjacent to land where biosolids have been applied. Based on the outcomes from this hazard assessment, as well as an understanding of the current concerns highlighted in literature, eight selected PPCPs and EDCs were chosen for a survey of Australian biosolids, 4-nonylphenol (4NP), 4-t-octylphenol (4tOP), bisphenol A (BPA), triclosan (TCS), 17β-estradiol (E2), estrone (E1), estriol (E3) and 17α-ethinylestradiol (EE2). Four target compounds, 4NP, 4tOP, BPA and TCS, were then selected for a more detailed dissipation study. This assessed the rate and pattern of dissipation of the compounds, when applied to soils as part of biosolids addition, and how these rates and patterns compare between the laboratory and the field. The standard procedure of “spiking” compounds into soil or biosolids was also tested to determine if

this method produced similar dissipation rates and patterns to those observed for the same compounds that were indigenous to biosolids. Finally, the recombinant yeast estrogen screen (YES) bioassay was used to determine if estrogenic activity was measurable in soils following the addition of biosolids and to determine the persistence of the estrogenicity.

The method used in the hazard assessment, i.e. the hazard quotient (HQ) approach, enabled a wide range of PPCPs and EDCs to be assessed in order to highlight specific compounds that may warrant further investigation. Overall, of the 45 compounds assessed, HQ values could not be calculated for ten, due to a lack of physiochemical and/or aquatic toxicity data, whereas 25 of the compounds had HQ values less than one and were therefore classed as posing a low or moderate hazard. There were ten compounds that were identified as posing a high hazard to aquatic ecosystems. These were the fragrance compounds, tonalide and galaxolide; the estrogen compounds, E2 and EE2; the antimicrobials agents, TCS and triclocarban; and the antibiotics, ciprofloxacin, norfloxacin, ofloxacin and doxycyline. It is recommended that future research in this area should focus on the compounds highlighted from this assessment or compounds with similar toxicological and physical properties. The hazard model used for this assessment can also be used to assess the hazard posed from a broader range of organic compounds, including additional PPCPs and EDCs, as more information becomes available.

The survey of 14 biosolids was conducted in order to gain an understanding of the range of concentrations of several selected PPCPs and EDCs in Australian biosolids. The selection of compounds for this survey was based in part on compounds that were highlighted in the hazard assessment, as well as some additional compounds due to concern over their potential environmental impacts. The compounds included in the survey were 4NP, 4tOP, BPA, TCS, E2, E1, E3 and EE2. Only 4NP, 4tOP, BPA and TCS

were detected in all of the samples. The compound E1 was detected in approximately 25% of the samples, whereas E2, E3 and EE2 were below their levels of detection in all of the samples. All of the detected compounds in Australian biosolids were within the range of what has been measured globally. Based on the maximum concentrations measured, the compounds 4NP, 4tOP, BPA and E1 would all be classed as posing a low hazard to aquatic ecosystems if the previous aquatic hazard assessment is applied. This is not the case, however, for TCS, where 8 of the 14 biosolids samples analysed in the survey would be classed as posing a high hazard to aquatic ecosystems. The results from the survey provide useful information on the concentrations of these compounds within Australian biosolids which can assist with future hazard and risk assessments and for the management of organic contaminants in biosolids within Australia.

The assessment of the dissipation of the compounds 4NP, 4tOP, BPA and TCS was a large component of this thesis. These four compounds were selected for this experimental component due to their high detection rates observed in the biosolids survey. The initial experiment was conducted to measure the dissipation of the compounds that were indigenous to biosolids under laboratory conditions. It was found that the pattern of dissipation for most of the compounds was best described by a biphasic model, which consisted of a phase that initially dissipated exponentially and a recalcitrant phase that did not dissipate. Although the mechanisms responsible for the presence of a recalcitrant fraction following the addition of biosolids to soil are not clear, some suggestions were made. For example, it may be due to limited oxygen penetration into biosolids aggregates or non-reversible sorption of compounds to the biosolids matrix. It does appear however that the biosolids matrix, which is often highly complex and consists of many components, may influence both the rate and pattern of dissipation of the compounds in soils.

When a similar dissipation experiment was conducted in the field, it was found that as a result of the sub optimal conditions for microbial degradation, the rate of dissipation of the same compounds was considerably slower than that observed in the laboratory. These differences were minimal for BPA, where the rate of dissipation was approximately 2.5-times slower in the field compared to the laboratory. For 4NP and 4tOP, dissipation occurred between 10- and 20-times slower in the field than in the laboratory. In comparison to the other three compounds, TCS appeared to show no significant dissipation over the duration of the field trial, whereas 30% to 50% dissipation of the initial concentration was observed in the laboratory. This highlights a specific concern regarding this compound and the potential for its accumulation in soils following repeated application of biosolids. It should also be noted that this was one of the compounds that was highlighted in the hazard assessment as warranting further research with regards to its potential for offsite migration with runoff water or leachate and resulting in adverse effects to aquatic ecosystems. The results obtained in the field trial presented in this thesis are however a site-specific example and variations in environmental conditions may lead to differing results.

The results obtained in the laboratory dissipation experiment, indicated that the biosolids matrix influenced the dissipation of the compounds. It was therefore hypothesised that the commonly used procedure of spiking in degradation/dissipation studies, may not necessarily produce similar results to the procedure where the compounds are indigenous to biosolids. This was tested in two ways: (i) spiking isotopically labelled surrogate compounds (i.e. BPA-d_{16} and TCS-$^{13}C_{12}$) into a biosolids amended soil and, (ii) spiking elevated levels of the same compound (i.e. non-labelled 4NP and 4tOP) into a biosolids amended soil. Although using both methods found differences in the dissipation rate of the spiked and indigenous compounds, using isotopically labelled surrogate spikes was found

to be a more sensitive and powerful way to test this hypothesis. The reason for this was that the indigenous and spiked compounds could be measured independently of each other, which enabled more comparisons to be made. For example, differences in the overall pattern of dissipation could be seen, in terms of the presence or absence of a recalcitrant fraction. In general, however, it was determined that the use of degradation experiments that involved spiking, yielded both faster rates of dissipation and, particularly in the case of BPA, variations in the pattern of dissipation, in terms of the presence of a recalcitrant fraction. It was clearly observed for this compound that the indigenous BPA had a fraction that was recalcitrant, whereas the spiked BPA-d_{16} dissipated to a concentration that was below detection. These results raise concern about the accuracy of using spike degradation experiments in the risk assessment of PPCPs and EDCs and potentially other persistent organic contaminants in biosolids amended soils.

The final component of this thesis involved determining if estrogenic activity could be measured in soils following the addition of biosolids. Some of the estrogen compounds (i.e. E2 and EE2) were highlighted as posing a high hazard to aquatic ecosystems (Chapter 2), as they can exert adverse environmental effects at trace concentrations. Due to their low concentrations in biosolids they were not detected in any of the biosolids samples in the survey (except E1, which was measured in four of the 14 samples). The extracts from the samples collected over the initial period of the field trial were therefore tested for estrogenic activity using the YES bioassay. Although this assay does not provide information on concentrations of specific estrogenic compounds, it was clear that there was estogenic activity in the soils that had received biosolids and this remained for at least four months following application. Although the activity measured overall was low (ranging up to 3.3 µg 17β-estradiol equivalency/kg), it was still considered to pose a high hazard to aquatic ecosystems (using the same method as Chapter 2). The implication of

this result is that there is the potential for organisms, both terrestrial and aquatic, to be exposed to estrogenic activity as a result of biosolids addition to soils.

9.2. CONCLUSIONS

The key findings and conclusions from this thesis are summarised below.

- Of the 45 pharmaceuticals and personal care products (PPCPs) and endocrine disrupting compounds (EDCs) that have been detected in biosolids, the majority were classed as posing a low or moderate hazard to aquatic ecosystems, whereas ten compounds (tonalide, galaxolide, 17β-estradiol, 17α-ethinylestradiol, triclosan, triclocarban, ciprofloxacin, norfloxacin, ofloxacin and doxycycline) were classed as posing a high hazard. These ten compounds therefore warrant further investigation with regards to their potential to adversely affect aquatic ecosystems.
- The average concentrations of 4-nonylphenol (4NP), triclosan (TCS) and bisphenol A (BPA) in Australian biosolids were lower than global averages (by 42%, 12% and 62%, respectively), whereas, the average concentration of 4-t-octylphenol (4tOP) was higher (by 25%). However, overall the biosolids concentrations of these four compounds were similar to international values.
- The dissipation of 4NP, BPA and TCS, when added to a soil through the addition of biosolids, followed a biphasic pattern with a degradable and a recalcitrant fraction and the use of a single value (i.e. time taken for 50% of the initial concentration to dissipate, DT50), was not suitable in describing the dissipation pattern. It was therefore concluded that the dissipation of some organic compounds did not proceed to completion with a recalcitrant fraction persisting for many compounds for at least 32 weeks. The magnitude of the recalcitrant fraction differed between the compounds and the biosolids treatments. Due to the presence

of a recalcitrant fraction for many compounds, there is the potential for accumulation in soils if repeat applications of biosolids are used.

- The dissipation rates of 4NP, 4tOP, BPA and TCS was considerably slower in the field compared to rates observed in the laboratory. The dissipation of 4NP and 4tOP was 10- to 20- times slower in the field and for BPA dissipation was 2.5-times slower. TCS did not show any significant dissipation under field conditions whereas in the laboratory up to 50% dissipation was observed. Therefore, laboratory-based dissipation experiments that do not take into account field conditions are considered to be not appropriate when determining the persistence of organic compounds following biosolids addition.
- The use of spiking in dissipation studies produced differing results in terms of both rate and pattern (biphasic or first-order) to those observed when the same compounds were indigenous to biosolids. Therefore, it was concluded that the widely used practice of spiking for degradation/dissipation studies is not appropriate to measure the dissipation of organic compounds in biosolids amended soils.
- Detectable levels of estrogenic activity were measured in extracts from a field soil that had received biosolids addition, and that levels of activity measured may pose a high hazard to aquatic ecosystems. It was concluded that estrogenic activity does occur in biosolids amended soils and persists and therefore warrants further quantitative analysis.

9.3. RECOMMENDATIONS FOR FUTURE RESEARCH

The work that has been presented in this thesis can be used to assist in determining the potential risks posed to the environment from PPCPs and EDCs in biosolids and possibly other organic compounds. However, to conduct a full risk assessment more research is required. In addition, there are many important research questions that arise from the results presented throughout this thesis. These are summarised below.

1. In order to gain a full understanding of the potential risks associated with PPCPs and EDCs in biosolids, a greater understanding of the terrestrial toxicity of these compounds in required. In some cases, considerable aquatic toxicity data was available, which enabled us to conduct the aquatic hazard assessment. This aquatic data can be used as a starting point to select specific compounds that should be the focus of terrestrial toxicological studies. The results from the hazard assessment would also assist in this selection and it is suggested that the compounds that pose a high aquatic hazard be used as a guide for this research.

2. The dissipation experiments showed that many of the compounds examined had a recalcitrant fraction. The proportion of the recalcitrant fraction also appeared to vary between the two biosolids tested. It is difficult to draw conclusions on the effect of the biosolids matrix on these patterns as only two biosolids were used for the experiment. In order to better understand the influence that the biosolids matrix has on the dissipation of the compounds, similar laboratory experiments could be conducted on a much broader range of biosolids types, including biosolids with various aggregate sizes.

3. The field component of this study showed that dissipation occurred at a considerably slower rate in the field than that in the laboratory. This difference was likely to be influenced by unfavourable temperature and/or moisture availability during the annual seasonal cycle. This therefore indicates that under varying environmental conditions, the potential persistence of PPCPs and EDCs in soils following the land application of biosolids will vary. A greater understanding of the effect of the environmental conditions on the dissipation of the compound could be assessed in a series of controlled laboratory- and/or field-based experiments, under various temperatures and soil moisture contents. This type of study would enable a greater understanding of the environmental conditions that are likely to lead to accumulation of these compounds in soils following land application of biosolids.

4. The presence of a recalcitrant fraction for many of the compounds studied in this project raises that question of whether this fraction is bioavailable. If there is limited availability of these compounds to microorganisms, due to mechanisms including non-reversible sorption of the compounds to the biosolids matrix, then it is possible that the environmental risk of the compounds is reduced despite the chemicals being present.

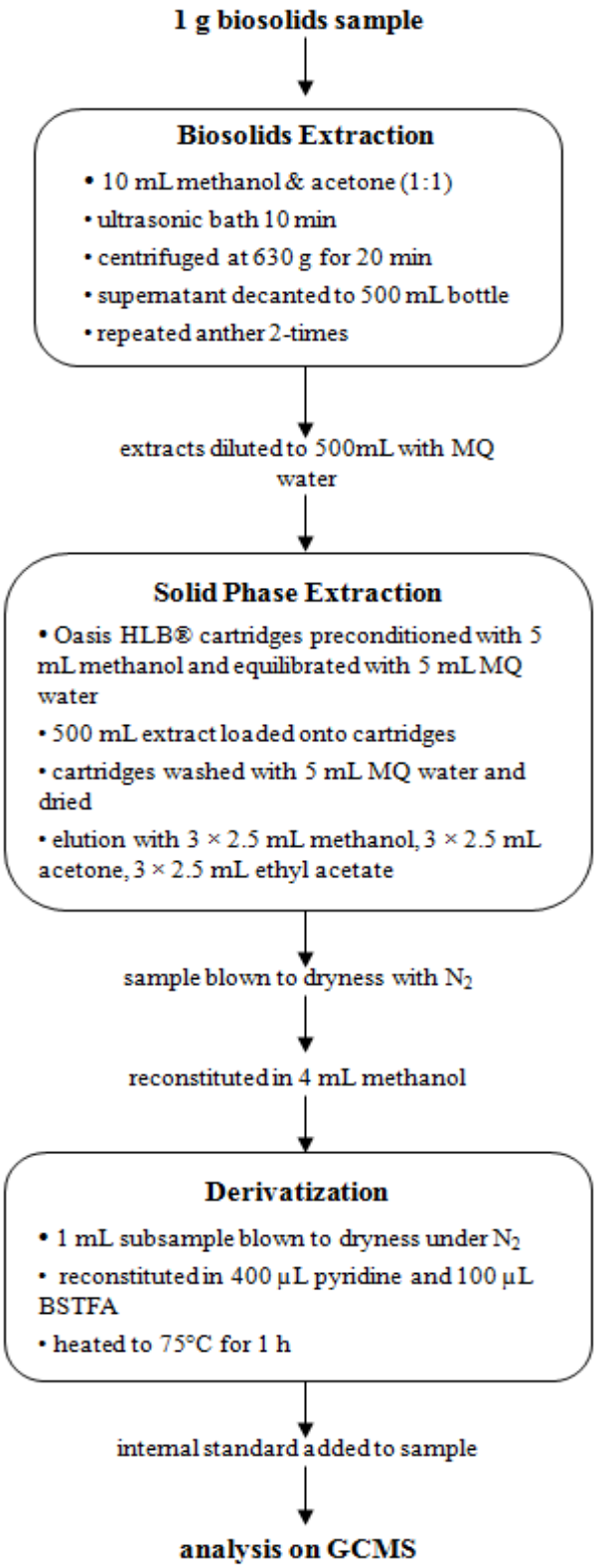
5. More specific research should also focus on the antimicrobial agent triclocarban (TCC). The reasons for this are two-fold, (i) this compound was highlighted in the hazard assessment as posing a high hazard to aquatic ecosystems, and (ii) the demonstrated resistance to degradation of the other commonly used antimicrobial agent that was assessed in this study, TCS. The reason for exclusion of TCC from the current body of work was difficulties in obtaining a reliable method for its detection

and quantification. Following the development of sound methods for this compound, more focussed work should be conducted.

Appendices

Appendix A

Flow diagram of biosolids sample preparation and extraction for analysis using GCMS



Appendix B

Recovery values for the labelled surrogate compounds, 4-n-nonylphenol-d_8 (4nNP-d_8), triclosan-$^{13}C_{12}$ (TCS-$^{13}C_{12}$), bisphenol A-d_{16} (BPA-d_{16}), estrone-d_4 (E1-d_4), 17β-estradiol-d_4 (E2-d_4) and 17α-ethinylestradiol-$^{13}C_2$ (EE2-$^{13}C_2$) in the 14 biosolids samples collected for the biosolids survey (Chapter 3). All recoveries are shown as percentages and the values from two spiked replicates are shown. Missing values indicate samples where recoveries could not be determined as concentrations were below detection limits.

Sample	**4nNP-d_8**		**TCS-$^{13}C_{12}$**		**BPA-d_{16}**		**E1-d_4**		**E2-d_4**		**EE2-$^{13}C_2$**	
	1	**2**	**1**	**2**	**1**	**2**	**1**	**2**	**1**	**2**	**1**	**2**
A	84	76	314	218	125	113	106	112	128	121	347	336
B	133	132	199	131	113	105	120	101	134	161	382	358
C	124	109	126	140	119	127	109	101	156	154	360	352
D	124	128	59	57	61	56	72	74	120	154	120	163
E	112	120	89	89	102	114	245	283	136	146	136	146
F	174	173	45	55	55	60	68	58	173	147	202	154
G	182	169	193	226	159	140	-	-	-	-	-	-
H	197	211	163	154	159	177	100	93	154	142	154	142
I	198	182	63	68	106	140	9.6	10	27	27	-	-
J	127	109	94	79	78	72	154	135	295	253	189	157
K	158	172	68	91	98	89	129	137	181	189	495	214
L	352	320	199	177	323	359	200	199	173	185	152	149
M	299	298	91	102	141	149	32	42	148	148	158	148
N	245	351	98	102	87	81	96	108	187	207	251	258