Monitoring Organic Contaminant Concentrations and Carbon Mineralization in Field Soils Receiving Alkaline-Stabilized Biosolids

by

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The undersigned hereby certify that they have read and recommend to the Faculty of Graduate Studies for acceptance a thesis entitled "Monitoring Organic Contaminant Concentrations and Carbon Mineralization in Field Soils Receiving Alkaline-Stabilized Biosolids" by Joseph Daniel Gillis in partial fulfillment of the requirements for the degree of Master of Science.

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DEDICATION

To my parents, for encouraging me to always do the best that I could, and to Richard and Clara, for allowing me to take the road less travelled.

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ABSTRACT

The application of municipal sewage biosolids to agricultural land is a common practice worldwide. Increasing attention is being directed at the presence of organic contaminants bound to the organic phase during wastewater treatment, which end up in the biosolids. The goal of this study was to investigate the decomposition of an alkaline-stabilized biosolid being used as an agricultural soil amendment containing unknown organic contaminants. A two year field trial and a 120 day laboratory soil incubation using increasing rates (0, 7, 14, 28, and 42 Mg ha^{-1}) of an alkaline-stabilized biosolid (ASB) were set up to monitor biosolid decomposition and concentrations of selected contaminants over time. The seven contaminants selected for monitoring (*p*-cresol, indole, 4-*t*-octylphenol, phenanthrene, triclosan, carbamazepine, and benzo[*a*]pyrene) represent a wide range of physico-chemical properties and fall under several different chemical classes. The decomposition of ASB in soil was examined in the incubation study. Almost half of the CO_2-C evolved from ASB amended soils occurred within the first 6 days, indicating that a relatively labile pool of carbon remains in ASB following the sewage treatment process. By day 121, between 71 to 78% of the total carbon added to soil had been evolved as CO_2-C. A new model developed during this study to describe carbon mineralization, a first order plus logistic function (FLOG), performed better than other commonly used models. The method chosen to analyze organic contaminants in soil was only able to determine four out of seven compounds reliably, with recoveries greater than 50% for 4-*t*-octylphenol, phenanthrene, triclosan, and benzo[*a*]pyrene. In treated soils, only triclosan was able to be detected and quantified. Average triclosan concentration in the incubation study ranged from a high of 143 ng g^{-1} on day 3 to a low of 26 ng g^{-1} by day 121, representing an 81% decrease over a roughly 4 month period under idealized conditions. In the field, triclosan concentrations following a Fall biosolids application in Oct. 2008 increased to detectable levels (29 to 47 ng g^{-1}) in all three plots measured in Nov. 2008, which remained elevated (29 to 66 ng g^{-1}) over the winter period in two out of three plots when sampled in May 2009. Following the Spring application in June 2009, measured triclosan concentrations in July 2009 samples from these same two plots were lower than predicted (33 to 48 ng g^{-1}) and eventually decreased to levels below the detection limit by the Oct. 2009 sampling.

LIST OF ABBREVIATIONS AND SYMBOLS USED

4-*t*-OP	4-*tert*-octylphenol
AE	Alberta Environment
ASB	alkaline-stabilized biosolid
BaP	benzo[*a*]pyrene
BFR	brominated flame retardant
CBZ	carbamazepine
DFO	double first order exponential model
EDC	endocrine disrupting compound
EQ	Environment Quebec
FLIN	first order plus linear model
FLOG	first order plus logistic model
FO	first order exponential model
GC-MS	gas chromatography with mass spectrometry
IND	indole
MSE	mean squares error
NSE	Nova Scotia Environment
OME	Ontario Ministry of the Environment
P-CRE	*para*-cresol
PAH	polycyclic aromatic hydrocarbon
PBDD	polybrominated dibenzyl dioxin
PBDE	polybrominated diphenyl ether
PCB	polychlorinated biphenyl
PHE	phenanthrene
POP	Persistent Organic Pollutant
PPCP	Pharmaceuticals and Personal Care Products
SPE	solid phase extraction
SSE	sum of squares error
TCS	triclosan
USEPA	United States Environmental Protection Agency
WWTP	wastewater treatment plant

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CHAPTER 1: INTRODUCTION

1. Introduction

Evidence of environmental contamination by anthropogenic organic compounds has been found in a number of environmental matrices worldwide (Bargagli 2008). Historically, water bodies used for waste disposal have created a persistent source of water pollution as particulate-bound contaminants disassociate from the sediment over time, and bioaccumulate in the environment impacting the food chain (She et al. 2002). Most of the wastewater from urban sources in Canada is now serviced by at least primary treatment, which produces a solid waste product known as sewage sludge (Health Canada 2004). Following treatment to reduce labile carbon content or stabilize the material by another approved method, the product is termed a 'biosolid' in Nova Scotia, which must meet guidelines for pathogens, metals content, dioxins and furans, and PCBs (NSE 2010).

Biosolids contain nutrients and improve soil properties and crop yields, which makes land application a desirable solution to deal with the growing volume produced annually (Cooper 2005; Mantovi et al. 2005). Land application of biosolids is a common disposal option (Singh and Agrawal 2008), and in China, land application of raw sewage sludge from some of the nation's largest cities has been taking place for the last several decades (Wang 1997). However, research has also shown that sewage sludges can contain undesirable components, including heavy metals, pathogens, and organic contaminants (Health Canada 2004).

Wastewater treatment can be effective at removing a percentage of certain organic compounds from water, although this is often a result of compounds accumulating in the solid fraction. For example, a mass balance study of triclosan (a commonly used antimicrobial compound) in an activated sludge wastewater treatment plant (WWTP) showed that up to 98 ± 1% of triclosan was removed from the liquid phase. However, there was a 6400-fold increase in triclosan concentration (by weight) in the sludge when compared to influent water (Heidler and Halden 2007). In contrast, other compounds are not removed as efficiently during wastewater treatment. In another study on the removal

of antimicrobial compounds, only 11% of lincosamides and 25% of sulfonamides were removed during activated sludge wastewater treatment (Watkinson et al. 2007).

The prevalence of organic contaminants in sewage sludge products applied to land is beginning to emerge. A recent review (Harrison et al. 2006) found data for as many as 516 organic compounds in sewage sludges, 83% of which were not on the USEPA list of priority pollutants. The available data are not sufficient to determine whether an environmental risk exists (Harrison et al. 2006). Recently, there has been a surge of global interest in sludge research, particularly in surveying organic contaminant levels in sludges using gas and liquid chromatography with mass spectroscopy (Cai et al. 2007, Gomez-Rico et al. 2007, Sánchez-Brunete et al. 2008), and trying to identify emerging pollutants that may potentially be present in sludges for future research (Eriksson et al. 2008).

1.1. Thesis Objectives

This project was initiated to evaluate a biosolid produced in the Halifax Regional Municipality (N-Viro Soil™, an alkaline-stabilized biosolid, ASB) as a soil amendment for agriculture. The primary goal was to analyze soil samples treated with biosolids for a suite of organic contaminants using gas chromatography-mass spectroscopy. To achieve this, a method was identified from the literature that claimed to be rapid, cost effective, and able to quantify a range of priority contaminants in agricultural soil. A 120 day aerobic soil and biosolid incubation and a two-year field trial using four rates of ASB were initiated to meet these objectives. This chapter presents a review on biosolids, organic contaminants in soil, and methods for analysis of these contaminants by GC-MS. The dynamics of decomposition of the biosolid are presented in Chapter 2, while Chapter 3 contains a discussion of method development trials undertaken prior to analysis of treated soil. Finally, Chapter 4 discusses the presence of triclosan in soil from the incubation and field samples. Triclosan is a broad-spectrum antibacterial compound and was the only compound out of seven that was reliably detected.

2. Biosolids in Canada

According to Health Canada (2004), 93% of urban Canadians were serviced by wastewater treatment plants in 1994, and of these, about 40% were tertiary treatment, 33% secondary treatment and 20% primary treatment. Atlantic Canada had the worst record, with only 50% of the urban population served by wastewater treatment (Health Canada 2004). Since that time, the percentage has likely increased with the implementation of advanced primary treatment plants in the Halifax Regional Municipality. The best way to deal with the increasing volume of sewage sludge produced is a topic of debate in many areas.

2.1. What is a Biosolid?

In the scientific literature, the residual solids from wastewater treatment are usually referred to as sewage sludge (Lega et al. 1997; Schnaak et al. 1997; Wang 1997; Harrison et al. 2006), although the word 'biosolid' has been used to mean either raw or stabilized sewage sludge (Cooper 2005; Singh and Agrawal 2008). References to stabilized sludge products are also inconsistent in their terminology. Some authors refer to alkaline-stabilized sewage sludge (Logan and Harrison 1995), others to alkaline-stabilized biosolids (Melakeberhan and Noel 2006). The Health Canada *Canadian Handbook on Health Impact Assessment* states that biosolid is "a term more politically correct than 'sludge'" (Health Canada 2004). The Government of Alberta states that biosolids were "formerly called sewage sludge" (AE 2001), a view shared by several other provinces and the USEPA (OME 1996; USEPA 1999; EQ 2004). Nova Scotia and British Columbia make a distinction, however, between untreated and treated sludge, in which they define the latter as biosolids (Queen's Printer 2007; NSE 2010). A recent paper from British Columbia defined biosolids as residual solids "...after steps have been taken to curtail pathogen risks" (Bright and Healey 2003).

In Canada, biosolids regulations fall under provincial jurisdiction. Nova Scotia has developed guidelines for the land application of biosolids, revised in 2010, which dictate that all biosolids applied to land in the province must be stabilized using an approved method (NSE 2010). Some of these methods include composting, aerobic or anaerobic digestion, and alkaline-stabilization. Biosolids are classified into two groups, Class A and

Class B, based on fecal coliform levels and heavy metal concentrations (NSE 2010). Class A biosolids have significantly lower limits for heavy metals (2 to 7 times lower) than B, and more stringent limits on fecal coliform levels than Class B biosolids. The most recent guidelines established in Nova Scotia set limits of 0.017 and 0.050 ng g^{-1} in Class A and B biosolids respectively for dioxins and furans, as well as a limit of 800 ng g^{-1} for PCBs in Class A biosolids. For Class B biosolids, anyone seeking approval for application must monitor selected alkylphenols and ethoxylates, flame retardants, pharmaceuticals and personal care products, hormones, steroids, and "other substances" in soil, and these parameters must be reported by the biosolids producer for every 10,000 Mg of material produced (NSE 2010). The land application of Class A biosolids is not designated as an activity requiring approval under the Environment Act and can be applied at any time, although regular monitoring is required by the company to ensure that the biosolids meet the Class A guidelines (NSE 2010). Since spreading of Class A biosolids is not deemed an activity requiring approval, it is only recommended that the same practices be adopted as for Class B biosolids (NSE 2010), which means that monitoring for these broad groups of contaminants in soil is not required when spreading Class A biosolids.

2.2. Alkaline-Stabilized Biosolids

According to the Nova Scotia definition, a biosolid has to meet certain conditions to be deemed 'stabilized'. Guidelines state that enough alkaline material must be mixed with sewage sludge so that it reaches pH 12 within 2 hours of mixing and must maintain this pH during storage. Alternatively, the volatile solids content of the sludge must be decreased by 38% (NSE 2010). However, our analysis of N-Viro Soil™ produced and stored in the Halifax Regional Municipality found a pH of 9.5, which likely dropped during storage. A number of alkaline agents have been used to stabilize biosolids, such as quicklime, cement and lime kiln dust, and pulverized coal ash (Poon and Boost 1996). WWTPs typically must acquire these services or technology from a private company as the processes are patented (USEPA 1999). Two examples are N-Viro Soil™ and Agri-soil, which combine quicklime and cement kiln dust with sewage sludge. N-Viro Soil™

uses an accelerated drying process to increase the solids content, while Agri-soil composts the mixture (Christie et al. 2001).

Alkaline-stabilized biosolids have established benefits to agriculture, both as a fertilizer source and for their liming capabilities. Application rates of 1.5, 3.0 and 4.5 Mg ha^{-1} N-Viro soil™ have been shown to significantly increase soil pH and yields of wheat and triticale (Cooper 2005). Christie et al. (2001) showed similar results in spring barley using Agri-soil. Alkaline-stabilized biosolids can also improve soil physical properties. An application rate of 500 Mg ha^{-1} N-Viro Soil™ to a physically degraded mineral soil, a rate used for land reclamation or horticulture, significantly lowered the bulk density and increased the hydraulic conductivity of the soil, noticeably improving the tilth (Logan and Harrison 1995). Analysis of 28 N-Viro Soil™ products from the United States, England and Australia determined that the physical properties of all of the products were generally similar, despite the sludge originating from different levels of sewage treatment and the use of different alkaline amendments (Logan and Harrison 1995). The maximum levels reported of As, Cd, Cu, Mo, Ni, Pb, Se, and Zn were below the limits for a Class A biosolid in Nova Scotia, and the minimum levels were all lower than the Exceptional Quality limits for heavy metals (NSE 2010). Values were not reported for Co, Cr, or Hg, which would be required in Nova Scotia.

2.3. Sources and Types of Organic Contaminants in Wastewater Treatment

Municipal wastewater treatment must deal with wastewater from a number of industrial and residential sources, which contribute different types of contaminants to the wastewater stream. Industry contributes many solvents, surfactants, and organic byproducts of chemical processes (Soares et al. 2008). Households can be a significant source of pharmaceuticals and personal care products (PPCPs) to the wastewater stream, as well as other chemicals, including flame retardants, cleaning products, and anything else that goes down the drain (Kolpin et al. 2002). In urban centers, storm water collection can contribute a large amount of contaminated water and sediment to the wastewater stream after rain events (Badin et al. 2008). Research has shown that polycyclic aromatic hydrocarbons (PAHs) can be present in storm water, along with pesticides, phthalates, and nonylphenols, accumulated from surfaces the water comes in

contact with (Eriksson et al. 2007). Another potential source of contaminants to the wastewater stream is the leachate collected from modern sanitary landfills, which is sometimes treated at wastewater treatment plants. Landfill leachate can contain any number of organic contaminants (Barnes et al. 2004; Choi and Lee 2006). Some of the typical groups of compounds detected in biosolids will be discussed individually, with respect to their sources and fate in WWTPs, as well as risks associated with their introduction to the environment. Collectively, the compounds have been referred to as xenobiotic organic compounds (Eriksson et al. 2008), or anthropogenic waste indicators (Kinney et al. 2008).

2.3.1. Persistent Organic, Toxic, and Priority Pollutants

Persistent organic pollutants (POPs) are a group of compounds that have known negative impacts on human and environmental health, and are generally quite mobile in the environment. Under the Stockholm Convention on Persistent Organic Pollutants, twelve pollutants termed the "dirty dozen" were targeted for reduction or elimination from use and release into the environment, including a number of pesticides, and the polychlorinated biphenyls, dibenzodioxins, and furans (PCBs, PCDDs, and PCDFs respectively, USEPA 2002). PCBs, PCDDs, and PCDFs have similar structures based around two benzene rings, differing in the linkage between the two phenyl rings. The toxicity of these pollutants vary, and each is assigned a Toxic Equivalency Factor (TEF) relative to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD), the most potent compound in any of the three classes, which is assigned a value of 1 (Schecter et al. 2006). Other compounds including some polycyclic aromatic hydrocarbons (PAHs), brominated flame retardants (BFRs), polybrominated dibenzodioxins (PBDDs), and furans (PBDFs) can have slight dioxin-like activity (Eljarrat and Barcelo 2003).

The USEPA has their own lists of pollutants that they recognize: the Toxic Pollutants and Priority Pollutants. The list of Toxic Pollutants contains sixty-five entries, some of which are specific compounds and some which are broad groups (NARA 2009). A number of the POPs are on this list, including PCBs, TCDD, aldrin, and dieldrin. The Priority Pollutants are a separate list of 126 specific compounds that the USEPA regulates

and has developed and published analytical methods for (USEPA 2008). This list contains a number of specific PAHs, PCBs, pesticides, and other compounds.

PCDDs and PCDFs are produced through a number of processes, including the incineration of chlorinated wastes, emissions from motor vehicles, and the production of other chlorinated compounds (Rogers 1996). They can enter the wastewater stream in industrial wastewater, can potentially be produced from chlorinated phenols in biological transformations, and may be present in trace amounts in bleached toilet paper (Rogers 1996). The fate of individual compounds in sewage treatment depends on a number of factors, including the physico-chemical properties of the compound. For example, in a study on the fate of PCDDs and PCDFs in a WWTP, some compounds increased in concentration between the incoming and outgoing water, while others decreased in concentration (Oleszek-Kudlak et al. 2005). A decrease in aqueous concentration would be expected due to degradation or sorption to the solid fraction, but increases were attributed to transformations and dechlorination by microbes yielding lower chlorinated compounds.

2.3.1.1. Phenanthrene and Benzo[a]pyrene

The PAHs are a group of compounds made up of two or more benzene rings fused together. Phenanthrene is a three- membered ring system while benzo[*a*]pyrene has five rings. PAHs are commonly found as mixtures in such things as coal, ashphalt, and in the smoke from wood, tobacco, and other organic matter (Mumtaz and George 1995). Beginning around the 1800s, there has been an increase in soil PAH concentrations in many areas, which is likely due to atmospheric deposition from combustion of fossil fuels (Jones et al. 1989). In archived soil samples of control plots from a long-term field experiment, Jones et al. (1989) observed increases over time in the concentration of a number of PAHs, with higher molecular weight PAHs generally showing higher concentration factors. PAHs can enter the wastewater stream through a number of sources, including industrial wastewater, as well as after rainfall events in stormwater and sediment runoff containing PAHs (Oleszczuk 2006). PAHs have a strong tendency to sorb to organic matter in the aqueous phase, and PAH water solubility decreases with increasing molecular weight (Johnsen et al. 2005).

Under ideal aqueous conditions, PAH availability as a substrate for microorganisms is generally limited by the flux of PAHs into the aqueous phase, which is used for growth and for maintenance (Johnsen et al. 2005). In soil, PAH degrading microbes are separated from their substrate and also rely on transfer in the aqueous phase, but there are more competing factors such as sorption to soil particles and organic matter, and small pore sizes which slow the rate of transfer and degradation (Johnsen et al. 2005). A number of PAHs are known to cause adverse health effects, such as cancer in vertebrates and invertebrates, although toxicity information is only available for a limited number of PAHs including both phenanthrene and benzo[*a*]pyrene (CCME 2008). Benzo[*a*]pyrene is metabolized to compounds containing diol and epoxide groups that have the potential to interact with regions of DNA and form adducts (CCME 2008). Benzo[*a*]pyrene adducts on areas of DNA encoding tumor suppressing genes (the P53 gene) can alter the stability and function of tumor suppressing proteins, promoting uncontrolled cell growth and leading to cancer (Denissenko et al. 1996).

2.3.1.2. p-Cresol

Cresols are methyl-substituted phenols that can occur in either of the ortho, meta, or para positions. They are found in wood preservatives like creosote, and have been observed in groundwater near wood treatment facilities using creosote (Rosenfield and Plumb 1991). Cresols are also produced during wood combustion (Edye and Richards 1991) and are dispersed into the environment in smoke. Many railway ties and bridges are treated with creosote, so cresols may enter wastewater dissolved or sorbed to organic matter in storm runoff coming in contact with treated wood surfaces or smoke residues. They are slightly soluble in water (Lide 2005) and with a log k_{ow} of about 1.9 (Xie et al. 2008), the majority of *p*-cresol is expected to be sorbed to organic matter during sewage treatment. *Para*-cresol is also produced by certain microorganisms, including those from the *Clostridium* genus during anaerobic degradation of the amino acid tyrosine (Yu et al. 2006). It is commonly found in biosolids (Kinney et al. 2006) and in soils receiving biosolid amendments (Burkhardt et al. 2005; Kinney et al. 2006). However, it is degraded by soil microorganisms under both aerobic (O'Reilly and Crawford 1989) and anaerobic (Bossert and Young 1986) conditions. Health effects from cresol exposure in humans

include respiratory failure, damage to kidneys, pancreas, or spleen, nausea, abdominal pain, headache, mental confusion, or other adverse effects (CDC 1988).

2.3.1.3. Indole

Indole is a naturally occurring heterocyclic amine whose structure is found in a number of physiologically active molecules including the amino acid tryptophan and the tryptamines, a class of natural and synthetic compounds with psychoactive properties (Shulgin and Shulgin 1997). It is a volatile compound that is an odour component of poultry manure and other fecal materials (Burnett 1969; Smith et al. 1977). Indole and other nitrogen-containing aromatics are often found alongside PAHs and have been associated with pollution from petroleum and combustion products which can indicate human activity (Krone et al. 1986). Indole can be anaerobically co-metabolized to indolic acid in batch experiments using specific bacterial strains (Safinowski et al. 2006), but it is considered to be a recalcitrant form of nitrogen in soils (Olk 2008). The aromatic nature of indole allows it to covalently bond with other soil compounds and can undergo humification (Olk 2008). Eisele (1986) showed that indole can be accumulated by poultry and cows and was detectable in eggs, milk, and body tissues. In humans, high blood concentration of indole may lead to damage to blood cells, kidneys, and other organs including the bowels (Eisele 1986).

2.3.2. Brominated Flame Retardants

Flame retardants are chemicals commonly added to plastic materials to make them more resistant to combustion. Flame retardants are either chemically bonded to the materials (reactive flame retardants), or mixed into the product (additive flame retardants). The latter group is much more likely to leach since they are not chemically bonded to the material they are supposed to protect (Alaee et al. 2003). Polybrominated diphenyl ethers (PBDEs) are a class of additive flame retardants typically used in products containing polystyrenes and polyurethanes, but they have also been found in the environment and in adipose tissue of different animals, including humans (de Wit 2002). PBDEs have up to ten substitutable hydrogens, although steric hindrance, among other factors, means that only a limited number of possible congeners are formed (Alaee et al.

2003). BDE-209, the main component of the commercially available flame retardant deca-BDE, is a fully substituted congener found at the highest concentrations in sludge compared to the other PBDE congeners, and concentrations are observed to be increasing, both in sewage sludge and in human tissues (Clarke et al. 2008). Studies have shown that chronic exposure to deca-BDE can lead to thyroid problems in mice and humans (Legler and Brouwer 2003). These results are not surprising, considering that the PBDEs are structurally very similar to some of the persistent organic pollutants and can exhibit dioxin-like activity (Eljarrat and Barcelo 2003). Additionally, PBDDs and PBDFs can be formed during the combustion of plastic containing brominated flame retardants (Ebert and Bahadir 2003).

Ricklund et al. (2008) performed a mass balance of deca-BDE in a WWTP and found that less than 1% leaves the plant in effluent water, with most ending up in sludge. About 50% was retained in the primary sludge, and 10% in the secondary sludge. Thirty percent of the mass was unaccounted for, however, which could be due to biological transformations or errors in calculating the different mass flows (Ricklund et al. 2008). In another study on the fate of PBDEs in a WWTP, it was found that 4.7% of the PBDEs entering the WWTP were released in the effluent water, with over 95% ending up in the sludge (Peng et al. 2008). The contribution of BDE-209 to the congener profile decreased from influent to effluent water, while the contribution of less substituted congeners increased. This was thought to be due to a higher affinity of BDE-209 for the suspended solids, which end up in the sludge.

2.3.3. Pharmaceuticals and Personal Care Products

The term ‘Pharmaceuticals and Personal Care Products’ has been used to describe all of the parent forms, metabolites, and degradation products of human and veterinary drugs entering the environment, as well personal care products, which are used on the body to alter smell, appearance, or other functions (Daughton and Ternes 1999). Many recent studies are focusing on PPCPs, which have since been detected in a number of environmental matrices including surface and groundwater (Kasprzyk-Hordern et al. 2008; Zhang et al. 2008). PPCPs are used in amounts equal to agrochemicals, but are introduced into the environment continuously through sewage discharge, whereas

agrochemicals are only applied periodically (Daughton and Ternes 1999). While some compounds may degrade with time in the environment, continuous discharge can cause these compounds to act like a persistent pollutant at low concentrations. Pharmaceuticals are designed to interact with the biochemical systems of different organisms, and it has been hypothesized that they place aquatic organisms at an increased risk of harm due to chronic exposure over a number of generations (Daughton and Ternes 1999). This proved to be the case when Kidd et al. (2007) spiked an experimental lake multiple times with a birth control medication resulting in dramatic effects on fish populations.

Active pharmaceutical ingredients are excreted either unchanged or as metabolites in human and animal wastes, but pharmaceuticals also enter the wastewater stream through direct disposal by a number of sources (Ruhoy and Daughton 2008). Hospitals and nursing homes, dental and veterinary clinics have been identified as sources of pharmaceuticals in the wastewater stream (Ruhoy and Daughton 2008). Personal care products can enter the environment directly, through volatilization of synthetic musks or sunscreen being washed off in water (Daughton and Ternes 1999). These compounds also enter the wastewater stream daily from residential wastewaters (La Farré et al. 2008).

As with any other group of contaminants, the fate or partitioning behaviour of PPCPs in sewage treatment and environmental matrices is dependent on the physico-chemical properties of the individual compound, such as lipophilicity, polarity, water solubility, and vapour pressure (La Farré et al. 2008). Additionally, degradation of certain compounds by biological or other means can lead to a diverse mixture of parent compounds and their transformation products, which in some cases are more hazardous or recalcitrant than the parent compound. For example, 4-nonylphenol, an endocrine disrupting compound found in sewage sludge, is a transformation product of nonylphenol ethoxylates. Nonylphenol ethoxylates are highly effective surfactants used in a large number of personal care products and consist of a linear or branched 4-nonylphenol substituted with an ethoxylate chain of varying length (CCME 2002). Nonylphenol ethoxylates have shown greater recalcitrance with shorter ethoxylate chains, but 4-nonylphenol, an endocrine disruptor, can be produced under aerobic field conditions (Soares et al. 2008).

2.3.3.1 . 4-t-Octylphenol (4-t-OP)

A member of the para-substituted alkylphenols, this compound is also recalcitrant in the environment and has similar sources and fates as 4-nonylphenol (4-NP). It is produced through incomplete degradation of octylphenol ethoxylates, which are typically used in lower quantities than other alkylphenol ethoxylates (Johnson et al. 1998). 4-t-OP has been found at lower concentrations than 4-NP in sewage sludge (Kinney et al. 2006), but it has more potent estrogenic properties than nonylphenol (Johnson et al. 1998; Bøgh et al. 2001). Their hydrophobic nature causes alkylphenols to partition into the organic phase during wastewater treatment, and they are detectable at $\mu g\ g^{-1}$ concentrations in sewage sludge (Kinney et al. 2006). Octylphenols are lipophilic thus accumulating in fatty tissue, and may be released in relatively high concentrations during catabolysis (Bøgh et al. 2001). They are also potent endocrine disruptors in animals, increasing the production of interleukin-4 by mice T-cells, a signalling molecule which induces production of IgE by B-cells, leading to higher serum concentrations, allergic inflammation, and sensitivities to environmental allergens (Lee et al. 2004). It also has estrogenic and antiandrogen activity, which can cause developmental anomalies in animals exposed during early life stages (Paris et al. 2002). Recently, a number of alkylphenols have been detected in human breast milk with potential implications regarding environmental exposure of infants to a number of contaminants, a topic which still needs further studies to clarify risks (Ademollo et al. 2008). Exposure to other endocrine disruptors such as bisphenol-A (BPA) and diethylstilbestrol (DES) in utero has been associated with increased risk of breast cancer later in life (Doherty et al. 2010).

2.3.3.2. Triclosan

Triclosan is a very common broad spectrum antibacterial component of a wide range of personal care products, including soaps, shampoos, toothpaste, mouthwash, and skin creams, generally ranging between 0.1-0.5% by weight (McAvoy et al. 2002). It is a relatively hydrophobic compound (log k_{ow} approximately 4.8), soluble in deionized water at approximately 10 μg/mL, and resistant to hydrolysis (McAvoy et al. 2002). However, recent concerns have emerged with the phototransformation of triclosan occurring under sunlight in aqueous situations, leading to production of chlorinated phenols and dioxins

(Latch et al. 2005). Triclosan is readily sorbed by the organic matter during sewage treatment and able to be degraded by microorganisms, leading to high removal rates (95%) from water during sewage treatment (Bester 2003). Although triclosan is considered safe for humans in its current capacity, it is ubiquitous in water bodies and can have negative impacts on aquatic organisms. For example, Orvos et al. (2002) detected a decrease in algae biomass with increasing doses of triclosan compared to the control. They also showed a decrease in early life stage fish survival at a triclosan concentration of 71 µg/L, the highest concentration tested. Sublethal effects on fish, including jaw locking, loss of equilibrium, and erratic swimming behaviour were also observed (Orvos et al. 2002). Ishibashi et al. (2004) observed a decline in percent hatchability and an increase in time to hatching in Medaka fish eggs exposed to an aqueous concentration of triclosan at 313 µg/L, and zero hatchability at concentrations 625 µg/L and greater. Increased hepatic and gonadal vitellogenin production in male and female Medaka was observed at concentrations of triclosan between 20-200 µg/L (Ishibashi et al. 2004). Vitellogenin is associated with the maturation of oocytes in female fish, and the detection in male gonads indicates feminization. Kidd et al. (2007) showed dramatic increases in whole-body homogenate vitellogenin concentrations in male and female fathead minnows after spiking an experimental lake continuously at 5-6 ng/L with the synthetic birth control medication 17-α-ethynylestradiol. Just one year after spiking, the population of fathead minnows crashed in the treated lake and had not recovered 2 years after the treatment was stopped, while population in a control lake remained relatively stable. While there have been no similar studies done with triclosan, it is one of the most frequently detected compounds in water bodies (Kolpin et al. 2002) and similar ecological effects may yet be shown.

2.3.3.3. Carbamazepine

Carbamazepine is a widely used antiepileptic drug and is one of the most commonly detected pharmaceuticals in sewage treatment plant effluents and natural water bodies (Daughton and Ternes 1999; Vernouillet et al. 2010). It has a relatively low removal efficiency during wastewater treatment (up to 20 or 30%) and has been detected at higher concentrations in untreated and treated wastewater than in untreated biosolids

(Miao et al. 2005). Despite a low removal rate during wastewater treatment, carbamazepine is still detected in biosolids at concentrations in the range of 10 to 1000 ng g^{-1} (Kinney et al. 2006; Lapen et al. 2008). Carbamazepine has been detected in surface water runoff (Topp et al. 2008) and tile drainage water (Lapen et al. 2008) in plots treated with liquid municipal biosolids. It has been detected in both groundwater (Heberer 2002) and surface water bodies (Kolpin et al. 2002). There are documented effects of carbamazepine on aquatic organisms used as biomarkers from different trophic levels, including a significant reduction in reproduction of the algae *Chorella vulgaris*, inhibition of the plant *Allium cepa*, decreased bioluminescence by the bacterium *Vivrio fischeri*, and increased immobilization of *Daphnia magnia* (Jos et al. 2003). Vernouillet et al. (2010) also showed biochemical disruptions in an aquatic food chain consisting of a primary producer (*Pseudokirchneriella subcapita*, an alga) and two consumers (the crustacean *Thamnocephalus platyurus*, and the cnidarian *Hydra attenuata*).

3. Challenges in Analyzing Environmental Samples for Organic Contaminants

Analysis of environmental samples for trace organic contaminants of concern is an important monitoring tool to determine the impact of human activities on natural systems. Many synthetic and naturally occurring organic compounds are released into the environment through human use, with wastewater collection and treatment systems considered a primary source to water bodies in treated effluent (Kolpin et al. 2002) and to soils during the land application of wastewater sludges or irrigation with treated effluent (Bright and Healey 2003). Reports of organic contaminants in sewage sludge have been around for decades (Webber and Lesage 1989; Pham and Proulx 1997), but with the advance of analytical instrumentation and decrease in analytical costs, more researchers worldwide are documenting levels of contaminants in sewage sludge and receiving waters (Boyd et al. 2003; Cai et al. 2007). Studies have also looked at the effect of sewage sludge application on contaminant levels in soil (Kinney et al. 2006), surface (Topp et al. 2008) and sub-surface (Lapen et al. 2008) drainage water, and potential transfers to soil biota (Kinney et al. 2008).

At the heart of each of these papers however is a sample preparation method, by which the analytes are extracted from the particular environmental matrix, interferences

removed, and remaining sample processed into a form that can then be analyzed by the instrument of choice. The extraction method is often optimized to a particular group of compounds, and each step in sample processing can potentially have a great influence on the final determination of analyte concentrations. It is therefore important to take certain precautions during sample handling and analysis to ensure that any results are valid and reproducible, and not an artifact of the analytical method.

The physico-chemical properties of a compound affect its fate during sewage treatment and in the environment by determining partitioning behaviour of the compound between the atmosphere, the organic material of the sludge and soil, and the aqueous phase (Clarke et al. 1995). These properties include vapour pressure, polarity, water solubility, and octanol-water partition coefficient which can also determine the analytical instrument used for separation. For example, gas chromatography (GC) requires that the analyte be naturally in the gas phase, or be a volatile liquid or solid (Grob and Barry 2004). Non-volatile analytes can be derivatized by adding a nonpolar functional group to make the compound volatile enough for determination using GC (Grob and Barry 2004), which adds another sample processing step and potential source for analyte loss or introduction of contamination. A method can be targeted at a large group of compounds with similar properties and structures like the polybrominated diphenyl ethers (PBDEs), compounds used as flame retardants (USEPA 2007a). Other methods target compounds with diverse structures and physico-chemical properties, such as pharmaceuticals, which are grouped by their use or effects on the body rather than chemical similarities (Ternes 2001). The following section briefly reviews some of the various approaches taken to extracting different groups of compounds from soil and some considerations during sampling, handling, and preparing the extract for analysis. Potential sources of contamination or analyte loss during sample processing are discussed in each section.

3.1. Soil Sampling and Handling for Trace Organic Compounds

3.1.1. Soil Sampling

The soil matrix is perhaps the most complex environmental compartment, consisting of a backbone of mineral soil particles held together by organic molecules produced by plants, animals, and soil microbial biomass (Bronick and Lal 2004). It is a

very heterogeneous material, with an active microbial population controlled in part, at the spatial scale of centimetres to millimetres, by substrate quality (soil organic matter or inputs from plants or soil amendments) and soil chemistry, while at larger scales differences in soil type and land use patterns can determine microbial activity (Ettema and Wardle 2002). Such heterogeneity makes taking a representative sample difficult, especially for factors that may vary over several centimetres. Organic contaminants introduced during the land application of sewage sludge may not be mobile in soil pore water if strongly sorbed to organic matter or soil particles (Lapen et al. 2008), and their distribution in the soil initially may be limited to the vicinity of the amendment. It is not realistic to try to capture an entire field in a single soil sample, so an approach commonly taken is to combine a number of random samples from different locations in the same experimental unit into a composite or gross sample, which is then homogenized and sub-sampled for laboratory analysis (Skoog et al. 2004). Ploughing mixes the soil and amendment, and would make a homogenized sample more representative of the bulk material.

On no-till fields, the soil amendment either sits on top of the ground or is lightly incorporated using a 'minimal tillage' implement. In a nonhomogeneous arrangement, the amendment and the contaminants do not decompose as rapidly as when homogenized in soil (Heselhøe et al. 2001), and the amendment can be visible on the surface after several months. This can introduce a number of complicating factors into achieving a representative sample. For one, mixing an organic amendment into soil (which would happen during a composite sample from a no-till plot) causes a rapid initial flush of microbial respiration due to organic compounds being consumed, which tapers off over time as substrate is depleted (Stott et al. 1983). Small, high-energy organic molecules are used first (within days), followed by more complex molecules and structural polymers (months-years) once the energetic pool of carbon is depleted (Lorenz et al. 2007). Some of the organic compounds mineralized within days would also be extractable using many of the procedures described later, and would be present as excipients or potential analytes in the extract. Depending on the target analytes, they may or may not be degraded to a meaningful extent during the time from sampling to analysis. Soil samples are commonly stored at -4°C to -10°C until analysis to minimize microbial activity (Kinney et al. 2006),

and should be stored in a cooler with ice packs immediately after sampling for transport from the field to the lab. Another complicating factor in no-till plots is that the visibility of the soil amendment on the surface could introduce sampling bias, either for or against sampling on top of the amendment. In a large field it may be difficult to lay out a grid, but there are practical techniques that can reduce potential bias.

3.1.2. Problems Posed by the Soil Matrix

Soil contains organic material and mineral soil particles that can interact with analytes during the extraction process. The soil organic matter (SOM) is considered to be the non-living and recently dead organic residues, which are acted on by the soil microbial biomass (Wolf and Wagner 2005). High-energy compounds in SOM are consumed rapidly by microorganisms adapted for fast growth and utilization of resources, and as high-energy resources decline, other groups of organisms adapted for less energetic compounds become active (Fontaine et al. 2003), over time creating a more and more stable substrate. During decomposition, certain chemical structures are more stable and resist transformation, accumulating in older SOM. These include aromatic and aliphatic structures, which contribute to the sorption of hydrophobic organic compounds in soil (Chefetz and Xing 2009). Humification is a stabilization process by which complex molecules such as proteins, polysaccharides, and lignin are transformed by chemical, physical, and biological processes into a heterogeneous mass of organic material (Zech et al. 1997). The humification process leads to many different surface functional groups able to interact with analytes, ranging in polarity (Huang et al. 2003). It has been suggested that analytes could become chemically bonded to the SOM (humified) if present in soil for an extended period of time (Löffler and Ternes 2007). A significant amount of SOM is also extracted along with analytes, leading to a high background response from the instrument relative to the concentration of potential analytes (Burkhardt et al. 2006). For this reason, soil extracts often require an extensive cleanup and concentration procedure to produce a sample suitable for analysis.

The mineral soil particles can also bind analytes, especially clays which have a large surface area with many exposed oxide groups available for binding (He et al. 2006). The contribution of clay particles in soil to the binding of certain organic contaminants

(e.g. nitro-aromatics and triazine pesticides) can be equal to or exceed that of the SOM (He et al. 2006). Metal oxides in soil contribute a partial charge from polar hydroxyl groups available for binding, which becomes more strongly positive at lower pH as hydroxyl groups are protonated (Dubus et al. 2001). Sorption of certain molecules containing carboxylic acid groups in soil is due to an interaction with OH and OH_2^+ groups on mineral particles (Dubus et al. 2001). To determine the loss of analytes due to sorption to the soil matrix, a spiking experiment should be conducted using each analyte to assess method performance when trying a new method or making modifications to an existing method (USEPA 2007c). Non-spiked samples and samples spiked with a known mass of each analyte can be extracted to determine the recovery of each analyte from the soil matrix for the particular method.

3.2. Extraction

3.2.1. Sample Preparation and Solvent Choice

The particle size of the soil sample should be reduced before extraction to allow for better solvent coverage by increasing the surface area of the sample. Using a ball mill, mortar and pestle, or sieve can reduce the particle size to less than 1 mm, a value used in US EPA methods (USEPA 2007a, 2007c). For organic contaminant analysis, electric grinders with plastic parts (e.g. the cover) should be avoided to reduce potential contamination. The grinding apparatus can bind analytes, and should be thoroughly cleaned with a mild detergent solution and rinsed with solvent in between runs to prevent cross-contamination (Mitra 2003). The solvent from this rinse can be analyzed to estimate analyte losses due to the grinding procedure (Mitra 2003).

Soil samples can be extracted at field or air-dried water holding capacity, but if the sample contains moisture it can inhibit the extraction. A non-miscible solvent like hexane cannot penetrate water that is tightly bound to the surface of the soil matrix, and analytes trapped between the water and soil surface will not be extracted (Mitra 2003). In these cases, a drying agent such as anhydrous sodium sulphate is added in excess to homogenized soil to remove any moisture, allowing the solvent to come in contact with analytes bound to the soil surface (Mitra 2003; USEPA 2007a, 2007c). Another solution is to use a water-miscible solvent such as methanol or isopropanol, or a co-solvent

mixture with water, which is able to penetrate surface water to reach trapped hydrophobic analytes (Banjoo and Nelson 2005; Burkhardt et al. 2005). If looking for moderately non-polar analytes, this approach has the added advantage of being more selective than using a highly nonpolar solvent by reducing the amount of nonpolar SOM extracted, and can lead to a cleaner sample (Burkhardt et al. 2005).

Solvent choice can also depend on the target analytes, the chosen extraction technique (e.g. Soxhlet, ultrasonic, pressurized liquid), the cost and availability of solvents, and on the toxicity of a particular solvent to lab workers and the environment (Banjoo and Nelson 2005; Bossio et al. 2008). The optimal solvent choice is a balance between these and other factors, such as the requirements of the desired cleanup method, the instrument used for analysis, and the method performance (Banjoo and Nelson 2005; Burkhardt et al. 2005). Solvents can be exchanged after extraction to suit a particular cleanup or instrument by evaporating the extract almost to dryness (~100 μL) using a nitrogen evaporator and adding a fixed volume of the second solvent, repeating twice more to ensure the majority of the first solvent is evaporated (USEPA 2007c). The extract should not be evaporated to dryness unless gravimetrically determining the mass of extracted residue, as volatile analytes can be lost and exposure to the atmosphere could lead to oxidation of certain analytes (USEPA 2007c). It is also good practice to extract multiple times with smaller solvent volumes and combine the extracts rather than once with a large volume of solvent, since many analytes are not extracted with high efficiency after only one treatment (Skoog et al. 2004; USEPA 2007c). An equilibrium is established as compounds dissolve, eventually reaching a point where the rate of analyte adsorption to soil and dissolution in the solvent are equal. Removing this solvent and replacing it with fresh changes the equilibrium and allows more of the analyte to be extracted.

3.2.2. Extraction Techniques.

Several techniques exist for the extraction of organic contaminants from soil samples. They vary greatly in their purchase and operational costs, time required for processing, volume of solvent required, and extraction efficiency for a particular compound or group of compounds from soil (Mitra 2003). In general, increasing the

temperature and pressure, reducing sample size, and agitating the sample will increase extraction efficiency (Mitra 2003). Several common extraction procedures used in soil analyses are Soxhlet (Abrha and Raghavan 2000; Cai et al. 2007; USEPA 2007a, 2007c), Accelerated Solvent Extraction or Pressurized Liquid Extraction (ASE or PLE) (Abrha and Raghavan 2000; Burkhardt et al. 2005; Kinney et al. 2006), Microwave-Assisted Extraction (MAE) (Liu et al. 2004; Rice and Mitra 2007), and Ultrasonic-Assisted Extraction (UAE) (Banjoo and Nelson 2005; USEPA 2007b; Bossio et al. 2008).

Soxhlet extraction is the 'benchmark' approach for the extraction of semi-volatile organic contaminants from soil (Mitra 2003), and is the technique on which USEPA methods are based (USEPA 2007a). Schematics of the Soxhlet apparatus are available in several published sources. Briefly, a reservoir of solvent is heated and the vapours condensed, draining into the sample container and covering the sample. When the container is full, excess solvent drains back into the reservoir, taking extracted compounds with it. The sample is constantly doused with recycled solvent through evaporation/condensation, which ensures a thorough extraction. Relatively few parameters affect the efficiency of Soxhlet extraction, making it a robust extraction technique, but large solvent volumes (~300 mL) and a lengthy extraction time (>24 hrs) limit the use of Soxhlet extraction when a high sample throughput is required (Mitra 2003).

ASE or PLE both describe the same technique using elevated temperature and pressure to reduce the sample time required while still providing an efficient extraction procedure. The procedure is automated, reducing solvent consumption and thus is popular in laboratories analyzing many samples (Burkhardt et al. 2006), although the system is expensive to purchase. The sample is placed in a tube-shaped sample holder (extraction cell) with an entry and exit for solvent to flow. The container is housed in an oven while a fixed amount of solvent is pumped into the extraction cell, then valves seal and pressure is allowed to increase as the extraction cell is heated. The increased pressure raises the boiling point of the solvent, allowing ASE to reach much higher temperatures than Soxhlet extraction (Mitra 2003). Extraction of nonpolar, high molecular weight compounds such as PAHs and PCBs can be achieved with ASE using water and an organic cosolvent such as methanol or isopropanol (Burkhardt et al. 2006).

Where PLE heats the sample by convection using an oven, MAE applies heat through two different mechanisms using microwave radiation. Dipole rotation occurs when molecules with a dipole align themselves with an electromagnetic field (EMF), which occurs at a rate of 4.9×10^9 times per second (Mitra 2003). Molecules with larger dipoles respond more vigorously to an EMF, which generates heat through frictional forces between molecules. Ionic conduction occurs when ions move in a solution in response to an EMF, which also generates heat through friction (Mitra 2003). Heating can occur through an interaction between components of either the solvent system or the sample with microwave radiation.

UAE (also called sonication) applies a physical force to the sample, bombarding it with ultrasonic waves that disturb the sample matrix and allow better contact with the solvent (Mitra 2003). When sound waves with frequencies greater than 15 kHz travel through water, bubbles of gas form and collapse as a result of the changing pressure (called cavitation), exerting extreme temperatures (2-5 kK) and pressures of several hundred atmospheres in the vicinity of the bubble (Kotronarou et al. 1992). UAE can be done using an ultrasonic probe placed in contact with the solvent, or by placing a number of samples in an ultrasonic water bath. Extraction temperature can also be increased by heating the water bath, which can improve the recovery of certain analytes (Bossio et al. 2008). Using two or more extraction cycles with a fresh aliquot of solvent each time is recommended, but the short extraction time (several minutes) means that this can be accomplished easily (Mitra 2003). Sonication is an extraction method becoming more popular due to the low cost relative to other methods and comparable extraction efficiencies while using smaller sample sizes and solvent volumes (Mitra 2003). However, there is potential for certain analytes to degrade when subjected to sonication due to thermal decomposition and reaction with hydroxyl radicals formed through homolytic cleavage of water molecules that occurs during cavitation (Kotronarou et al. 1992).

3.3. Sample Cleanup

Soil samples generally contain a wide variety of compounds that are not of interest to the analyst, but have similar physico-chemical properties to compounds

targeted by a particular method and are simultaneously extracted. Certain extraction techniques like Soxhlet are exhaustive, extracting many background matrix compounds from the SOM that must be removed before analysis (Burkhardt et al. 2005). Sample cleanup traditionally has used liquid-liquid extractions to isolate analytes based on partitioning behaviour between non-miscible solvents (USEPA 2007c), but this produces a large amount of solvent waste that must be disposed of (Burkhardt et al. 2005). Column chromatography using silica or alumina columns can be used as an alternative or supplement to liquid-liquid extraction depending on the degree of cleanup required (USEPA 2007c), but are generally time consuming to prepare. Automation of extraction techniques and development of techniques able to process batches of samples have increased sample throughput, and cleanup techniques have become more rapid as well. Solid phase extraction (SPE) is a newer technique that uses manufactured disks or cartridges containing a sorbent for analytes or excipients to produce a cleaner extract (Sigma-Aldrich Co. 1998). The sorbent can have a number of functionalities, for example: OH, C18, NH_2 (Sigma-Aldrich Co. 1998), or specialized copolymeric materials such as polystyrene-divinylbenzene (PS-DVB) (Zaugg et al. 2007). SPE can be done on a single cartridge using a syringe pump, or up to 96 at one time using a vacuum manifold, greatly decreasing sample processing time (Sigma-Aldrich Co. 1998).

3.3.1. Liquid-Liquid Extraction

The mechanism of separation in liquid-liquid extraction is a difference in solubility of the analytes and excipients between two immiscible solvents. An aqueous phase and a nonpolar organic phase are used in a separatory funnel to separate polar and nonpolar compounds. Analytes amenable to GC would tend to stay in the nonpolar phase, with the more polar excipients partitioning into the aqueous phase. The concentration of analytes in a particular phase at equilibrium occurs when the rate of transfer of a compound from the organic to aqueous phase is equal to that from the aqueous to organic phase (Moldoveanu and David 2002). As was true for extracting compounds from soil, most compounds are not extracted with high efficiency after a single extraction, so the solvent layer containing the excipients can be changed for fresh solvent and the extraction repeated (Moldoveanu and David 2002).

Another liquid-liquid extraction approach separates acidic and basic compounds from those that are neutral by adjusting the pH of the aqueous phase. Non-polar compounds containing ionisable groups can be made more soluble in the aqueous phase by adjusting the pH so that basic groups become protonated and gain a positive charge, or acidic groups are deprotonated gaining a negative charge (Moldoveanu and David 2002; Mitra 2003; USEPA 2007a). Neutral compounds and those unaffected by the pH change stay isolated in the organic phase. Changing the pH of the aqueous phase back to neutral allows extraction of the remaining acidic or basic compounds into fresh organic solvent if desired (Moldoveanu and David 2002).

3.3.2. Column Chromatography and SPE

The mechanism of separation in column chromatography and SPE is through liquid-liquid partitioning or solid-liquid adsorption depending on the composition of the sorbent (Moldoveanu and David 2002). It is used as a cleanup and concentration tool by binding the analytes, excipients, or both, which are eluted from the column using a different solvent (Sigma-Aldrich Co. 1998). To be successful, the partitioning behaviour of the target compounds must be strongly in favour of the solid phase (Moldoveanu and David 2002). This is achieved through selection of a proper sorbent and extraction solvent depending on the SPE method (Moldoveanu and David 2002). SPE can take several forms. The excipients can be removed on the solid phase and the eluant collected and used in analysis, or the analytes can be retained by the solid phase and excipients in the eluant, with the analytes later eluted from the column with a stronger solvent (Sigma-Aldrich Co. 1998; Moldoveanu and David 2002). More complex methods involve binding the analytes and excipients to the solid phase (loading the column) and eluting either the analytes or excipients from the column using an appropriate solvent (Sigma-Aldrich Co. 1998; Moldoveanu and David 2002), or combining two types of sorbents to first bind the compounds and then elute with a different solvent through a material that removes excipients (Mitra 2003; Bossio et al. 2008). The extraction solvent should be chosen to encourage the partitioning of the desired compounds onto the solid phase material. A milder solvent combined with a stationary phase with a higher affinity for the compounds of interest will create conditions favourable for adsorption to the SPE material. Ideally, all

of the desired compounds should be bound on the solid phase during column loading, and all should be removed during elution, but this is not always the case (Mitra 2003). Larger extract volumes can cause partial elution of bound compounds during column loading, a phenomenon known as breakthrough (Moldoveanu and David 2002).

Columns containing silica, alumina, or Florisil (magnesium silicate) gels are commonly used to remove excipients from an extract (USEPA 2007c). All three contain many active hydroxyl groups on the surface, which can interact with polar excipients and bind them while nonpolar analytes pass through relatively unhindered (Mitra 2003). Alumina, silica, and Florisil must be activated by heating to remove water prior to dry packing of the column (USEPA 2007c). Water present in samples will also bind to these sorbents, so a layer of drying agent (such as sodium sulphate) must be included above the sorbent layer to prevent inactivation of the column by water that may be present in the extract (USEPA 2007c). Florisil is also available as a SPE cartridge or disk for more rapid sample preparation (Sigma-Aldrich Co. 1998), although a drying agent should still be added if the extract could contain water (Bossio et al. 2008).

Other SPE materials use bonded silicas containing a variety of alternative functional groups, similar to GC or HPLC columns. Nonpolar characteristics can be imparted to the column through Si–O–R bonds with alkyl (C2, C8, C18), phenyl, or cyclohexyl groups (Sigma-Aldrich Co. 1998; Moldoveanu and David 2002). These increase the sorption capacity of the column for nonpolar compounds. Other polar functional groups can also be substituted, including diol (2OH), amino (NH_2), or cyano (CN) groups depending on the requirements of the target analytes (Sigma-Aldrich Co. 1998; Moldoveanu and David 2002). More recent advances include the use of organic polymers such as PS-DVB as a non-polar backbone, to which compounds with polar functional groups can be added to provide a multi-functional SPE material that is resistant to drying (Hennion 1999).

3.4. Analysis by Gas Chromatography with Mass Spectrometry (GC-MS)

For trace analysis of complex samples, GC with MS provides a sensitive detection method that provides additional confirmatory molecular identification in the mass spectra (in full scan mode) not present in other detectors (Clench and Tetler 2000). Mass

spectrometry uses a high-energy electron beam to create unstable molecules which then break into predictable fragments based on the structure of the molecule (Clench and Tetler 2000). The fragments carry a charge, and are accelerated through an electromagnetic field (EMF) which alters the trajectory so that only ions with a specific mass to charge ratio can enter the detector. In the universal mode, called full scan mode, the EMF is varied so that the instrument scans through all masses on each sampling time. In the selective mode, called selective ion monitoring, only a few mass fragments are monitored throughout the analysis. This reduces the amount of time spent monitoring unnecessary masses and increases the sampling rate, lowering average background noise and increasing the precision associated with each measurement (Clench and Tetler 2000). The mass spectra for a particular molecule is reproducible under constant operating conditions and can be used to help identify sample components as long as there are no other components co-eluting at the same time as the analytes. Using selected ion monitoring can allow pseudo-separation of two co-eluting components in a complex sample by selecting a molecular fragment for monitoring that is present in only one of the co-eluants. As long as the molecular fragment can only come from one of the co-eluants, it can be used for quantification in a complex sample (Stashenko et al. 1997). However, this requires knowledge of anything that may be co-eluting in the sample.

4. Conclusions

Wastewater treatment produces a solid organic residual called sewage sludge or biosolids, which is often used as a soil amendment. However, the composition of modern wastewater can have detectable concentrations of toxic organic compounds that end up in biosolids, with potentially damaging consequences for organisms in environmental matrices such as soil and water. Analysis of environmental samples (especially soil) for organic contaminants is a complicated process, but is becoming easier and more rapid as new sample preparation technologies emerge. Newer methods are moving towards lower solvent usage, smaller sample sizes and automation to reduce environmental impacts and improve precision. There are often a number of possible approaches to obtaining a sample capable of analysis by gas chromatography. The optimum choice is a balance between analysis cost, difficulty, suitability of a particular technique to a matrix, and method

performance in the chosen application. For all techniques, it is important to monitor method performance using spiked samples, and to take measures to avoid sample contamination in all stages of analysis.

CHAPTER 2: DECOMPOSITION OF N-VIRO SOIL™ IN AN AMENDED FIELD SOIL

(Reproduced with minor modifications from Gillis, J.D. and Price, G.W. (2011) Comparison of a novel model to three conventional models describing carbon mineralization from soils amended with organic residues. Geoderma 160(3-4):304-310)

1. Introduction

Organic soil amendments such as manure, compost, and biosolids are important sources of fertility in agriculture and have the added benefit of increasing soil organic carbon pools (Lal 2004). The land application of municipal organic residuals is a common method of recycling nutrients, especially in places such as the Halifax Regional Municipality, Nova Scotia, Canada where landfilling and incineration of organic residuals are banned. Soil microbial biomass is mostly responsible for the cycling of carbon, nitrogen, phosphorus, sulfur, and other nutrients in soil as they decompose organic material (Van Veen et al. 1985). Labile compounds are generally limited in soil (Morita 1988), but organic amendments provide a diverse array of consumable substrates for soil microorganisms. The more labile components such as simple sugars and amino acids are consumed quickly (hours-weeks), while more stable components, such as structural polymers, can persist for years (Lorenz et al. 2007). The soil ecosystem is highly complex, and our understanding of the decomposition behavior of organic materials is still incomplete. Highly variable soil conditions, biotic and abiotic factors, as well as the nature of the organic amendment can alter decomposition dynamics in soil. There is a need to further understand this process for different types of organic waste residuals being directed to soil under varying environmental conditions if we are to predict any potential impacts of our soil management practices. Moreover, there is a need to interpret any results in the context of an ecological system, i.e. the interaction between organisms in response to a disturbance in their environment (Wardle and Giller 1996; Prosser et al. 2007; Andrén et al. 2008).

Chemical composition of the organic inputs and make-up of the microbial communities in the soil are important, interrelated factors affecting the dynamics of

carbon mineralization from amended soils. The active soil microbial community at a given site is governed in part by land use, which influences the nature of plant-derived carbon inputs and SOM quality (Waldrop and Firestone 2004). Lauber et al. (2008) found that land use alone as a predictor of microbial community composition is not sufficient, especially if soil properties and nutrient status vary significantly within that site. The addition of organic residuals to soil can alter the composition of the soil microbial community and consequently cause a shift in decomposition dynamics (Waldrop and Firestone 2004). As early as the 1920s, it was observed that adding different forms of nitrogen to soil resulted in different organisms appearing in the soil cultures compared to unamended soil (Winogradsky 1928). This observation gave rise to the recognition of different competitive strategies in the soil environment. More recently, Abaye and Brookes (2006) compared three soils under different land use management strategies, each amended with three different organic inputs. Their results indicate that the active microbial population can determine initial carbon mineralization dynamics in the short term, but as the composition of the soil microbial biomass shifts over time, in response to substrate addition, the substrate quality of the organic amendment can dominate mineralization dynamics.

Sewage biosolids are produced from the solid residuals of municipal wastewater treatment. Some biosolids may undergo a biological digestion process at the treatment plant to decrease the labile carbon content prior to land application. Biosolids can receive further treatment such as composting, thermal drying, or alkaline stabilization to meet regulatory requirements prior to land application. Differences in the treatment processes affect the quality of the carbon substrate for soil microorganisms. Bernal et al. (1998a) incubated solids from different stages of sewage treatment in soil and found a decrease in CO_2 evolution with biological digestion and composting compared to undigested solids. Similarly, Fernández et al. (2007) found that thermally-dried sewage biosolids are significantly more active than composted biosolids, based on the amount of CO_2 evolved from the amended soil. Alkaline-stabilized biosolids, such as N-Viro Soil™, are produced by adding quicklime (CaO), cement kiln dust, or other alkaline materials that undergo an exothermic reaction with water. Supplemental heating is sometimes used to achieve higher temperatures. This raises the pH and temperature in order to inhibit microbial

activity and kill pathogens (Logan and Harrison 1995). Consequently in many areas, these biosolids are used as a soil liming agent but little research on the decomposition dynamics of these alkaline-stabilized biosolids has been conducted.

Modeling is a useful tool in decomposition and ecological studies, especially when it is not possible to directly observe or manipulate the microbial populations. In these cases, as long as the system is adequately described, the model provides some predictive capability and allows evaluation of the system function under theoretical scenarios (Gertsev and Gertseva 2004). Typically, carbon dioxide evolution curves are used to monitor decomposition activity and are a measure of either the net cumulative respiration or respiration rate of the soil microbial biomass under aerobic conditions, and take a variety shapes. Mathematical models containing informative theoretical parameters, such as the quantity of mineralizable carbon and half-life of the material in the exponential model (Table 1), are used to describe CO_2 evolution data but no single model is applicable across all disturbances in soil. For instance, alterations to the first order exponential model have been used to separate the carbon sources into two or more sub-pools representing easily available and more resistant carbon sources within the pool of potentially mineralizable organic carbon (Voroney et al. 1989). However, authors will fit several models and choose the best one based on statistical measures of model adequacy (Bernal et al. 1998b; Berndt 2008). In cases where the curve has inflection points, exponential and linear models often fail to capture this feature.

The objective of this study was to examine the carbon mineralization dynamics from an alkaline-stabilized biosolid applied at increasing rates to an agricultural field soil. Furthermore, we compared four models describing cumulative CO_2-C evolution from the amended soil using statistical tests of model adequacy. Three of the models have been used previously in the literature and the fourth, a first order exponential plus logistic model (Table 1), is proposed as a possible alternative that could be particularly useful in cases where curves have inflection points.

2. Materials and Methods

2.1. Sampling and Handling

A gleyed and fragic humo-ferric Podzol (Webb et al. 1991) with a crop history of carrots and soybeans was sampled from the Ap horizon, using 30 cm Oakfield soil probes, at the Bio-Environmental Engineering Center in Bible Hill, NS, Canada (45°23' N, 63°14' W). Soil was air dried prior to homogenization by 2 mm sieving, re-wetted to 18% GWC and pre-incubated for 10 days prior to biosolids amendment. The soil was amended with an alkaline-stabilized biosolid (ASB), N-Viro Soil™, which was also homogenized by 2 mm sieving. The ASB was applied wet but at dry weight equivalent rates. The ASB originates from an advanced primary treatment process (settling with chemical flocculation of additional solids), followed by an addition of cement kiln dust and quicklime mixture with supplemental heating. Chemical properties of the agricultural field soil and ASB are listed in Table 2.

Table 1
Selected models describing cumulative carbon dioxide evolution from soil over time.

Model	Form	Parameter description (units)
FO	$C_t = C_0(1-e^{-k_0 t})$	C_t = cumulative CO_2-C evolved at time t (mg g^{-1}) C_0 = size of potentially mineralizable carbon pool (mg g^{-1}) k_0 = first order rate constant (day^{-1})
FLIN	$C_t = C_1(1-e^{-k_1 t}) + k_2 t$	C_1 = size of labile pool (mg g^{-1}) k_1 = labile pool rate constant (day^{-1}) k_2 = CO_2-C evolution rate from linear pool (mg g^{-1} day^{-1})
DFO	$C_t = C_1(1-e^{-k_1 t}) + C_2(1-e^{-k_3 t})$	C_2 = size of recalcitrant first order pool (mg g^{-1}) k_3 = recalcitrant pool rate constant (day^{-1})
FLOG	$C_t = C_1(1-e^{-k_1 t}) + \frac{C_3}{1+e^{-\frac{t-k_4}{k_5}}}$	C_3 = size of delayed logistic pool (mg g^{-1}) k_4 = location of inflection point (days) k_5 = distance from inflection point to ¾ maximum (days)

Table 2
Chemical properties of agricultural field soil and alkaline-stabilized biosolid (ASB). Values are expressed on a dry weight basis.

	Soil	ASB
Gravimetric Water Content (%)	18	37
Total Carbon (g kg^{-1})	14	109
Total Nitrogen (g kg^{-1})	1.1	6.8
Inorganic Nitrogen, $NH_4^+ + NO_3^-$ (g kg^{-1})	0.104	ND
M-3 Calcium (g kg^{-1})	2.5	71
M-3 Phosphorus (g kg^{-1})	0.32	2.5
M-3 Potassium (g kg^{-1})	0.29	2.6
M-3 Magnesium (g kg^{-1})	0.23	1.4
M-3 Copper (g kg^{-1})	0.018	0.041
pH (0.1 M $CaCl_2$)	5.3	9.5
Electrical Conductivity (mS cm^{-1})	0.071	4.5

ND = Not Determined

2.2. Experimental Design

Carbon mineralization from ASB-amended soil was monitored in an aerobic incubation (Hopkins 2008). Treatments consisted of five amendment rates: 0, 2.08, 4.16, 8.32, and 12.48 mg ASB g^{-1} soil in 1 L glass jars containing 130 g dry weight (d.w.) of soil. A total of 220 jars were included in the study for destructive sampling at eleven pre-determined intervals, on days 0, 3, 6, 9, 12, 15, 22, 29, 55, 88, and 121. Each treatment had four replicates which were incubated at 20°C in a controlled environment chamber. Evolved CO_2-C was measured titrimetrically during the pre-determined intervals using 25 mL, 1 M NaOH traps, however to prevent anaerobic conditions from forming, additional sampling periods were added on days 45, 71, and 103 (totaling 14 time periods for CO_2-C). Six blank jars were included during each time interval to account for background CO_2-C concentration. Traps were manually titrated with standardized 0.5 M HCl using a phenolphthalein end-point indicator. Background soil respiration was subtracted from treated soils to examine only evolved CO_2-C resulting from the ASB amendment rates. Soil water content was adjusted periodically by spraying soil with a mist of water until the weight of the soil plus jar was equal to the initial weight (Hopkins 2008).

2.3. Soil and Biosolid Properties

Total carbon and nitrogen of the soil and ASB were determined using a LECO CN2000 combustion analyzer (LECO Corporation, St. Joseph, MI). Solid samples were air dried and homogenized in a Retsch MM300 Ball Mill prior to C and N analysis. pH and electrical conductivity of the soil and ASB were determined on an Accumet XL50 meter. Solids pH was determined in a 0.01 M $CaCl_2$ suspension at ratios of 1:2 and 1:10 air dry solids:solution for soil and ASB, respectively (Hendershot et al. 2008). Electrical conductivity was determined in 1:5 fresh solids:deionized water (Miller and Curtin 2008). Soil inorganic nitrogen (NH_4^+-N + NO_3^--N) was extracted using a 2 M KCl solution at a ratio of 1:3 soil:solution and analyzed on a Bran & Luebbe AutoAnalyzer 3 (Seal Analytical Inc., Mequon, WI). Soil and ASB Ca, P, K, Mg and Cu were determined by Mehlich III extraction followed by ICAP analysis (Soil and Feed Testing Laboratory, Prince Edward Island Department of Agriculture and Forestry, Charlottetown, PEI).

2.4. Statistical Methods

Four functions were evaluated to model background-subtracted cumulative CO_2-C evolution (Table 1). Nonlinear regression was performed using the PROC NLIN procedure in SAS 9.1 with the Gauss-Newton method of iteration (SAS Institute Inc. 2008). The procedure sequentially improves initial parameter estimates provided by the analyst until there is no meaningful improvement in sum of squares error (SSE), the variance in the data set not accounted for by the model (Bates and Watts 1988). The models were evaluated based on the lowest mean squares error (MSE) and meeting model assumptions of normally distributed error terms with constant variance. MSE is the SSE divided by the error degrees of freedom ($n - p$, n = total degrees of freedom, p = # of model parameters), and as a measure of model fits it penalizes according to the number of parameters in the model. Normal probability plots of the residuals and the Anderson-Darling test were used to check normality, and scatter plots of the residuals vs. fits and residuals vs. time to check constant variance.

After fitting each model to CO_2-C evolution from each amendment rate, parameter estimates for a given model were compared across ASB amendment rates. Pairwise comparison of parameters was achieved using nested models with incremental parameters

(Bates and Watts 1988). In this procedure, data from two curves were combined into a single data column. A dummy variable was generated in a new column with 0 for the first curve and 1 for the second. The model was adapted as follows:

$$C_t = (C_1 + i_1 X)e^{-(k_1 + i_2 X)t}$$

where X is the dummy variable, i_1 and i_2 are the difference in parameters C_1 and k_1 respectively between the two models. The model was fit using the same procedure as the initial analysis, and if i_n was significantly different from zero for any parameter, then differences in the parameters between the two ASB amendment rates were deemed to be significant at a 95% confidence level. Letter groupings were assigned by pairwise comparison.

2.5. Development of FLOG Model

The FLOG model (developed by me) is composed of an exponential and a logistic function which represent two separate pools of potentially mineralizable organic carbon, where $y(t) = y_1(t) + y_2(t)$. Assuming the mineralization rate (rate of CO_2-C evolution) of the exponential (labile) pool is proportional to the amount of carbon remaining, the labile pool can be represented by the differential equation:

$$\frac{dy_1(t)}{dt} = k_1 \cdot \left[y_1(\infty) - y_1(t)\right]$$

where k_1 is the proportionality constant and $y_1(\infty)$ is the total amount of mineralizable carbon in the labile pool, $y_1(t)$. This equation can be integrated by separating variables, resulting in the first order exponential model:

$$\int \frac{dy_1(t)}{\left[y_1(\infty) - y_1(t)\right]} = \int k_1 \cdot dt$$

$$-\ln \left| \left[y_1(\infty) - y_1(t)\right] \right| = k_1 \cdot t + C$$

$$y_1(t) = y_1(\infty) - C \cdot e^{-k_1 t} \qquad y_1(0) = 0;$$

$$y_1(t) = y_1(\infty) \cdot \left[1 - e^{-k_1 t}\right]$$

where C is the integration constant, which equates to $y_1(\infty)$ assuming no carbon has been evolved from this pool at $t = 0$.

The logistic pool represents a carbon source that is not mineralized to a large extent until some period of time has passed, requiring the growth of more specialized microorganisms but also becoming depleted over time. We assume that the mineralization rate of carbon from this pool will follow a bell curve not centered at time 0, but rather at a later time (k_4). The mineralization rate is assumed to be proportional to both the amount of CO_2-C evolved (representing growth of organisms) and to the fraction of carbon remaining in the logistic pool (representing resource depletion). The differential equation is as follows:

$$\frac{dy_2(t)}{dt} = k_6 \cdot y_2(t) \cdot \left[1 - \frac{y_2(t)}{y_2(\infty)}\right]$$

where k_6 is the proportionality constant and $y_2(\infty)$ is the total amount of carbon in the logistic pool. The equation can be integrated by separating variables and using partial fractions, resulting in the logistic equation:

$$\int \frac{dy_2(t)}{y_2(t) \cdot \left[y_2(\infty) - y_2(t)\right]} = \int \frac{k_6}{y_2(\infty)} \cdot dt$$

$$\ln | y_2(t) | - \ln | \left[y_2(\infty) - y_2(t)\right] | = k_6 \cdot t + C$$

$$\frac{\left[y_2(\infty) - y_2(t)\right]}{y_2(t)} = e^{-k_6 t - C} \quad \therefore \quad y_2(t) = \frac{y_2(\infty)}{1 + e^{-k_6 t - C}}$$

$$y_2(k_4) = \frac{y_2(\infty)}{2} \quad \therefore \quad C = -k_4 \cdot k_6; \quad k_6 = \frac{1}{k_5}$$

$$y_2(t) = \frac{y_2(\infty)}{1 + e^{-\frac{t - k_4}{k_5}}}$$

where k_6 is the inverse of parameter k_5 from the FLOG model (Table 1), and C is the integration constant. Assuming that when $t = k_4$ the function $y_2(t)$ is equal to half of the maximum pool size [$y_2(\infty)/2$], which makes C equal to $-k_4/k_5$. The combined differential equation for the FLOG model is:

$$\frac{dy(t)}{dt} = k_1 \cdot \left[y_1(\infty) - y_1(t)\right] + \frac{1}{k_5} \cdot y_2(t) \cdot \left[1 - \frac{y_2(t)}{y_2(\infty)}\right]$$

The fact that the logistic model approaches but never actually reaches zero (at -∞), means that at $t = 0$, $y(t)$ is going to be greater than zero (equal to $y_2(\infty)/[1+exp(k_4/k_5)]$). In theory, there should be no carbon evolved at $t = 0$, but in most cases the error should be relatively minor. Including the point (0, 0) during regression encourages the model to come close to that value, but there will still be some error in using the model to predict $y(0)$. However, model estimates occurring later in time should be unaffected by this slight anomaly.

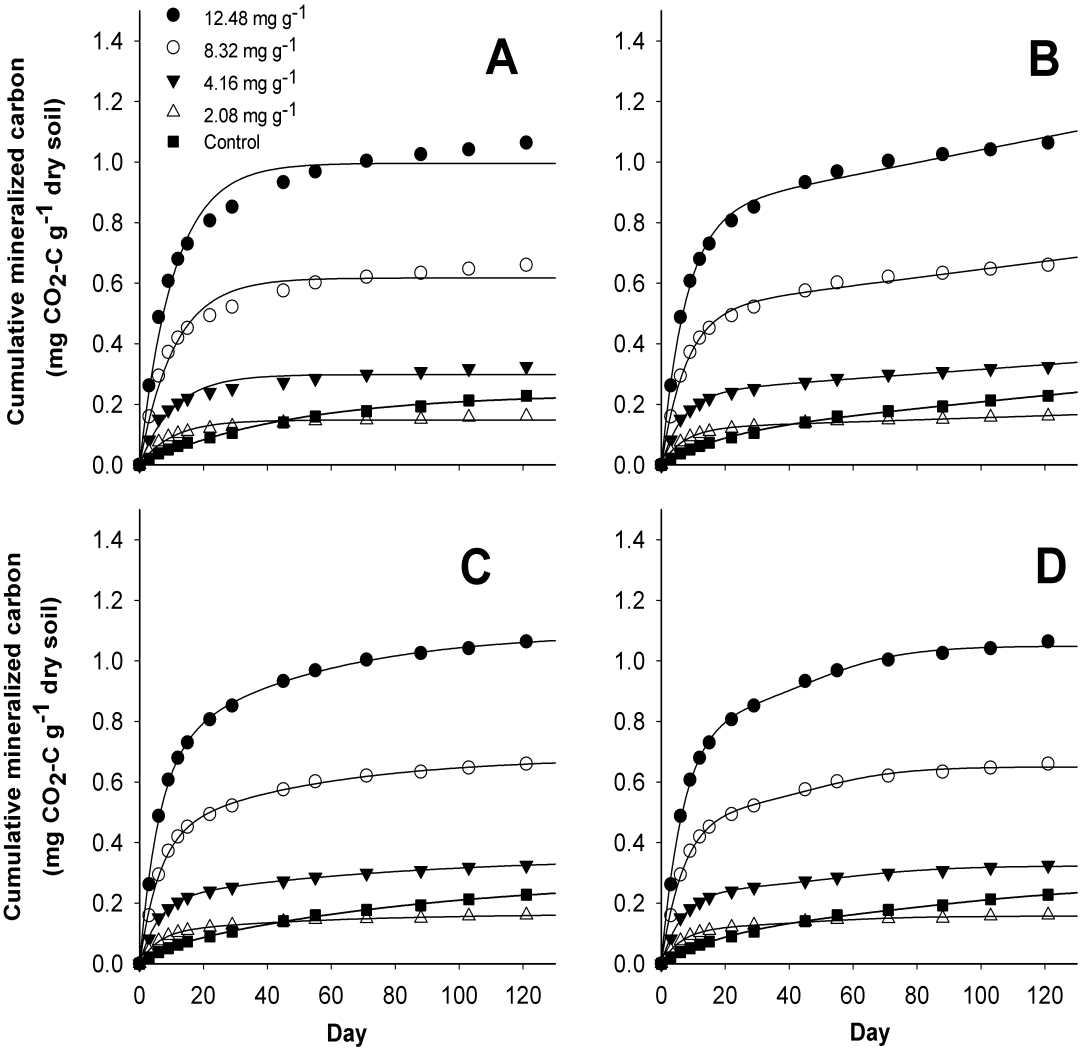

Figure 1. Cumulative carbon mineralization from soil at increasing biosolid amendment rates (mg biosolids g^{-1} soil d.w.). Curves are modeled using the FO model (A), FLIN model (B), DFO model (C), and FLOG model (D).

3. Results and Discussion

3.1. Carbon Mineralization and Model Fits

The rate of CO_2 evolution from all ASB amendment rates decreased over time, exhibiting the fastest rate in the first sampling time (Figure 1), which is consistent with exponential decay. By day 6, between 33-36% of the total carbon added to amended soils had been evolved as CO_2-C, and by day 121 this had risen to 71-78%. Almost half (44 to 47%) of the total CO_2-C evolved from ASB amended soils occurred within the first six days, indicating that a relatively labile pool of carbon remains in ASB even after the sewage treatment process. However, the FO model performed poorly compared to the other three (Table 3), having the highest MSE and a failure to meet the assumption of constant variance in all cases. Visual examination of the FO model fits confirmed that it inadequately described the carbon mineralization dynamics of the ASB amendment (Figure 1). The three remaining models, each containing two theoretical pools of carbon, fit the data better than the single pool FO model (Table 3). The three models had comparable MSEs, with the DFO model consistently producing the lowest value, indicating the closest fit, for all treatments and the control. The FLOG model had a lower MSE than the FLIN model in all cases except the control soil, despite having two additional parameters. Constant variance was not met in two cases for the FLIN model, and normality was violated in one case for the DFO. Overall, the FLOG model performed best statistically and the FLOG and DFO models had the overall best fits for all ASB amended soils. It is interesting to note that the FLOG model performed relatively poorly in the unamended soil, where both the FLIN and DFO model produced a lower MSE (Table 3). This suggests that the decomposition process taking place in the ASB amended soils, which the FLOG model describes well, is dissimilar from the unamended soil.

None of the models presented here best described carbon mineralization under all circumstances. Murwira et al. (1990) compared the first order (FO) and first order plus linear (FLIN) models (Table 1) to describe carbon mineralization from sand amended with cattle manure over a range of moisture contents. The authors selected the FO model over FLIN based on the SSE and the adjusted R^2, although there was no mention of

Table 3
Comparison of statistical measures of model adequacy of selected functions used to model cumulative CO_2-C evolution from soil. Values are MSE, with a lower value indicating a better fit. All models are significant with $p<0.0001$.

	Treatment Rates (mg ASB g^{-1}soil d.w.)				
Model	**Control**	**2.08**	**4.16**	**8.32**	**12.48**
FO	5.3×10^{-5} A	6.1×10^{-5} A	3.0×10^{-4} A	8.9×10^{-4} A	2.2×10^{-3} A
FLIN	1.0×10^{-5} A B	8.2×10^{-6} A	2.4×10^{-5} A B	1.4×10^{-4} A	3.6×10^{-4} A B
DFO	6.1×10^{-6} A B	3.8×10^{-6} A B	1.1×10^{-5} B	5.8×10^{-5} A B	1.4×10^{-4} A B
FLOG	1.2×10^{-5} A B	7.6×10^{-6} A B	1.9×10^{-5} A B	7.7×10^{-5} A B	1.9×10^{-4} A B

A - Normality assumption met B - Constant variance met

model assumption checks in their analysis. Bernal et al. (1998b) found that in some cases the FO model resulted in a higher MSE and non-random distribution of the error terms, and preferred the FLIN model in those cases. A double first-order (DFO) model has also been used to describe the decomposition of organic residues (Voroney et al. 1989), being preferred in a number of recent examples (Fernández et al. 2007; Berndt 2008). Berndt (2008) found the DFO model produced the lowest SSE of the models compared and passed statistical checks of model adequacy when modeling decay of turf grass thatch. Although the FO model is sometimes preferred for its simplicity, the more complex models perform better statistically under the scenarios shown.

Numerical values for parameter estimates varied depending on which model was used to describe the data, but there were statistically significant patterns when parameters were compared across amendment rates (Table 4). Parameters representing carbon pools increased with ASB amendment rate in an approximately linear relationship with the amount of carbon added. A noticeable difference in the distribution of carbon between the two pools in the DFO and FLOG models was also observed. The DFO model consistently produces a smaller labile pool of carbon but a larger recalcitrant pool of carbon than the FLOG model (Table 4).

Rate constants for ASB amended soils were not significantly different across amendment rates, but all ASB amended rate constants were significantly higher than the control soil (Table 4). In amended soils, half-lives derived from first order rate constants

Table 4
Comparison of parameter estimates for FO, FLIN, DFO, and FLOG models. For a given parameter in each model, estimates with the same letter are not significantly different across amendment rates based on pairwise comparison using nested models with incremental parameters (α=0.05).

Model	Para-meter	Amendment Rate: Control		2.08		4.16		8.32		12.48	
FO	C_0	0.235	D	0.148	E	0.298	C	0.617	B	0.996	A
	k_0	0.022	B	0.097	A	0.094	A	0.094	A	0.093	A
FLIN	C_1	0.103	E	0.121	D	0.238	C	0.512	B	0.832	A
	k_1	0.053	B	0.142	A	0.145	A	0.129	A	0.130	A
	k_2	0.0011	B	0.0003	D	0.0008	C	0.0013	B	0.0021	A
DFO	C_1	0.036	E	0.101	D	0.209	C	0.417	B	0.677	A
	C_2	0.252	B	0.065	D	0.146	C	0.266	B	0.418	A
	k_1	0.140	A	0.178	A	0.171	A	0.162	A	0.165	A
	k_3	0.012	A	0.019	A	0.013	A	0.020	A	0.021	A
FLOG	C_1	0.153	D	0.119	E	0.242	C	0.506	B	0.822	A
	C_3	0.104	AB	0.038	C	0.081	B	0.144	A	0.227	A
	k_1	0.037	B	0.148	A	0.143	A	0.136	A	0.137	A
	k_4	91.3	A	46.4	B	54.5	AB	47.8	B	47.5	B
	k_5	28.5	A	16.2	B	17.4	B	13.8	B	14.1	B

for the DFO model ranged between 3.9 to 4.3 days for the labile C pool and 33 to 53 days for the recalcitrant C pool. Half lives for the first order (labile) component of the FLOG model ranged from 4.7 to 5.1 days. The parameter k_5 is the time interval between the inflection point (50% max height) and the point at 75% of the maximum height of the logistic pool, so twice that value, 28-35 days, would correspond to 50% of the total logistic pool size. This is slightly less than the value predicted by the DFO model, and a smaller range. The parameter k_4 ranged from 46 to 55 days, indicating the time at which 50% of the carbon in the logistic pool was mineralized. In combination, k_4 and k_5 characterize the speed and timing of carbon mineralization from a slightly recalcitrant pool of carbon.

3.2. Theoretical Considerations

At times, cumulative carbon mineralization or rate curves do not appear to follow typical exponential kinetics and sometimes exhibit inflection points (e.g. Zibilske 1987, 1997; Bernal et al. 1998b; Abaye and Brookes 2006; Cayuela et al. 2009). This phenomenon cannot be reproduced by a single or double exponential model whose rate of

change is always decreasing with time, and modeling these data with traditional models can be difficult to interpret. It is believed that part of these secondary increases in carbon mineralization rates result from the growth of a different subset of microorganisms which are able to attack the more stable carbon sources through enzymatic cleavage of macromolecules (Abaye and Brookes 2006; Cayuela et al. 2009).

Previous work in modeling substrate utilization by microorganisms has shown that the logistic curve is a good approximation for substrate utilization particularly in cases where the added substrate is co-utilized and not used as a primary source of energy for growth by the microorganisms (Schmidt et al. 1985). This would be the case for macromolecules and more recalcitrant compounds which are thought to be acted upon by a different subset of microorganisms that are adapted for less energetic conditions by producing extracellular depolymerizing enzymes and normally feed on SOM (Fontaine et al. 2003). These types of microorganisms (*K*-selected) have also been implicated in the priming effect induced by some soil amendments, where enzymes normally targeting SOM also act on organic amendments; therefore, the increased availability of substrate may trigger the growth of that microbial population (Fontaine et al. 2003). Trace amounts of added substrates ($\mu g\ g^{-1}$) have also been shown to trigger increases in soil microbial respiration that can amount to several times the carbon added in 'trigger solutions' (De Nobili et al. 2001).

Microorganisms have the ability to sense changes in substrate using extracellular enzymes. Klonowska et al. (2002) found that certain fungi can produce different forms of extracellular lignin degrading enzymes *in vitro*, which are thought to have different functions in the environment. In that study, one form of the enzyme, with a higher affinity for phenols but lower oxidative potential, was produced under all growth conditions tested and was believed to have acted as a sensing enzyme. Another enzyme, induced by addition of copper and *p*-hydroxy benzoate to mimic the original enzyme substrate, had a much higher oxidative capacity but a lower specificity which maximizes resource accumulation when the substrate is known to be available (Klonowska et al. 2002). In the soil environment, Cayuela et al. (2009) showed increases in the extracellular enzyme activity of casein-protease and β-glucosidase, enzymes responsible for the cleavage of proteins and polysaccharides, respectively, in response to additions of complex animal

and plant residues. Geisseler and Horwath (2009) saw similar responses from depolymerizing enzymes to simple soil amendments with additions of pure cellulose, NH_4^+, sodium caseinate, and gluten. These inducible responses to substrates take time to develop, which is inconsistent with the mathematics of using an exponential model to represent the more recalcitrant carbon pool.

Above ground ecological theory suggests that *r*-selected, or ruderal species, compete by utilizing a niche created by a disturbance. They have a faster rate of reproduction and are able to acquire resources faster under optimal conditions (Grime 1977). Soil microbial *r*-strategists are typically found in the rhizosphere of a root for example, where there is disturbance by the growing root and highly labile carbon compounds are abundant (De Angelis et al. 2009). At the opposite end of the spectrum, *K*-selected or stress tolerant species would tend to invest more energy into sustenance, by producing extracellular enzymes, rather than reproduction, and are better suited to survival in an oligotrophic environment (Fontaine et al. 2003). The middle ground consists of species that have what is termed a 'competitive' growth strategy, which dominate over ruderal species in productive regions in the absence of a disturbance (Grime 1977). It is possible to find fungal species that can be classified under each of these three strategies (Grime 1977; Kendrick 2000).

The FLOG model presented in this paper appears able to represent some of the different strategies used by soil microorganisms in response to complex carbon inputs. The exponential component represents a rapidly mineralized carbon pool whose rate depends mostly on substrate concentration, particularly since soil microbial biomass is known to exist in a state of metabolic readiness to quickly respond to labile substrates (Morita 1988; De Nobili et al. 2001). The contribution of ruderal and competitive microbial species to CO_2-C evolution is likely captured by the first order exponential component and is represented by the initial flush of respiration from labile carbon sources in the soil amendment. As the environment becomes more stressful due to carbon resource depletion, ruderal and competitive species numbers decrease and create a niche for organisms better suited to the harsher environment (Grime 1977). However, it should be noted that under these harsher conditions additional carbon becomes available from turnover within the microbial biomass and is subsequently released as CO_2-C, but is not

separated in any of the models presented here. The general logistic function represents a delayed carbon pool whose mineralization rate depends on substrate concentration and a shift in the composition of microorganisms (towards stress tolerant or *K*-selected) capable of enzymatic degradation of more recalcitrant carbon from the amendment. The empirical data set from our study shows that the FLOG model is at least as good as other commonly used models, and serves as a useful starting point for future research with complex organic residuals applied to agricultural soils.

Recently, Rovira and Rovira (2010) made a similar argument about the double exponential model in their more detailed mathematical analysis of models describing forest litter decomposition over several years. They proposed a single pool exponential model, with a rate constant that varies as a function of time, which allows for adaptation of the model to different environmental conditions. This approach had the benefit of being able to fit highly variable data sets extracted from the literature (Rovira and Rovira, 2010). They also state that even though the double first order model can appear to provide a better fit, it may not necessarily represent the actual decomposition process occurring at the site.

The use of the FLOG model in this study is based on a multiple pools of carbon concept but also recognizes the need to adapt the model to fit varying circumstances and assumes that the more recalcitrant pool of carbon will not follow exponential kinetics. The recalcitrant carbon pool is thereby represented by a different function rather than assuming a variable rate constant. Both approaches have merit in attempting to address similar challenges in modeling disruptions in soil carbon dynamics after additions of organic residues.

To demonstrate the versatility of the FLOG model, hypothetical cumulative CO_2-C evolution and mineralization rate curves were generated by varying parameters of the logistic portion of the model, k_4 and k_5, while keeping C_1, C_3, and k_1 constant (Figure 2). Parameters C_1 and C_3 were both fixed at 0.5 μg g^{-1}, while k_1 was fixed at 0.16 d^{-1}. Changing just two parameters from the logistic model produced cumulative respiration curves ranging in appearance from almost exponential to a curve with two inflection

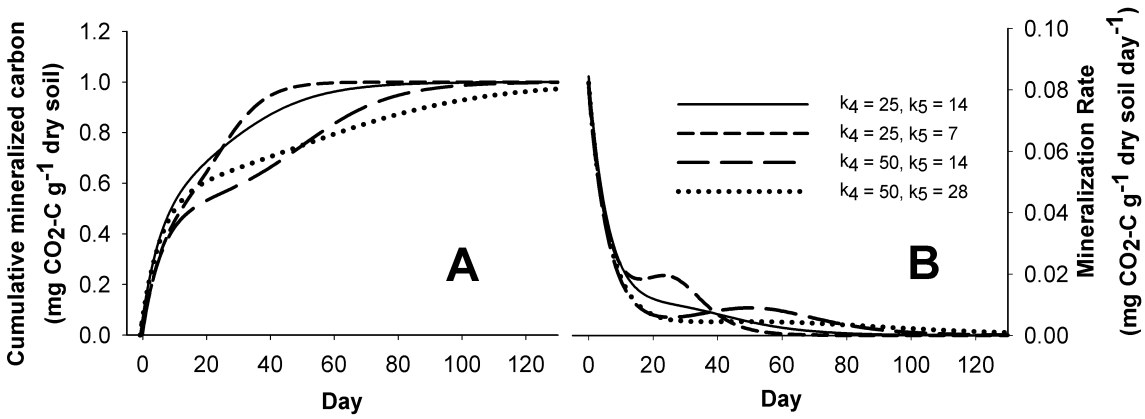

Figure 2. Simulated CO_2-C evolution (A) and mineralization rate curves (B) using the FLOG model (Table 1). Parameters C_1, C_3, and k_1 were held constant at 0.5 mg g^{-1}, 0.5 mg g^{-1} and 0.16 d^{-1} respectively, while k_4 and k_5 were varied as indicated in the legend.

points, as well as being able to highlight secondary increases (local maxima) in carbon mineralization rate curves.

Using a logistic model to describe the decomposition of a recalcitrant carbon pool appears to be a good approach to describe CO_2 evolution from amended soils. It is particularly useful where exponential models are not a good fit because of the inflection points or due to organic amendments known to contain more recalcitrant carbon sources requiring growth of specially adapted microorganisms. As this chapter shows, the FLOG model is suitable for CO_2 evolution curves that appear exponential in nature (Figure 1), as well as having the potential to fit curves containing inflection points caused by secondary increases in the mineralization rate (Figure 2). The FLOG model introduces two parameters which are unique to the logistic function and can be estimated graphically. The parameter k_4 indicates the position of the inflection point in the logistic portion, which corresponds to the peak in mineralization rate for that pool (Figure 2), while k_5 characterizes the steepness of the logistic function and is the time interval between the inflection point and 75% of the maximum respiration from this pool. This model allows the timing of a secondary respiration event to be captured and described mathematically. It may be possible to establish a relationship between these parameters and factors affecting microbial community functioning, such as temperature and soil chemistry, which may then allow these properties to be incorporated into the FLOG model. The

model could also be useful for characterizing different types of amendments with varying composition. The depolymerization of complex organic compounds has recently been identified as a likely rate limiting step in nitrogen mineralization as well (Schimel and Bennett 2004), so it may also be possible to use or adapt the FLOG model to studies on nitrogen dynamics in soil.

4. Conclusions

The objective of this chapter was to examine the carbon mineralization dynamics from a soil amended with increasing rates of an alkaline-stabilized municipal biosolid. As a result of this study, a new model describing carbon mineralization from ASB amended soils was developed. The new model, a first order exponential plus logistic function (FLOG), was based on the recent application of above ground ecological theories regarding competitive strategies among organisms to the soil environment. The FLOG model performed better than simple first order and first order plus linear models except in the unamended control soil, having a lower MSE and meeting model assumptions in all cases. The FLOG model performed well compared to other common models, with the advantage of being able to generate curves ranging from almost exponential in appearance to curves containing inflection points. This study highlights a need for future research in order to establish clear links between the timing of the delayed carbon pool and changes in soil enzyme activities, microbial community composition, or environmental parameters such as temperature and soil chemistry.

CHAPTER 3: EVALUATION OF AN ULTRASONIC EXTRACTION METHOD FOR DETERMINATION OF DIFFERENT CLASSES OF PRIORITY ORGANIC CONTAMINANTS IN AN AGRICULTURAL FIELD SOIL

1. Introduction.

Analysis of environmental contaminants is of great importance in monitoring potential impacts of human activities. Extraction of organic contaminants from solid matrices, such as soil and biosolids, generally requires intensive extraction and multi-step cleanup methods. Increasingly, a number of methods are being reported in the literature describing single extraction procedures to detect and quantify a range of organic environmental contaminants, which can potentially save time and effort (Burkhardt et al. 2005; Bossio et al 2008). Before implementing a method on a large scale however, it should be validated within the laboratory to determine expected operating characteristics, such as the linear range of analyte concentration, within- and between-run variability, and spiking/recovery performance in each matrix to which the method will be applied (Thompson et al. 2002).

It was noted in Chapter 1 that soil is a particularly difficult matrix from which to extract analytes due to the large number of reactive sites on the mineral surface and association of analytes with the heterogeneous soil organic matter. The reduced analyte recovery, a deviation from the true instrument response that would normally be observed for the quantity of analyte present, is referred to as bias and its effects should be quantified when validating a method (Thompson et al. 2002). Knowing this information enables the correction of results to reflect the reduced extractability of analytes. However, compounds introduced by spiking may not reflect the true behaviour of native analytes, and there is no consensus on whether to report corrected or uncorrected values (Thompson et al. 2002). Regardless, the recovery of spiked compounds is still an important piece of information describing the performance of a method (Thompson et al. 2002).

The precision of a method is an important parameter to document initially, for the purpose of quality control monitoring and as a measure of repeatability in the results. Many factors can cause variability in the results, including the analyst, gravimetric and

volumetric errors, and instrument calibration for example. While it is assumed that some variability exists due to uncontrollable factors, knowing the inherent variability in the analytical system can help determine if some other factor is causing additional variation in the results that should be investigated. It is also the basis for statistically derived operational parameters such as the limits of detection (LOD) and quantification (LOQ) (González and Herrador 2007). Variability in the response is also suspected to change as a function of analyte concentration, therefore it is recommended that analyte concentration be included as a factor under investigation when validating a method (Thompson et al. 2002).

There are a number of operating characteristics in a method that must be established in order to consider a method as validated (Thompson et al. 2002). The range of linearity for the relationship between analyte concentration and instrument response must be determined, and the adequacy of the calibration curve linear model must be assessed using statistical tests. This is done using a range of independently prepared standards, spaced uniformly across the concentration range being examined, and should be close to the detection limit of the instrument. According to the IUPAC Harmonized Guidelines for Single-Laboratory Validation of Methods of Analysis, six or more calibration points should be chosen, and the range of independently prepared standard concentrations investigated should be from 0 to 150% of the native concentrations expected in the chosen test material (Thompson et al. 2002). The published method upon which this study is based (Bossio et al. 2008), had method detection limits ranging between 0.06 and 0.11 $\mu g\ g^{-1}$ soil. Concentrations of 1.3 and 4.7 $\mu g\ g^{-1}$ soil of *p*-cresol and indole, respectively, were measured in a soil with a 20 year history of receiving biosolids (Bossio et al. 2008). These values were used as the basis for the expected concentration range during initial method development.

Additionally when validating a method, bias resulting from matrix effects needs to be quantified in each test material using a spiking and recovery study. Inherent variability of the method within each run and the overall variability between runs need to be determined over time with independently replicated samples that are run on several different days. Ruggedness tests should then be carried out once the method is validated, where different characteristics of the method, such as extraction time, temperature, or

other factors potentially affecting the results, are varied to quantify the effect of small changes.

This chapter describes the evaluation of an ultrasonic-assisted extraction method for the determination of seven organic contaminants from soil. This was required before implementing the method to analyze real samples. The operating characteristics such as the linear range of analyte concentration and limits of detection and quantification determined by spiked analyte recovery at levels below those detected in biosolid-amended soil are presented. Results from ruggedness tests on a number of steps in the method are shown as well. The method was chosen since it claimed to provide a rapid and cost-effective extraction of a range of analytes from soil. However, it was later discovered that some details necessary to replicate the method within our laboratory were not always clear. It should be noted that the method was not finalized during the ruggedness testing trials, which were conducted first, since we were attempting to replicate a method from a paper that was not always described clearly. Chapter 4 describes the results of dedicated studies applying the ultrasonication extraction method and GC/MS analysis to incubation and field soils amended with biosolids to more accurately capture trends over time.

2. Materials and Methods

2.1. Chemicals and Materials

The analytes *p*-cresol (99.5% purity), indole (99.0%), 4-*t*-octylphenol (99.3%), phenanthrene (98.1%), triclosan (97.0%), carbamazepine (99.0%), and benzo[*a*]pyrene (96.0%) were obtained in solid form from Sigma-Aldrich (Oakville, ON). The phenanthrene-d_{10} internal standard (98.7%) was purchased from CDN Isotopes Ltd. (Pointe-Clare, QC). Anhydrous sodium sulphate, silica sand, and HPLC grade acetone, dichloromethane, diethyl ether, and isopropanol were purchased from Fisher Scientific (Ottawa, ON). HPLC grade water was prepared using a Millipore water filtration system. Strata-X (6 mL 500 mg), Strata FL-PR (6 mL 1 g), empty polypropylene 60 mL solid phase extraction cartridges, and a 12-hole SPE manifold were purchased from Phenomenex (Torrance, CA). Oasis HLB and Oasis Florisil SPE cartridges were purchased from Waters (Milford, MA). Extracts and standards were analyzed on an Agilent 5975 GC-MS with autosampler in negative ion mode using an Agilent DB-5 30m

capillary column. Oven temperature program and operational parameters are listed in the Standard Operating Procedures (Appendix I).

2.2. Soil Sampling and Processing

Soil samples used in spiking trials were obtained from unamended control samples from the incubation study (Chapter 2). A more detailed description of soil handling in the incubation study is provided in Chapter 2. Briefly, a field soil sampled from the top 30 cm at the Bio-Environmental Engineering Centre in Bible Hill, N.S., was air dried 2 mm sieved, and re-wetted to 18% GWC prior to incubation. Soil samples were extracted wet according to the Standard Operating Procedures (Appendix I) unless otherwise noted. Analytes were identified by their retention time and the presence of the quantitative and confirmation ions, and also using a standard mass spectrum library when possible. Analytes and the internal standard in calibration solutions were all able to be identified using the library, while only triclosan was able to be identified by the library (up to 96% match after background subtraction) in biosolid-amended soils.

2.3. Ultrasonic Extraction and Cleanup

A detailed description of the method used is provided in Appendix I. Unless otherwise noted, 10g w.w of soil was placed in a 50 mL culture tube and extracted with 3×10 mL aliquots of 80:20 IPA:H_2O. Extraction was assisted by a 10 min ultrasonic bath cycle followed by centrifugation and collection of the supernatant. To the 30 mL of extract, 20 mL of a 0.1 M pH 7 phosphate buffer was added and the mixture was loaded onto a Strata-HLB column pre-conditioned with 80:20 DCM:DEE, and dried under vacuum. The loaded HLB column was stacked onto a Strata-Florisil column pre-conditioned with acetone and containing 4 g of anhydrous sodium sulphate added to the barrel. The analytes were eluted by 3×5 mL aliquots of 80:20 DCM:DEE and collected in a test tube. 10 µL of 4 mg L^{-1} phenanthrene-d_{10} internal standard were added to each test tube and evaporated to 1 mL using a nitrogen evaporator. The concentrated extracts were transferred to 2 mL gas chromatography vials and analyzed by GC-MS on the same day as extracted. Extracted ion chromatograms of the quantitation ion were used to determine concentration in the extract.

2.4. Statistical Methods

Calibration curves prepared during each separate extraction and the combined calibration curves in Figures 3 to 9 were fit using linear regression analysis in Minitab 15 (Minitab Inc., State College, USA). Analysis of ruggedness testing experiments was done separately for each compound in SAS 9.1 (SAS Institute Inc., Cary NC) using PROC MIXED with multiple means comparison by Duncan's method. The ANOVA assumption of normally distributed error terms was tested using the Anderson-Darling test and normal probability plots of the residuals, while constant variance of the error terms was checked by plotting the residuals vs. fitted values.

3. Results and Discussion

3.1. Linear Range of Analyte Concentration

The results from all calibration curves prepared during method development that used an internal standard were combined on a single graph to assess the stability of the calibration curve between runs (Figures 3 to 9). A calibration curve was prepared for each separate extraction, although the concentration range varied since the concentration of spiked samples to measure recovery in each run was lowered over time to more accurately represent concentrations observed in real samples. Even though method parameters were changed during the process, preparation of the standard curves remained consistent. Calibration curves prepared when the concentration of spiked analyte was approximately 20 ng g^{-1} are presented separately since they captured much lower concentration ranges.

Calibration curves for all compounds appear approximately linear within the concentration ranges examined (0.005 to 30 μg mL^{-1}). Studies that used a calibration curve with a lower range of concentrations (points indicated as July 7, Sept. 2, Sept. 30, and Nov. 2, in Figures 3 to 9) indicate that strong linear relationships could be obtained using the combined data for *p*-cresol, indole, 4-*t*-octylphenol, and phenanthrene, although the data for carbamazepine, triclosan, and benzo[*a*]pyrene were more variable but still linear within that range of concentrations.

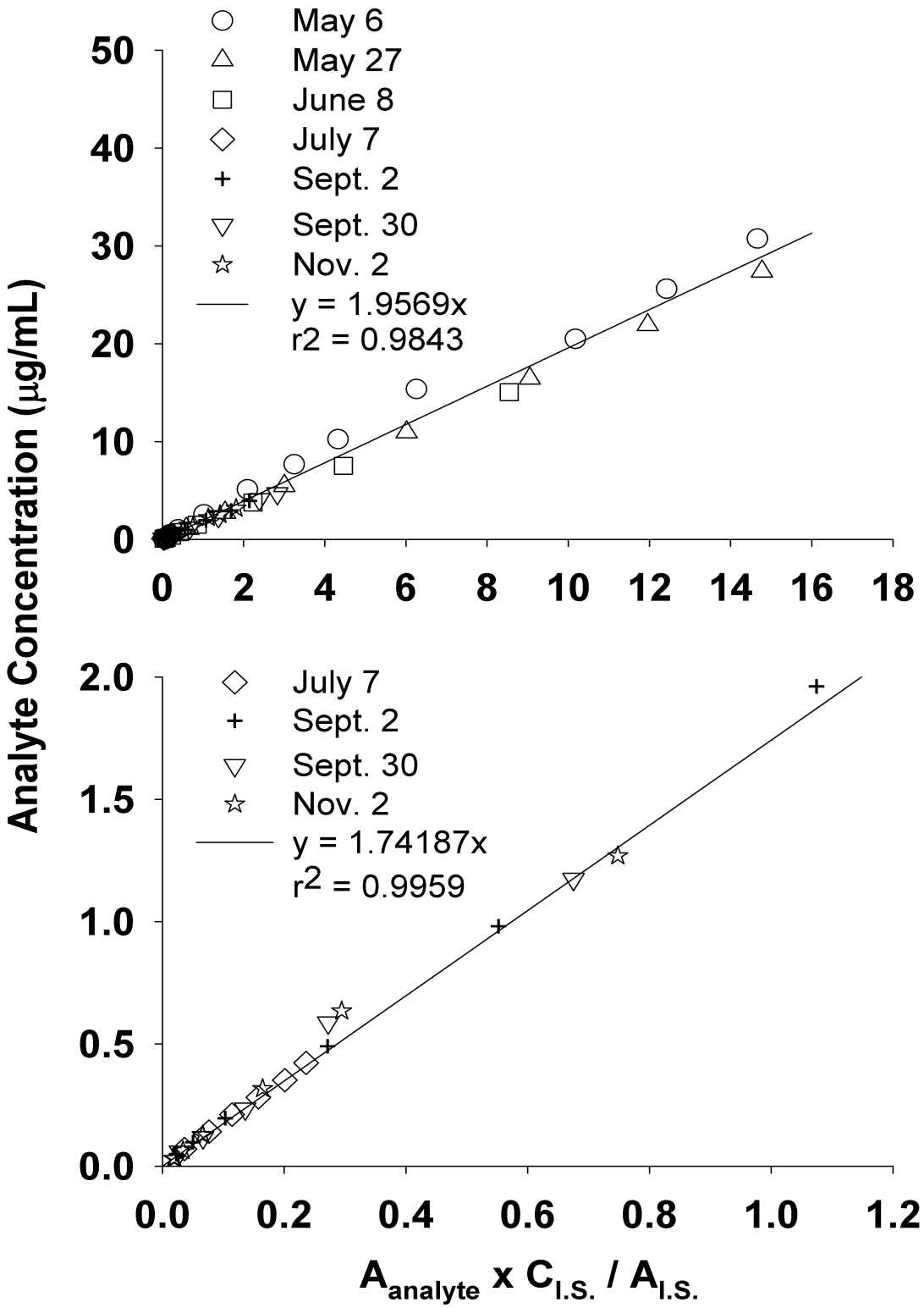

Figure 3. Calibration curve for *p*-cresol using combined data from method evaluation studies containing internal standard.

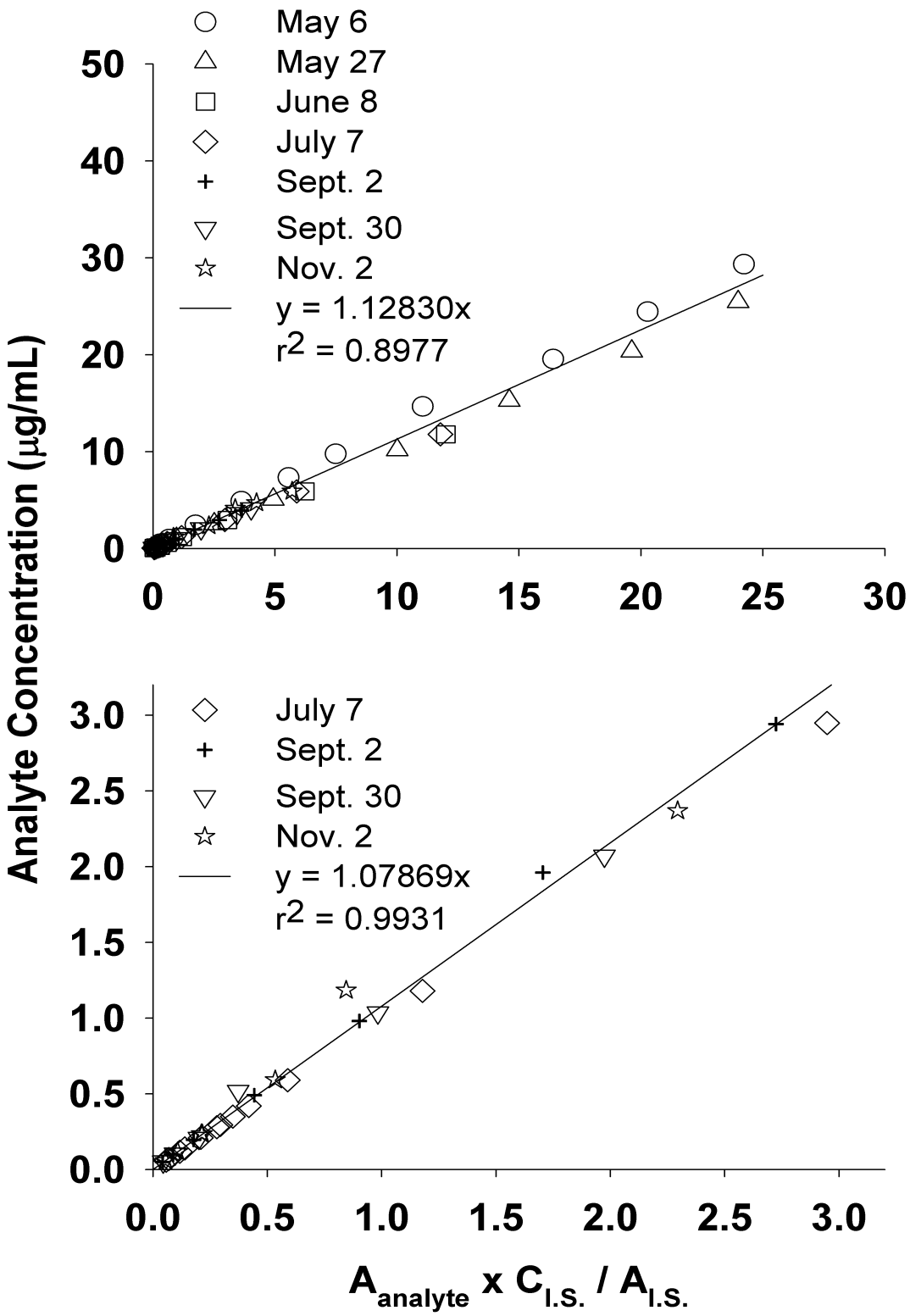

Figure 4. Calibration curve for indole using combined data from method evaluation studies containing internal standard.

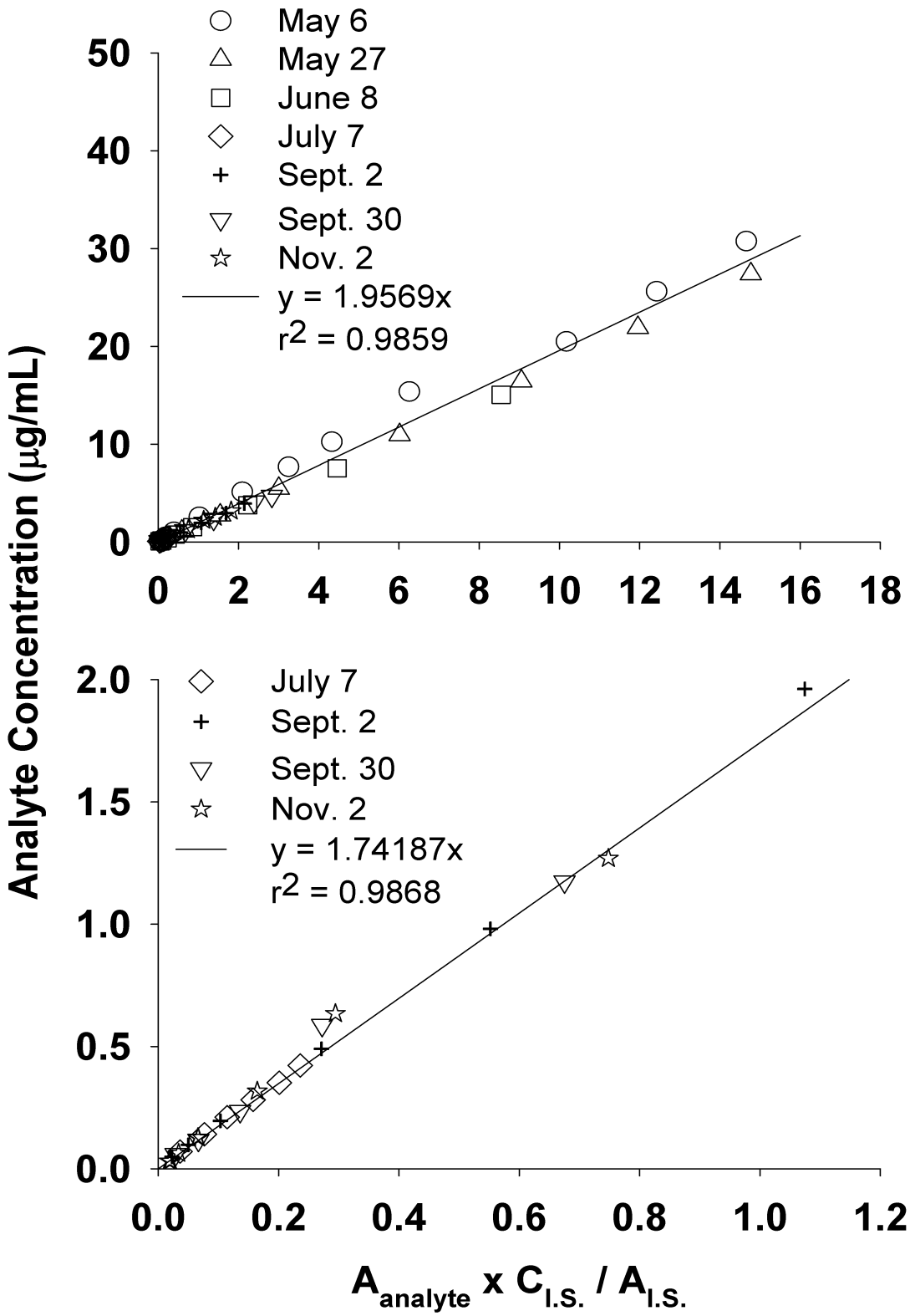

Figure 5. Calibration curve for 4-*t*-octylphenol using combined data from method evaluation studies containing internal standard.

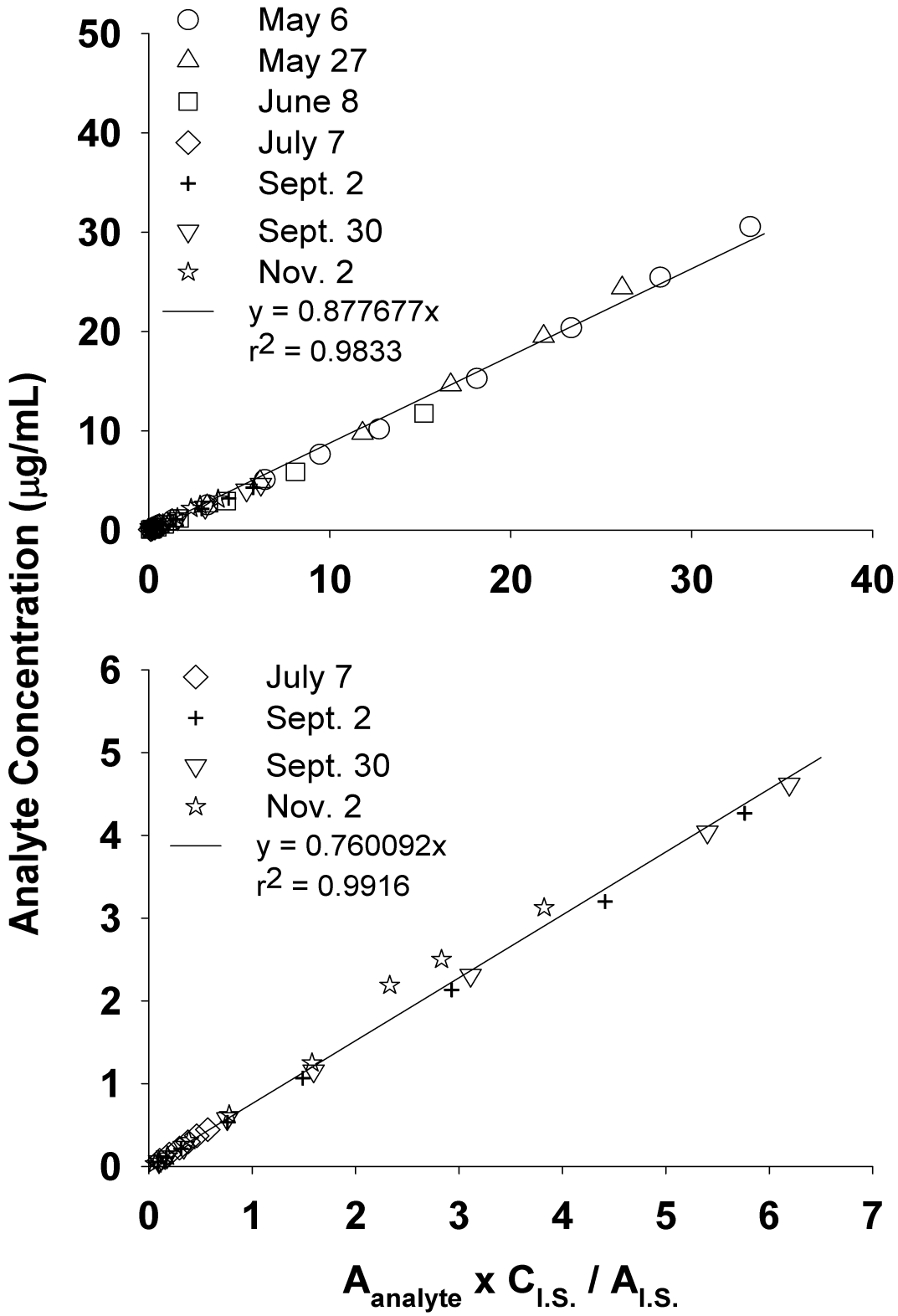

Figure 6. Calibration curve for phenanthrene using combined data from method evaluation studies containing internal standard.

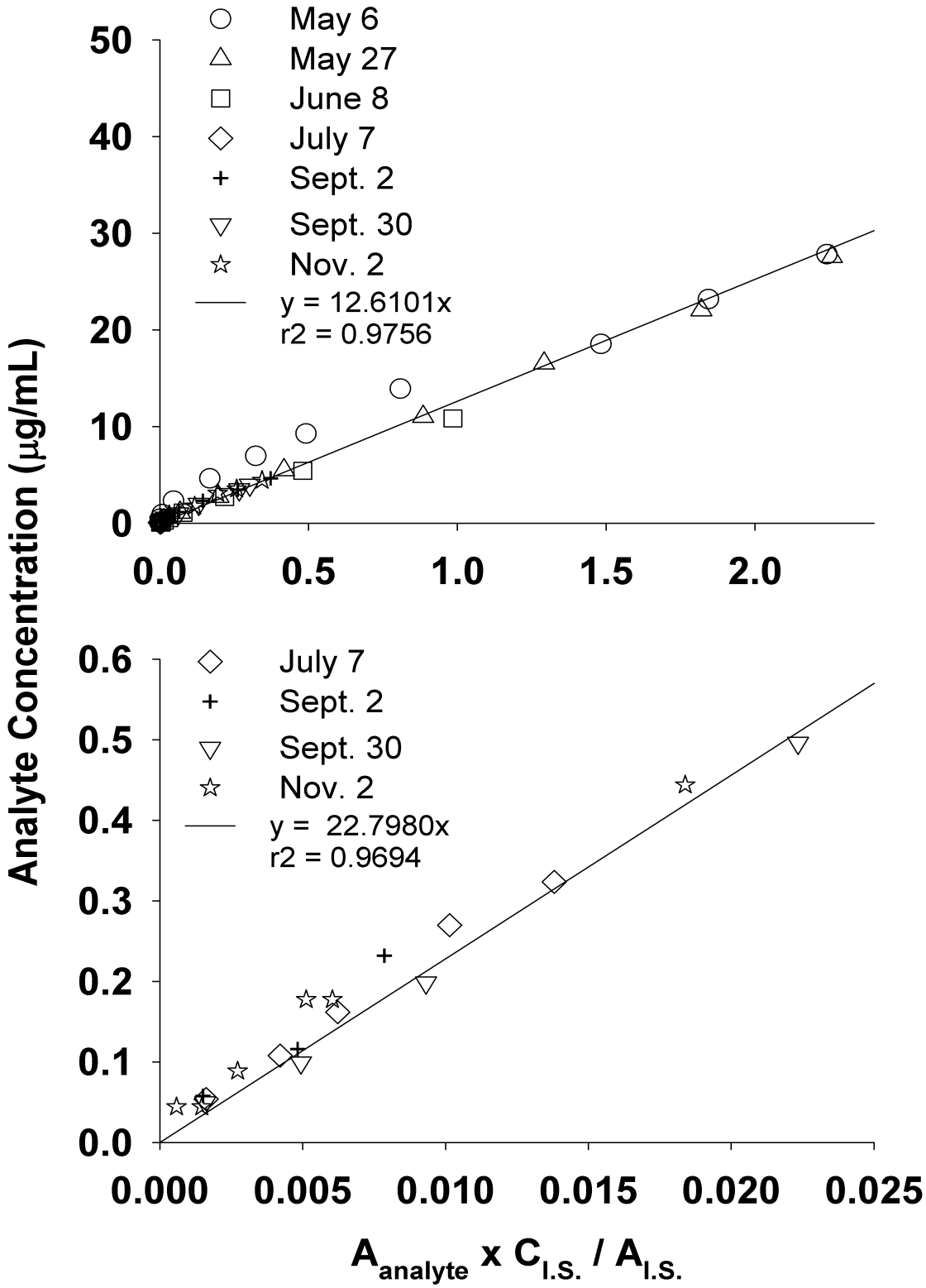

Figure 7. Calibration curve for triclosan using combined data from method evaluation studies containing internal standard.

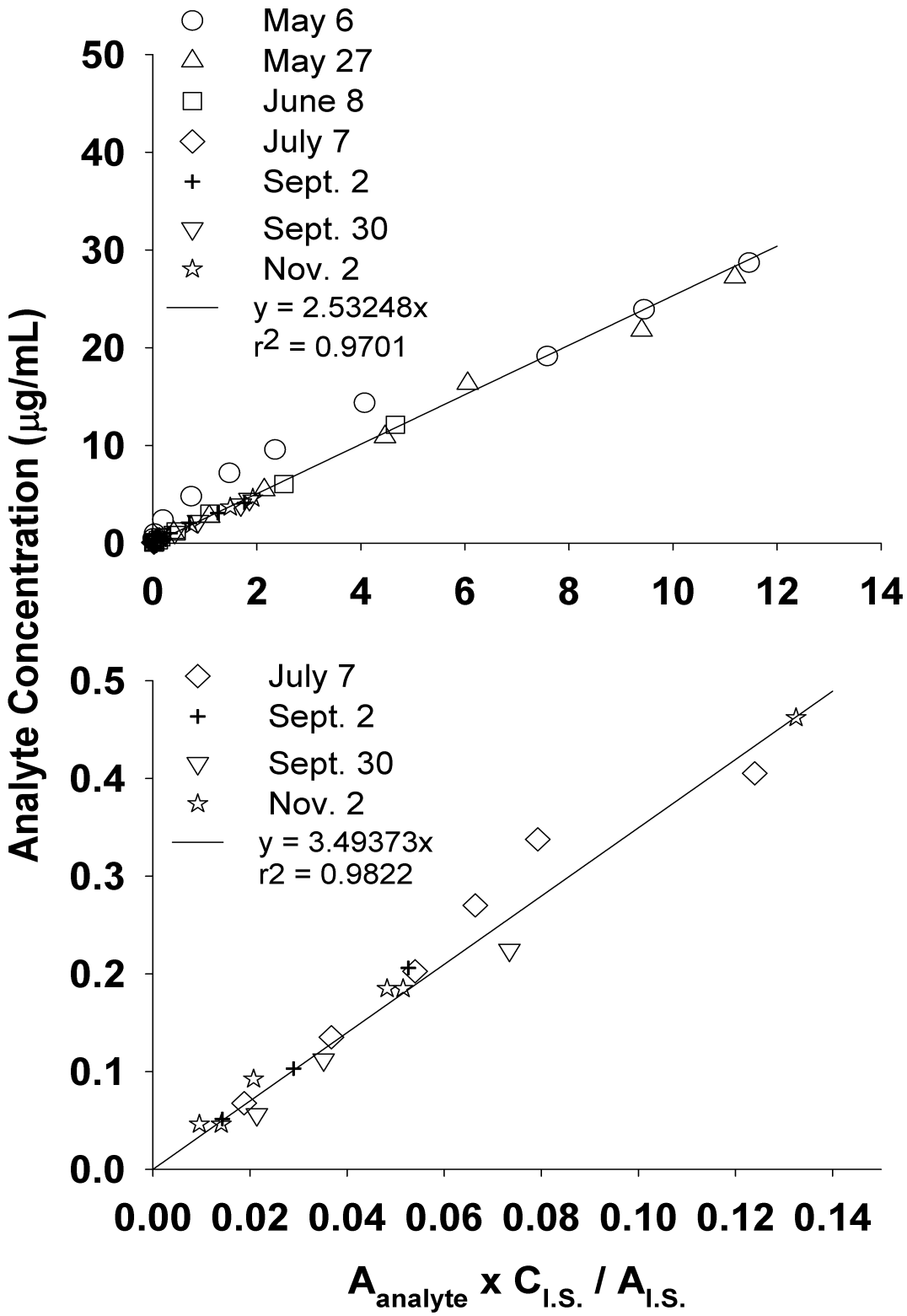

Figure 8. Calibration curve for carbamazepine using combined data from method evaluation studies containing internal standard.

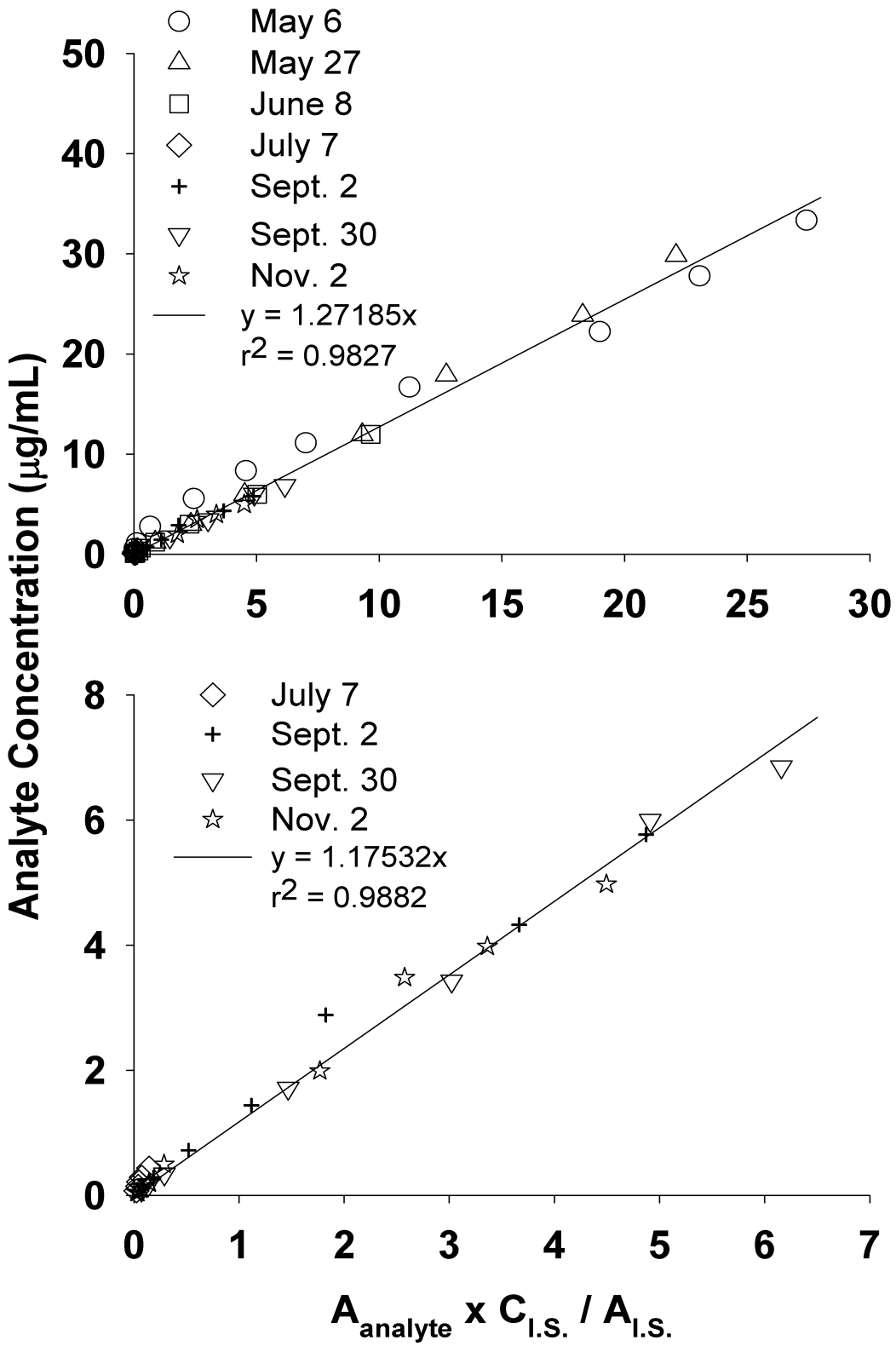

Figure 9. Calibration curve for benzo[*a*]pyrene using combined data from method evaluation studies containing internal standard.

3.2. Precision and Recovery

As part of validating a method within a laboratory, it should be documented through spiking and recovery trials that the analyst can consistently recover a known amount of analyte from soil, as well as determining the lowest level with which results can be reliably reported. To achieve this, a spiking and recovery trial in soil was designed using a concentration less than observed in soil containing biosolids during preliminary testing. Nine replicates of 10 g d.w of moist control soil were spiked at 20 to 30 ng g^{-1} soil with 1 mL of standards in acetone. Three 10 g blank control soils were prepared, although one was spilled during extraction. Samples were extracted according to the SOP.

Recovery of *p*-cresol, indole, and carbamazepine were low throughout preliminary method development, and could not reliably be determined using this method. However, the remaining compounds showed recoveries greater than 50%, although as high as 165% in the case of triclosan (Table 5). These results demonstrate that analyte concentrations listed under the LOD and LOQ for 4-*t*-octylphenol, phenanthrene, triclosan, and benzo[*a*]pyrene can be reliably detected and quantified using this method. Higher variability in recovery from spiked samples for some compounds, such as 4-*t*-octylphenol, triclosan, and benzo[*a*]pyrene, led to limits of quantification up to two times higher than spiked concentrations. Recoveries of p-cresol, indole, and carbamazepine, were less than 50% during preliminary testing, so an attempt was made to elucidate factors that may be contributing to the lower recoveries compared to those reported in Bossio et al. (2008). Percent recoveries for analytes spiked at 5 μg g^{-1} in 10 g of soil during method development in that study are: *p*-cresol 62.5, indole 81.6, 4-*t*-octylphenol 74.9, phenanthrene 56.9, triclosan 59.0, carbamazepine 67.6, benzo[*a*]pyrene 50.0. Recoveries of spiked analytes in biosolid-amended soils during application of the method to real samples were: *p*-cresol 82.7, indole 79.9, 4-*t*-octylphenol 81.0, phenanthrene 89.4, triclosan 84.2, carbamazepine 78.8, benzo[*a*]pyrene 87.6 (Bossio et al. 2008).

3.3. Ruggedness Testing

Recovery of most compounds in the first study that we conducted (data not shown) were much lower than the recoveries reported in Bossio et al. (2008) for ashed silica sand, and it was clear after the initial study that certain parameters of the extraction

Table 5
Retention time (minutes) and quantifying and qualifying ions[a] (m/z), spiking concentration, limits of detection and quantification (LOD and LOQ, ng g^{-1} soil) calculated as Mean + 3× and 10× Standard Error (n=9), and percent recovery for each compound.

	PCRE	IND	4tOP	PHE	TCS	CBZ	BaP
Retention Time	9.62	15.37	22.24	24.39	28.18	30.51	34.66
Quantitation Ion	107	117	135	178	288	193	252
Qualifying Ion 1	108	90	107	176	290	236	126
Qualifying Ion 2	77	89	-	89	218	165	250
Spiked Conc.	28	28	21	30	22	27	29
LOD[b]	31	34	26	32	25	32	39
LOQ[b]	38	47	37	38	32	50	63
Recovery (%)	17	25	54	87	165	16	104

[a] – From Bossio et al. 2008.
[b] – LOD and LOQ are based on amounts recovered corrected using recovery in spiking study.

process were not clearly described in the paper or implied and not described. This included such things as: whether the buffer was added as a mixture to the extract or used as a wash; whether analyte concentration affects recovery at environmentally relevant concentrations; if the Florisil cartridge was retaining some analytes in our situation; if the flow rate during SPE affected analyte recovery; and whether different brands of SPE cartridge (Phenomenex vs. Waters) were causing some of the deviation from the results reported in Bossio et al. (2008). A number of studies were undertaken to address these questions prior to final extraction of biosolids-amended soils.

3.3.1. Comparison of Phosphate Buffer Wash vs. Mixed With Sample

Recovery was poor for some compounds following our initial experiment, so a study was conducted to examine whether the phosphate buffer treatment (if mixed with extract or added to SPE cartridge after extract as an aqueous wash) affected the recovery of method compounds, since this was not clearly described in Bossio et al. (2008). Six 10 g samples of silica sand were weighed and spiked with 1 mL of 50 μg mL^{-1} method compounds in acetone (5 μg g^{-1} soil). The spiked samples were extracted by ultrasonic extraction, but during loading of the HLB cartridge three samples were loaded without

buffer which was added after. The other three samples were mixed with buffer before loading. Samples were extracted according to Standard Operating Procedure (Appendix I), with the following modifications: MS was operated in selected ion mode monitoring the quantitation ions of each compound; anhydrous sodium sulphate was ashed at 400 C for 4 hours; no internal standard was added to samples or standards; samples were evaporated to dryness following SPE and 1 mL of 80:20 DCM:DEE was added to each with a 1 mL glass volumetric pipette; no standard curve was prepared, samples were instead semi-quantified using a single point standard at 50 $\mu g\ mL^{-1}$; SPE flow rate was approximately 1 drop every 2 seconds; ultrasonic bath consisted of 1 cycle at room temperature and 2 cycles at 55°C.

Poor recovery was again obtained for *p*-cresol, indole, and carbamazepine (Figure 10), which indicates that the buffer treatment does not play a significant role in the poor recovery of these compounds. This left us with the following possibilities: that these three compounds are not being extracted by the solvent system, they are not being retained on the HLB solid phase extraction cartridge, that they are being retained by the Florisil cartridge, or that they are being lost during the evaporation step. A significant effect of buffer treatment on recovery was found with 4-*t*-octylphenol, with the wash treatment lowering recovery dramatically. While no statistically significant trends were observed for the remaining compounds, the wash treatment consistently lowered recovery of phenanthrene, triclosan, and benzo[*a*]pyrene by 10-20%, although high variability and a small sample size may have prevented statistical confirmation. This experiment confirmed that the proper buffer treatment was as a mix, and that acceptable recovery (between 50-90%) can be obtained for 4-*t*-octylphenol, phenanthrene, triclosan, and benzo[*a*]pyrene using this method. However, further testing was necessary to investigate other factors preventing good recovery of *p*-cresol, indole, and carbamazepine.

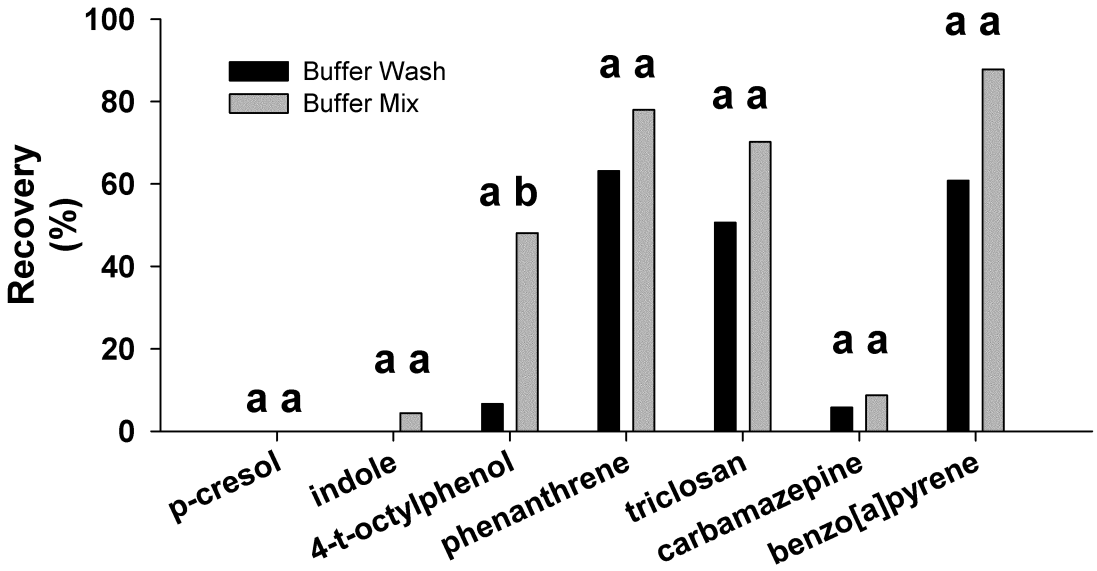

Figure 10. Comparison of buffer treatment (wash vs. mix) during extraction of sand spiked at 5 μg g^{-1}.

3.3.2. Investigating Effect of Analyte Concentration and Florisil Cartridge on Recovery

After ruling out the buffer treatment, our next attempt was a 2×2 experiment with three replicates that looked at whether the Florisil column was retaining *p*-cresol, indole, and carbamazepine, and what effect the concentration of analyte has on its recovery. Additionally, to eliminate extraction as a factor affecting recovery, two concentrations (2 and 20 μg/mL) of all seven method compounds were made up in the 80:20 IPA:H_2O solution to mimic a soil extract. However, the HLB + Florisil treatment was performed first on the SPE manifold, followed by the HLB without Florisil treatment, so the effect of any differences between runs is confounded with this treatment, and results from each are analyzed separately considering this limitation. Six 30 mL aliquots of extract mimic at each concentration were prepared in 50 mL extraction vessels, followed by 20 mL of phosphate buffer in each vessel. Twelve Supelco HLB solid phase extraction tubes were prepared and loaded with the buffered extract mimics according to the Standard Operating Procedure (Appendix I) with the following modifications: three tubes at each concentration were eluted through the Florisil cartridge as in SOP, and three tubes at each concentration were eluted without a Florisil cartridge underneath; MS was operated in

selected ion mode monitoring the quantitation ions of each compound; anhydrous sodium sulphate was ashed at 400°C for 4 hours; no internal standard was added to samples or standards; samples were evaporated to dryness following SPE and 1 mL of 80:20 DCM:DEE was added to each with a 1 mL gastight syringe; no standard curve was prepared for quantitation, instead samples were semi-quantitated using a single point standard at 20 ug/mL; SPE flow rate was approximately 1 drop every 2 seconds; ultrasonic bath consisted of 1 cycle at room temperature and 2 cycles at 55°C.

Much greater recoveries were obtained for *p*-cresol, indole, and carbamazepine than in the previous studies (Figure 11), indicating that the extraction portion of sample processing may play a significant role in the reduced recovery of these three compounds. There is also a significant effect of concentration on recovery of some compounds, with higher concentration leading to higher recovery for *p*-cresol, indole, 4-*t*-octylphenol, and phenanthrene in both Florisil treatments. However, the opposite effect was observed for triclosan in the No Florisil treatment and for carbamazepine in both Florisil treatments, but the magnitude of the effect was generally small (<10% difference in percent

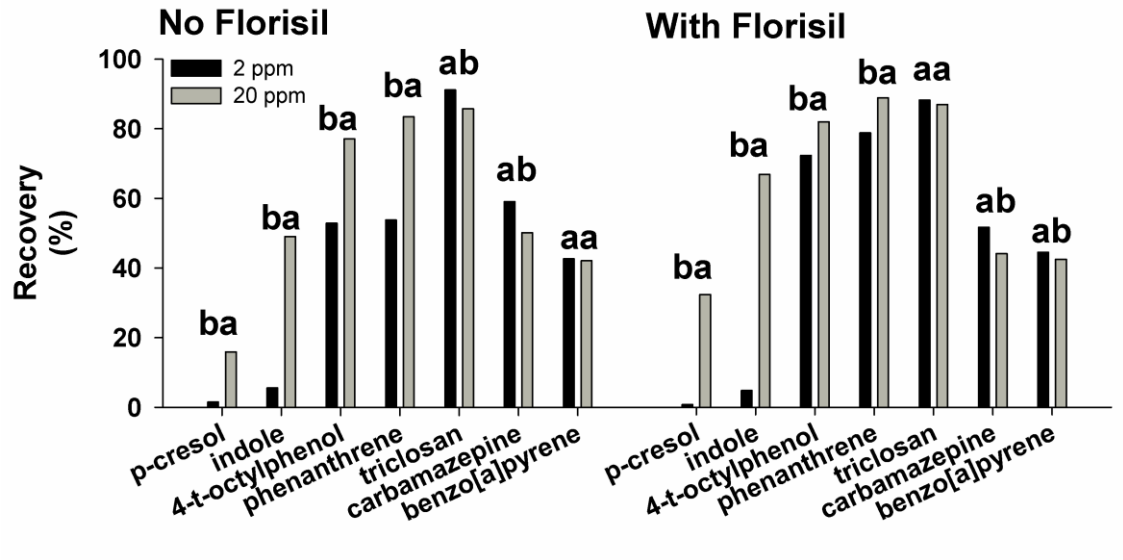

Figure 11. Effect of concentration on analyte recovery from spiked extraction solvent with and without Florisil cartridge cleanup.

recovery). There was also a noticeable decrease in the percent recovery of benzo[*a*]pyrene in this run compared to the first. This may be due to the spiked extraction solvent used in this study, which was prepared several days before it was used. It is possible that benzo[*a*]pyrene adsorbed to the container walls during that time since the solvent is relatively polar and benzo[*a*]pyrene is very hydrophobic.

3.3.3. Effect of SPE Cartridge Brand and Spiking Concentration on Percent Recovery

Throughout these method development trials we attempted to replicate a method from the literature for use in our laboratory. However, we chose a different HLB cartridge brand based on the cost relative to the number of samples being analyzed. An experiment to test the ruggedness of this method by changing the cartridge type and spiking concentration was performed according the SOP with no modifications. Treatments consisted of two spiking concentrations (0.2 and 2 μg/g soil) and two cartridge brands (Waters, Phenomenex) with three replicates. Since there are only 12 positions on the SPE manifold, no control soil was extracted along with samples. Average abundances for the control from previous trials, which are quite similar between runs, are subtracted from each value instead.

The two cartridge brands performed similarly, with only indole showing an effect of cartridge type at the lowest concentration (Figure 12). The effect of concentration on the recovery of most compounds was significant and quite large in some cases. Lower concentrations showed higher recoveries except in the case of phenanthrene, however, there was no representative control soil extracted along with these samples so this effect may also be due to the background abundance being different from the average value used. No analytes were detected in the solvent blank, indicating the glassware was clean.

3.3.4. Biosolids-Amended Soil Samples From Incubation.

Recovery of four out of seven compounds in all previous trials was generally good, so the decision was made to use this method to extract biosolids-amended soils to determine if any of the analytes with good recoveries were present in our unknown samples. For all of the studies biosolid-amended soil was extracted, an actual calibration

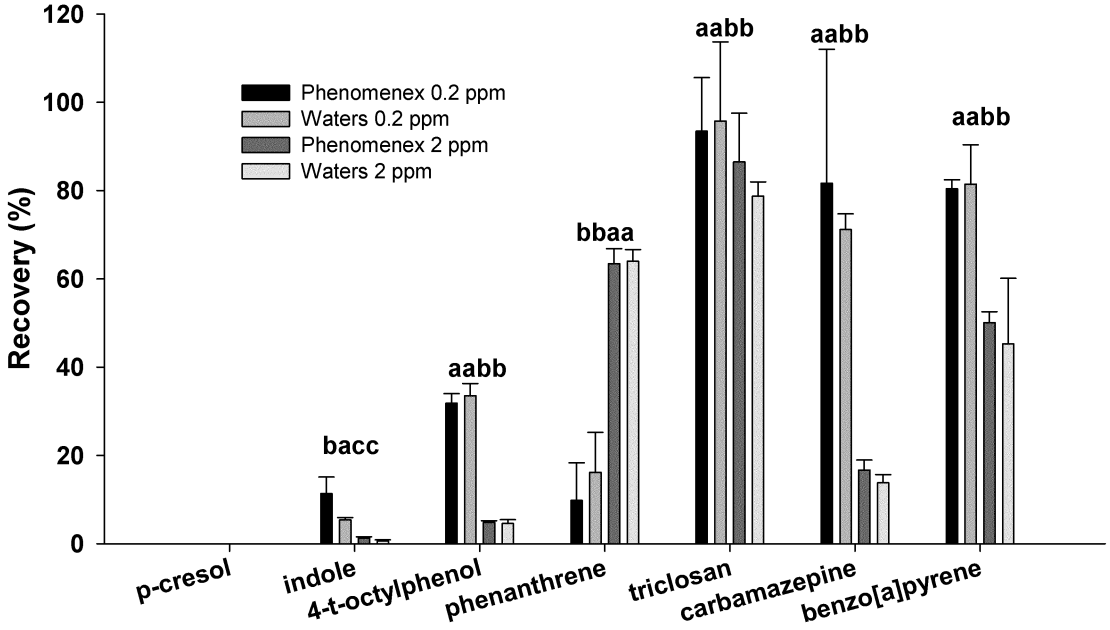

Figure 12. Comparison of Phenomenex vs. Waters SPE cartridges at two soil spiking concentrations (0.2 and 2.0 μg g^{-1} soil)

curve was constructed although an internal standard was not used for the first experiment. In total, soils from three time periods were extracted on four separate days.

Soil from incubation samples on day 0 from all amendment rates (0, 2.08, 4.16, 8.32, and 12.48 mg BS g^{-1} soil d.w.) were extracted along with four spiked control soils according to the Standard Operating Procedure (Appendix I), with the following modifications: MS was operated in selected ion mode monitoring the quantitation ions of each compound; no internal standard was added to samples or standards; samples were evaporated to dryness following SPE and 1 mL of 80:20 DCM:DEE was added to each with a 1 mL gastight syringe; SPE flow rate was approximately 1 drop every 2 seconds; ultrasonic bath consisted of 1 cycle at room temperature and 2 cycles at 55°C.

Samples from all amendment rates on day 3 were extracted on a separate day along with four samples of spiked control soil. It was during this study that an internal standard method was used for the first time. Samples were extracted according to the SOP with the following modifications: flow rate of SPE was approximately 1 drop every 2

seconds; stock solution used was several weeks old; samples were evaporated to dryness following SPE and 1 mL of 80:20 DCM:DEE was added to each with a 1 mL gastight syringe; ultrasonic bath consisted of 1 cycle at room temperature and 2 cycles at 55°C.

Samples from amendment rates 0, 4.16, 8.32, and 12.48 mg BS g^{-1}soil from day 3, plus four spiked controls, were re-extracted on a separate day according to the SOP with the following modifications: ultrasonic bath consisted of 1 cycle at room temperature and 2 cycles at 55°C.

Samples from amendment rates 0, 8.32, and 12.48 mg BS g^{-1}soil from day 6 were extracted on a separate day along with four control soils spiked 72 hrs and four spiked 1 hr prior to extraction. Samples were extracted according to the SOP with no modifications.

Triclosan was the only analyte detected in the incubation soils in all extractions, and an amendment rate effect on abundance was observed after subtracting background abundance of the control soils during the first two time periods (data not shown). However, only abundances from the highest amendment rate were captured within the linear range of the calibration standards. A general decrease in triclosan concentration over time was observed (Figure 14). Confirmation of triclosan was obtained by identification of quantifying and qualifying ions and comparison of the mass spectrum at the appropriate retention time in each sample with those from calibration standards. In

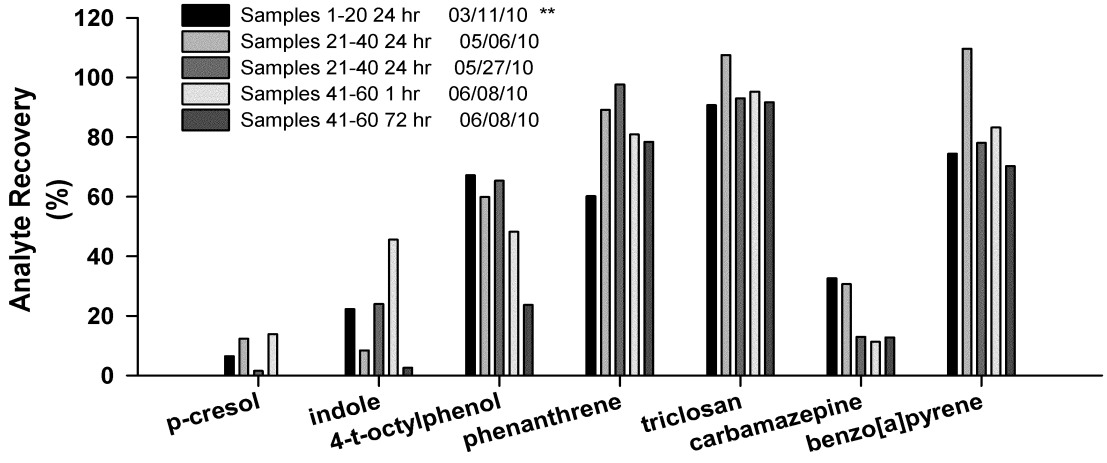

Figure 13. Percent recovery from spiked soils during extraction of incubation samples. ** No internal standard was used during sample preparation

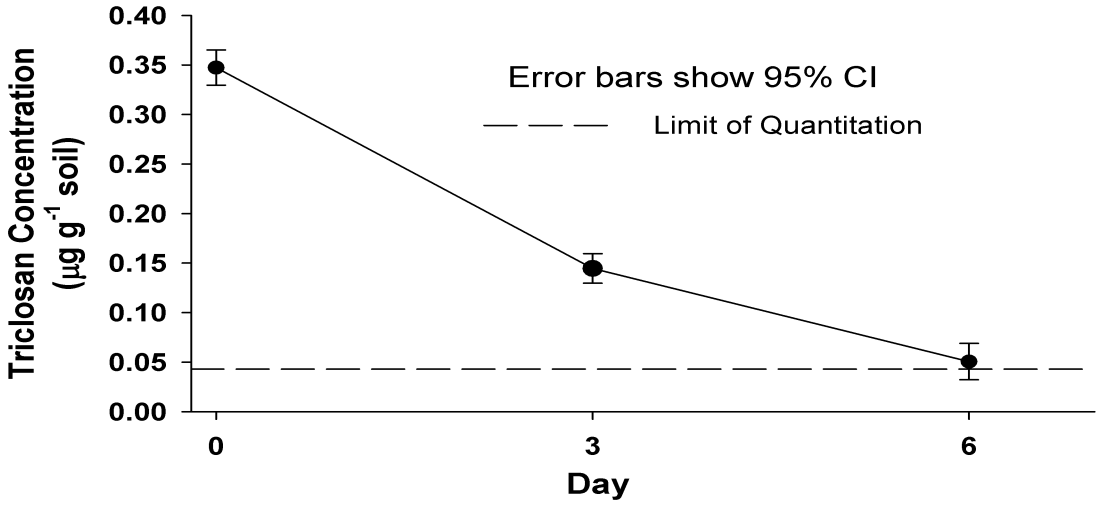

Figure 14. Triclosan concentration over time from incubation soil amended with 12.48 mg g^{-1} d.w. of ASB. Samples were extracted on separate days (Legend, Figure 13). Data for Day 3 is from mean of two separate extractions on the same soil. No internal standard was added in the Day 0 extraction.

addition, a standard reference library was used to identify analytes, which showed a greater than 95% match for all calibration standards but only triclosan was able to be detected using the library in amended soils. The data indicates that triclosan is present in biosolids-amended soils at detectable levels, and that triclosan may be removed by some mechanism (sorption or degradation) over time. Also, based on the direct comparison between 1 and 72 hrs prior to extraction, the time between spiking and extraction has a detrimental effect on recovery for some compounds, particularly for *p*-cresol, indole, 4-*t*-octylphenol, and benzo[*a*]pyrene (Figure 13). Failure to extract *p*-cresol, indole, and carbamazepine from soil using this method appears to be the primary reason for their low recoveries, and a reduction in recovery with increased time after spiking (for compounds such as *p*-cresol, indole, 4-*t*-octylphenol, and benzo[*a*]pyrene) indicates that interaction with components of the soil (degradation, sorption) could play a role in the reduced extraction efficiency of these compounds. To conserve resources, only samples from the control and highest amendment rates were extracted in subsequent runs.

3.4. Extraction and analysis of alkaline-stabilized biosolid

The method selected from the literature was developed to extract contaminants from soil, so an attempt was made to apply the method to the ASB since contaminants were expected to be in higher concentrations than soil. Two grams of dry ASB were extracted instead of 10 g soil, following the SOP. During the loading phase of the SPE cleanup however, most of the samples gelled in the tube and would not go through, even under the highest vacuum. Two samples worked (from biosolids that sat in an outdoor covered pile over-winter) but were not injected into the GC-MS to protect the instrument, since the quality of the sample cleanup may have been compromised. Samples from two batches of the biosolid were sent away for analysis by an independent laboratory (RPC, Moncton NB). Results are presented in Table 6.

Of the seven compounds, only 4-t-octylphenol and carbamazepine were not detected in the RPC analysis (Table 6). Recoveries from soil were higher than in our results for *p*-cresol, indole, and carbamazepine, but lower for phenanthrene and triclosan. Benzo[*a*]pyrene and 4-*t*-octylphenol showed similar recoveries from soil between their laboratory and ours.

Table 6
Summary of alkaline-stabilized biosolid samples analyzed by RPC Laboratory using GC-MS. Values listed are concentration ($\mu g\ g^{-1}$) with standard error listed in brackets. Within each compound, a different letter grouping indicates a difference in analyte concentration between ASB batches at a 95% confidence level. Recovery is based on spiked soil samples. ND = Not Detected.

Sample	PCRE	IND	4tOP	PHE	TCS	CBZ	BaP
1	8.3 (1.5)	3.5 (0.5)	ND	0.7 (0.2)	1.8 (0.2)	ND	0.1 (0.02)
2	47 (2.4)	1.8 (0.8)	ND	0.6 (0.1)	2.8 (0.2)	ND	0.3 (0.1)
Reporting Limit	0.50	0.50	0.50	0.05	0.05	0.50	0.05
Recovery (%)	55	60	51	47	74	69	78

4. Conclusions

A linear range of analyte concentration was established for all analytes targeted by this method. The method was able to reliably extract 4-*t*-octylphenol, phenanthrene, triclosan, and benzo[*a*]pyrene from soil spiked with standards and achieve recoveries greater than 50%. Three compounds reported to be recoverable by Bossio et al. (2008) using this method (*p*-cresol, indole, and carbamazepine) were not able to be recovered reliably during any of the experiments presented here. The extraction step appears to be a major source of analyte loss for *p*-cresol, indole, and carbamazepine, since mimicking the extract using spiked 80:20 IPA:H_2O improved recovery. Other important factors affecting the recovery of spiked analytes appear to be the spiking concentration and time between spiking and extraction, while the SPE cartridge brand did not affect the recovery, and the Florisil cartridge was not found to retain analytes. During extraction of incubation soils, only triclosan was able to be detected at levels above the LOQ and also be captured on the calibration curves. For this reason, the next chapter focuses only on triclosan concentration in soil from the incubation study and field samples.

CHAPTER 4: TRICLOSAN CONCENTRATION OVER TIME IN AN AGRICULTURAL SOIL AMENDED WITH A HIGH RATE OF AN ALKALINE-STABILIZED BIOSOLID

1. Introduction

Biosolids produced from municipal wastewater treatment processes have been used as soil amendments in North America for decades. Over the past twenty years, detection and quantification of toxic and priority pollutants, including flame retardants, pharmaceuticals and personal care products (PPCPs), and other compounds in biosolids and in amended soils (Webber and Lesage 1989, Pham and Proulx 1997, Bright and Healey 2003, Kinney et al. 2006), as well as surface and subsurface drainage water (Topp et al. 2008, Lapen et al. 2008), has led to increased regulation of these residuals in some regions by setting limits on specific contaminants (NSE 2010). In Nova Scotia, revised guidelines for storage and land-application of biosolids require reporting of industrial chemicals, alkylphenols and ethoxylates, flame retardants, pharmaceutical compounds, hormones and steroids, personal care products, and other substances in Class B (but not Class A) biosolids destined for land application, and set limits of 0.00017 and 0.8 mg kg^{-1} for dioxins/furans and PCBs, respectively, in Class A biosolids (NSE 2010). Also in 2010, the Canadian Council of the Ministers of the Environment (CCME) released an assessment of emerging contaminants in biosolids produced at eleven WWTPs in Canada (CCME 2010). Their data showed multiple emerging contaminants in biosolids from each WWTP tested, and found triclosan in every biosolid at concentrations generally in the range of 1 to 20 µg g^{-1}, and as high as 39 µg g^{-1} (CCME 2010). This suggests a significant route for transfer of triclosan, as well as other contaminants, into agricultural soils in Canada receiving biosolids from WWTPs. Lozano et al. (2010) reviewed a number of studies on triclosan fate during wastewater treatment, finding high removal rates (generally >90%) although it was not clear whether the mechanism was through degradation or sorption. The physico-chemical properties of triclosan (aqueous solubility = 12 mg L^{-1}, log k_{ow} = 4.8, log k_{oc} = 3.8-4.0) suggest sorption to organic matter in biosolids as a significant removal mechanism (Lozano et al. 2010). However, Heidler and Halden (2007) performed a mass balance study on triclosan fate in a WWTP and found

that although there was a 98% reduction between influent and effluent, almost half of the triclosan entering the plant was unaccounted for and assumed to be degraded.

Triclosan has drawn much attention as an environmental contaminant due to its prevalence in consumer products and frequent detection in surface water bodies (Kolpin et al. 2002). It is an antimicrobial compound that inhibits microbial lipid synthesis by targeting several genes that produce an enzyme (enoyl reductase) important in lipid synthesis, although mutations to these genes can impart triclosan resistance to bacteria possessing the mutated form (McMurry et al. 1998, Meade et al. 2001). Negative effects on aquatic organisms have been observed following exposure to various concentrations of triclosan. Orvos et al. (2002) detected a decrease in algae biomass and also showed a decrease in early life stage fathead minnow (*Pimephales promelas*) survival at triclosan concentration of 71 μg/L. They also observed sub-lethal effects on fish that included jaw locking, erratic swimming behaviour, and loss of equilibrium (Orvos et al. 2002). Other researchers observed slower hatching and lower percent hatchability in medaka (*Orzias latipes*) fish eggs exposed to an aqueous triclosan concentration of 313 μg/L, and zero hatchability at concentrations $\geq$625 μg/L (Ishibashi et al. 2004). Production of hepatic and gonadal vitellogenin in male and female medaka increased at concentrations of triclosan between 20-200 μg L^{-1} (Ishibashi et al. 2004).

One field study on triclosan fate in agricultural soils was conducted on 26 fields in northern Virginia that received either no biosolids, one year of biosolids application, or two or more years of application (Lozano et al. 2010). Biosolid application rates varied among farms, but the results showed no significant difference in triclosan concentration between control fields and those receiving a single application of biosolids, while a slight but significant increase (6.6 ng g^{-1}) was found in soils with single vs. multiple applications. Using initial Predicted Environmental Concentrations based on concentration in biosolids and the amendment rate from single applications, they estimated that 78% of triclosan was removed from soil after 7 to 9 months, and up to 96% removed after 16 to 21 months (Lozano et al. 2010). The authors stress the need for more detailed studies on degradation pathways and transformation products, which their study did not address. They report that this data, accompanied by the bioaccumulation of triclosan in earthworms after 31 and 156 days observed by Kinney et al. (2008), suggests

there may be an initial risk to organisms up to 16 to 21 months after biosolids application, which diminishes as soil concentrations approach background levels (Lozano et al. 2010). This chapter presents the soil triclosan concentrations from a four month soil incubation and field data from a soil amended annually with an alkaline-stabilized biosolid over a two year period. The field data incorporates two biosolid applications (Fall, Spring) over two years of sampling, while the incubation attempts to capture the dynamics of triclosan in soil under controlled conditions to minimize environmental variability.

2. Materials and Methods

2.1. Chemicals and Materials

Triclosan (97.0% purity) was obtained in solid form from Sigma-Aldrich (Oakville, ON). The phenanthrene-d_{10} internal standard (98.7%) was purchased from CDN Isotopes Ltd. (Pointe-Clare, QC). Anhydrous sodium sulphate, silica sand, and HPLC grade acetone, dichloromethane, diethyl ether, and isopropanol were purchased from Fisher Scientific (Ottawa, ON). HPLC grade water was prepared using a Millipore water filtration system. Strata-X (6 mL 500 mg), Strata FL-PR (6 mL 1 g), empty polypropylene 60 mL solid phase extraction cartridges, and a 12-hole SPE manifold were purchased from Phenomenex (Torrance, CA). Extracts and standards were analyzed on an Agilent 5975 GC-MS with autosampler in negative ion mode using an Agilent DB-5 30 m × 0.25 mm × 0.25µm 5% phenyl methylpolysiloxane capillary column. Oven temperature program and GCMS operational parameters are listed in the Standard Operating Procedures (Appendix I).

2.2. Soil Sampling and Processing

Soil was sampled from a field study using N-Viro Soil™, an alkaline-stabilized biosolid (ASB), as a soil amendment at the Bio-Environmental Engineering Center (BEEC) in Bible Hill, N.S., using annual amendment rates of 0, 7, 14, 28, and 42 t ha^{-1} ASB wet weight, split equally between Fall and Spring applications. Only samples from the highest amendment rate (42 t ha^{-1}) were analyzed for organic contaminants since the highest concentrations detected in the incubation soils during method development were found at this rate. ASB was broadcast manually across 5 m × 12 m plots, hand raked to

ensure even distribution, and incorporated by a tractor-mounted rototiller. ASB originated from different batches for each application. An aggregate sample of six Oakfield soil cores were taken from the top 15 cm in each plot, which were stored in a cooler with ice packs after sampling until returning from the field. Samples were placed in a freezer as soon as possible and stored until analysis. To avoid cross-contamination from the soil probe between plots, four cores were taken from the buffer area between plots to clean the probe before sampling the next plot. Samples taken from control plots each day serve as field blanks. Prior to extraction, field soil was not sieved; instead it was thawed until workable and mixed thoroughly before taking 5 and 12 g wet weight sub-samples respectively for moisture content and extraction. Incubation soil was obtained from the BEEC field at the start of the field trial but outside of the plot areas. Incubation samples extracted for organic contaminants were taken from the amendment rate equivalent to the 42 t ha^{-1} field samples (12.48 mg g^{-1} d.w) on days 0, 3, 6, 15, 55, and 121. A more detailed description of soil handling in the incubation study is provided in Chapter 2. Soil samples were extracted wet according to the Standard Operating Procedures (Appendix I).

2.3. Method Performance

During the extraction of incubation and field soils, soil from control treatments were spiked at approximately 20 ng g^{-1} soil to monitor recovery and method performance. The percent recoveries for triclosan in the incubation and field samples were 156% and 134% respectively. Triclosan was consistently detected at levels above the LOD and LOQ (25 and 32 ng g^{-1} respectively, from Chapter 3) in treated soils and also captured on the linear range of the calibration curve. Identification was achieved by comparing the retention time of the unknown peak to the calibration standard, and using the quantitation ion of 288, with qualifying ions of 290 and 218 to confirm identification. Additionally, triclosan was able to be identified with greater than 95% match in amended soils after background subtraction using the mass spectral library included with the instrument.

2.4. Statistical Methods

Incubation soils sampled over time were analyzed using Repeated Measures Analysis as part of PROC MIXED in SAS 9.1 (SAS Institute Inc., Cary NC). The best covariance structure, selected based on the lowest Aikake's Information Criterion (AIC), was Unstructured. ANOVA assumptions of normality and constant variance of the error terms were tested using normal probability plots and the Anderson-Darling test for normality, and scatter plots of the residuals vs. fits and vs. time to check constant variance. No transformation was required to meet model assumptions.

3. Results and Discussion

3.1. Triclosan Concentration in Soil

The concentration of triclosan measured over time in incubation soil ranged from as high as 170 ng g^{-1} on day 3 to 20 ng g^{-1} by the end of the incubation. There was a non-significant increase in the average concentration between day 0 and 3, but the average concentration decreased between each of the following time periods (Figure 15). There was an 82% reduction in triclosan concentration between the highest average levels measured on day 3 and lowest on day 121. The predicted concentration range of triclosan following application of the alkaline stabilized biosolid, based on the 12.48 mg ASB g^{-1} soil amendment rate and a triclosan concentration range of 4800 to 6500 ng g^{-1} ASB (CCME, 2010), is approximately 60 to 80 ng TCS g^{-1} soil. The highest concentrations measured in incubation soils are up to twice as high as predicted, although within an order of magnitude. When measured values are corrected based on the percent recovery, initial concentrations are quite close to the predicted range (not shown). For additional confirmation of these results, soil samples from days 0 and 121 from the incubation were analyzed by an independent laboratory (RPC, Moncton NB). Triclosan was detected in two of three samples from day 0 (in μg g^{-1}; RPC: 0.067, Us: 0.092, 136% difference; RPC: 0.194, Us: 0.134, 69% difference) but none from day 121. Their reporting limit was 0,05 μg g^{-1} however, which would have excluded concentrations from day 121 since our results indicate they are lower than the reporting limit. There was a rapid decrease in triclosan concentration after the third day of the incubation, although the study was not designed to investigate different removal mechanisms. Rapid carbon mineralization

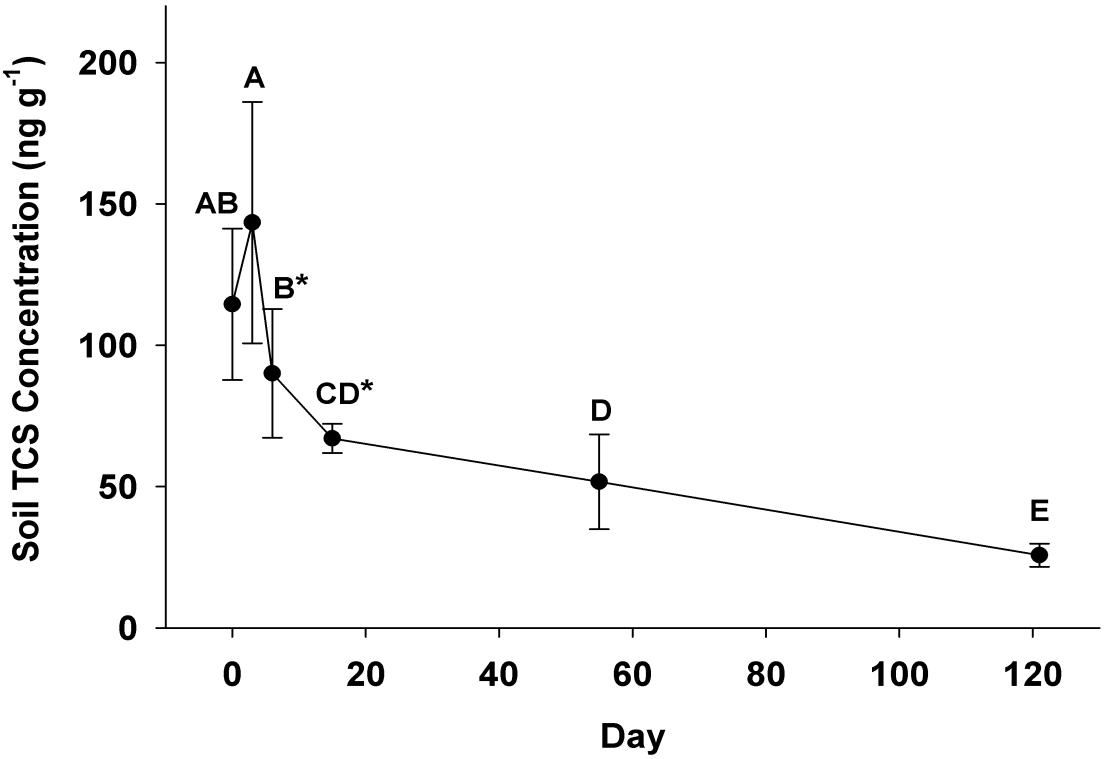

Figure 15. Triclosan concentration over time in amended incubation soils. Error bars show 95% C.I., and time points with the same letter are not significantly different based on repeated measures analysis.
* - p-value for difference of least squares means is 0.0524, or marginally significant.

Table 7
Triclosan concentration (ng g^{-1}) in the top 15 cm of field plots receiving annual biosolid applications. Application rate was 42 t ha^{-1} split equally between applications in Oct. 2008 and June 2009. Limits of detection (LOD) and quantification (LOQ) were 25 and 32 ng g^{-1} respectively; ND means not detected, and * indicates LOD < Conc. < LOQ.

Plot	May 08	Sept 08	Nov 08	May 09	July 09	Oct 09
6	ND	ND	32	29*	48	ND
23	ND	ND	47	66	33	ND
32	ND	ND	29*	ND	ND	ND

within the first few weeks in the same incubation study discussed in Chapter 2 may indicate that microbial transformation could be an important pathway for triclosan removal. However, no transformation products were identified, and without chemically labelled substrates to measure mineralization, microbial degradation of triclosan could not be confirmed.

A slight but non-significant increase in mean triclosan concentration was detected between days 0 and 3 in the incubation study (Figure 15). Although not statistically significant in this study, similar effects have been shown for other compounds during bioremediation of contaminated soil. For example, Miller et al. (2004) detected increases in the amount of pentachlorophenol extracted from a contaminated soil originating from a wood treatment facility. These increases were attributed to the production of biosurfactants by microorganisms, which release bound analytes in aged soil that are sorbed to organic matter (Miller et al. 2004). This may also be the case in our study, however, the time-scale of the increases were in the order of weeks to months in the study by Miller et al. (2004), whereas in this study the increase occurred within three days. Triclosan was added to soil sorbed to the organic matter in the biosolid, and almost 20% of the biosolid carbon added had been mineralized to CO_2-C after 3 days. The organic matter composition in biosolids presumably reflects the human diet and may consist of indigestible plant and animal residues including structural polymers like lignin, cellulose, and other polysaccharides, undigested protein and fats, polyphenolics, and other components and food additives not absorbed by the body during digestion (Saura-Calixto 2011). Plant residues contain a wide variety of functional groups that are capable of interacting with both polar and non-polar analytes (Chen et al. 2005).Therefore, it is also possible that the reduced amount of organic matter contributed to the higher mean triclosan concentrations observed on day 3 compared to day 0 in our incubation.

Triclosan concentrations detected in field soils were lower than in the incubation samples (Table 6), although the field application was split 50:50 between a Fall and Spring application. Incubation amendment rates were matched to the field, assuming incorporation into the top 15 cm of soil and a bulk density of 1600 kg m^{-3}. The amendment rate for the incubation was equivalent to 42 t ha^{-1}, which is three times the recommended rate for agriculture of 14 t ha^{-1}, while the field received two applications

(Fall, Spring) of 21 t ha^{1}, both representing 1.5 times the recommended rate. Also assuming that triclosan concentration in the biosolids is consistent between batches, the predicted concentration in field soil are approximately 30 to 40 ng g^{-1} after the first application, and 60 to 80 ng g^{-1} after the second application based on the range of values reported for N-Viro Soil™ by the CCME (2010). While measured triclosan concentrations after the first application in Fall are similar to the predicted (Nov. 08, Table 6), the concentrations measured after the Spring application (July 09, Table 6) are lower than expected, and decrease to levels below the detection limit by the end of the season. In most cases soil samples from the field were not taken until several weeks after biosolids application. The dynamics from the incubation study suggest that much of the triclosan could potentially be removed within these few weeks if temperatures are near 20°C, leading to measured concentrations lower than predicted.

Following the Fall 2008 application, a sharp increase in soil triclosan concentration was observed that remained elevated over the winter period. In contrast, the average soil triclosan concentration measured in months following the Spring application was actually lower than the values measured earlier in the season, returning to levels below the detection limit by the end of the study (Table 6). This may also be a consequence of the rapid triclosan removal rate seen in the incubation, since several weeks elapsed between ASB application and soil sampling during a time of suspected high microbial activity in the Spring of 2009. Assuming a consistent triclosan concentration in ASB batches, the fact that soil TCS concentration increased and did not drop over the winter period following Fall application but actually dropped rapidly the following Spring after the second ASB application, suggests a possible role for microorganisms in the removal of triclosan from agricultural soils. The activity of soil microorganisms decreases at colder temperatures, such as those seen in Nova Scotia during the winter, which would slow the decomposition of biosolids applied in the Fall. A rapid period of microbial turnover and decomposition is generally seen in Spring as temperatures warm and different microbial groups become active, feeding on labile substrates released following the crash of the winter microbial community and on plant residues left over from the previous year (Schmidt et al. 2007).

The soil triclosan concentrations detected remained very similar over the winter period after receiving biosolids, before returning to levels below the detection limit by the end of the following season. This supports the work by Lozano et al. (2010) who found that triclosan concentrations in soil from fields receiving one year of biosolids applications were not significantly different from the control fields, and soils receiving two or more years of application had only slightly higher triclosan concentrations than the one year treatment. While Lozano et al. (2010) suggest that there may be an increased risk of exposure for soil organisms to triclosan up to 16 to 21 months after application, this study reveals that triclosan may have the potential to persist at elevated concentrations over winter. Consequently, this may present an increased risk of exposure to organisms that are active early in the spring decomposition process if biosolids are applied in the Fall. It may also suggest that triclosan losses due to leaching over winter are minor, although drainage water was not collected to confirm this. Cha and Cupples (2010) suggest that triclosan has a low leaching potential based on modelling of their sorption and biodegradation data.

Lozano et al. (2010) report that up to 78% of triclosan was removed from biosolid-amended soils under field conditions after 7 to 9 months, and up to 96% removed after 16 to 21 months. The removal rate in our incubation study, 82% after 4 months under idealized conditions, is faster than the field results presented by Lozano et al. (2010), although this may be a consequence of the constant elevated temperature (20°C) and homogenized substrate (2 mm sieved) in a well mixed soil used in our incubation. The particle size of organic residuals applied to soil affects the decomposition rate of some organic contaminants. Smaller particle size causes faster decomposition due to a larger total surface area available for microbial attack and anaerobic sites at the center of large aggregates that inhibit aerobic degradation (Heselhøe et al. 2001). Ying et al. (2007) revealed that triclosan will degrade easily in soil under aerobic conditions, but not under anaerobic conditions. This suggests that aerobic microbial degradation is an important removal pathway for triclosan from soils (Ying et al. 2007). Cha and Cupples (2010) found that soil moisture contents between 10-15% produced similar removal rates of triclosan, as did the three different soil types they tested in a laboratory incubation of

spiked soils. However, there was an inhibitory effect of higher triclosan concentrations on the removal of triclosan from the system (Cha and Cupples 2010).

Identifying microorganisms involved in the transformation of triclosan in the field is important in understanding potential ecological impacts on the soil community from the use of biosolids in agriculture, especially since triclosan is a broad spectrum antibacterial compound (McMurry et al. 1998) and also an endocrine disruptor in animals (Ishibashi et al. 2004, Ahn et al. 2008). Meade et al. (2001) isolated two bacterial strains from compost, *Pseudomonas putida* and *Alcaligenes xylosoxidans*, that were capable of using triclosan as a sole carbon source. However, little or no research on triclosan-degrading soil microorganisms has been conducted, which may be due in part to the difficulty in culturing the majority of the soil community. A bacterial consortium isolated from activated sludge by Hay et al. (2001) was capable of triclosan degradation, measured using mineralization of ^{14}C-labelled triclosan and an increase in protein and chloride concentration to indicate microbial growth on triclosan. They suggest that the failure to isolate pure strains capable of utilizing triclosan for growth indicates that there may be multiple members of the consortium that cooperate to utilize the substrate (Hay et al. 2001), which may also be the case in soil.

To fully assess the potential ecological impacts of triclosan in the soil environment, it would be beneficial to identify soil organisms that may actually be harmed by the antimicrobial properties of triclosan, especially those involved in important nutrient cycling processes. As one example, the nitrification process relies on a few specific microbial groups that perform each step in the conversion of ammonium to nitrate, and is sensitive to stresses. Waller and Kookana (2009) showed that triclosan concentrations of 50 and 100 mg kg^{-1} in soil caused a decrease in nitrification measured by the substrate induced nitrification method. Although the mechanism remains unclear and the concentrations are much higher than observed in this study, the implications of a disrupted nitrogen cycle could mean an altered flow of nitrogen within agricultural ecosystems and affect organisms that depend on a supply of nitrate, including plants (Waller and Kookana 2009). There are numerous other microbial habitats in soil (such as the rhizosphere) that could potentially be altered by the presence of a broad spectrum antimicrobial compound, which is an area that lacks research and should urgently be

addressed. Soil macroorganisms such as earthworms, sometimes referred to as 'litter transformers' (Wardle 2002) process large amounts of soil and organic matter, and are at high risk of exposure from contaminated soils and substrates such as biosolids. Kinney et al. (2008) showed evidence of triclosan bioaccumulation in earthworms from a field receiving just a single biosolids application. At an amendment rate of 18 Mg ha^{-1}, soil and earthworm triclosan concentrations after 31 days were 160 and 1740 ng g^{-1} respectively, and 96 and 2610 ng g^{-1} after 156 days. Lin et al. (2010) saw evidence of DNA damage and biochemical changes indicating toxic stress in earthworms exposed to increasing concentrations of triclosan (as low as 1 μg g^{-1}) and after longer exposure times. Other members of the soil food web, including the micro- and mesofauna, may be similarly impacted by triclosan toxicity. Additionally, trophic levels not exposed to triclosan through direct consumption of contaminated substrate (e.g. predators rather than decomposers) may still be impacted if their prey bioaccumulate triclosan or if triclosan increases the mortality of prey organisms, decreasing their food supply (Wardle 2002). Although data describing triclosan impacts on soil organisms is in itself scarce, future ecotoxicological studies on triclosan in soil should attempt to consider multiple members of the soil food web from different trophic levels.

4. Conclusions

Triclosan, a commonly occurring antibacterial compound in many consumer products, was detected at levels greater than our limit of quantification in a biosolid and soil incubation and in soil from an agricultural field receiving biosolids. The amendment rate for the incubation was equivalent to 42 t ha^{-1}, which is three times the recommended rate for agriculture of 14 t ha^{-1}, while the field received two applications (Fall, Spring) of 21 t ha^{1}, both representing 1.5 times the recommended rate. Average triclosan concentration in the incubation study ranged from a high of 143 ng g^{-1} on day 3 to a low of 26 ng g^{-1} by day 121, representing an 81% decrease over a roughly 4 month period under idealized conditions. In the field, triclosan concentrations following a Fall biosolids application in Oct. 2008 increased to detectable levels (29 to 47 ng g^{-1}) in all three plots measured in Nov. 2008, which remained elevated (29 to 66 ng g^{-1}) over the winter period in two out of three plots when sampled in May 2009. Following the Spring application in

June 2009, measured triclosan concentrations in July 2009 samples from these same two plots were lower than predicted (33 to 48 ng g^{-1}) and eventually decreased to levels below the detection limit by the Oct. 2009 sampling. The results indicate that triclosan in Fall-applied biosolids may persist overwinter and could possibly present an increased risk to organisms active in the Spring decomposition process. However, removal of triclosan during the summer months, presumably by aerobic microbial degradation as indicated by the incubation study, led to levels lower than predicted following the Spring application, and levels below our detection limit by the end of the study. Further studies are necessary to evaluate effects on soil communities that may be altered by the presence of a broad-spectrum antibacterial compound.

CHAPTER 5: OVERALL CONCLUSIONS

1. Overall Conclusions

The purpose of this project was to evaluate a biosolid produced in the Halifax Regional Municipality (N-Viro Soil™, an alkaline-stabilized biosolid) as a soil amendment for agriculture. The primary research goal was to analyze soil samples treated with biosolids for a suite of organic contaminants using gas chromatography-mass spectroscopy. To achieve this, a method was identified from the literature that claimed to be rapid, cost effective, and able to quantify a range of priority contaminants in agricultural soil. A 120 day aerobic soil and biosolid incubation and a two-year field trial using four rates of ASB were initiated to meet this objective.

The decomposition of ASB in soil was monitored using CO_2-C evolution as part of the incubation study. The dynamics of carbon mineralization were modelled, and the model developed during this project performed better than others commonly used. While this was not originally an objective, inconsistencies between theory and practice that arose during data analysis led to a new modelling approach. Originally, the double first order model performed best, but was difficult to interpret. The recalcitrant pool in the DFO model has its fastest mineralization rate at the beginning, but recent research suggests that mineralization of recalcitrant carbon requires growth of specialized organisms. My approach was to introduce a delay in the mineralization of the recalcitrant carbon pool using a logistic function. This model performed comparably well against the DFO model in my data, despite having an additional parameter, and could produce curves with inflection points which the DFO model could not.

Before analyzing soil samples for organic contaminants, a method had to be obtained from the literature and reproduced within our own laboratory. The selected method was said to be capable of extracting seven analytes from soil: *p*-cresol, indole, 4-*t*-octylphenol, phenanthrene, triclosan, carbamazepine, and benzo[*a*]pyrene. Throughout preliminary method evaluation trials, only four of these compounds (4-*t*-octylphenol, phenanthrene, triclosan, and benzo[*a*]pyrene) could be reliably extracted from soil with recoveries greater than 50%. This was able to meet our objective to analyze for a suite of organic contaminants in soil. However, analysis of treated soils during preliminary work

only detected triclosan at levels greater than the method detection limit and with abundances that fell within the linear range of the calibration curve. Additionally, to conserve resources, only samples from the highest amendment rate were analyzed in dedicated trials to more accurately describe triclosan dynamics over time.

Analysis of incubation and field soil samples in dedicated studies detected triclosan at concentrations greater than our limit of quantification. The amendment rate for the incubation was equivalent to 42 t ha^{-1}, which is three times the recommended rate for agriculture of 14 t ha^{-1}, while the field received two applications (Fall, Spring) of 21 t ha^{1}, both representing 1.5 times the recommended rate. Average triclosan concentration in the incubation study ranged from a high of 143 ng g^{-1} on day 3 to a low of 26 ng g^{-1} by day 121, representing an 81% decrease over a roughly 4 month period under idealized conditions. In the field, triclosan concentrations following a Fall biosolids application in Oct. 2008 increased to detectable levels (29 to 47 ng g^{-1}) in all three plots measured in Nov. 2008, which remained elevated (29 to 66 ng g^{-1}) over the winter period in two out of three plots when sampled in May 2009. Following the Spring application in June 2009, measured triclosan concentrations in July 2009 samples from these same two plots were lower than predicted (33 to 48 ng g^{-1}) and eventually decreased to levels below the detection limit by the Oct. 2009 sampling. The results indicate that triclosan in Fall-applied biosolids may persist overwinter and could possibly present an increased risk to organisms active in the Spring decomposition process. However, removal of triclosan during the summer months, presumably by aerobic microbial degradation as indicated by the incubation study, led to levels lower than predicted following the Spring application, and levels below our detection limit by the end of the study. Further studies are necessary to evaluate effects on soil communities that may be altered by the presence of a broad-spectrum antibacterial compound.

APPENDIX I

STANDARD OPERATING PROCEDURES FOR ULTRASONIC EXTRACTION OF ORGANIC CONTAMINANTS FROM SOIL WITH SOLID PHASE EXTRACTION CLEANUP FOR ANALYSIS BY GAS CHROMATOGRAPHY-MASS SPECTROSCOPY

1. Scope of Method

This method is for the ultrasonic-assisted extraction of seven target organic contaminants from soil, followed by solid-phase extraction cleanup. Analytes are: *p*-cresol, indole, 4-*tert*-octylphenol, phenanthrene, triclosan, carbamazepine, and benzo[*a*]pyrene. Analysis is by gas chromatography with electron ionization mass spectrometry (GC-EIMS).

2. Matrix Characterization

Soil was sampled from an agricultural field with a crop history including carrots, soybeans, and most recently, corn. In the past, the field has been heavily manured. The soil is classified as a sandy loam, gleyed and fragic humo-ferric Podzol (Webb et al., 1991) with 1.5% TOC and pH 5.3. The biosolid is an alkaline-stabilized sewage sludge (N-Viro Soil™) produced in Halifax Regional Municipality, Nova Scotia. It has 11% TC, 62% solids, and a pH varying between 9.5 and 11. A more complete description is given in Chapter 2 (Table 2)

3. Standard Operating Procedures

3.1. Preparation of Solvent Mixtures

3.1.1. 80:20 Dichloromethane:Diethyl Ether – Read MSDS sheets before working with these solvents. Ensure that polyvinyl alcohol gloves are worn at all times when dispensing dichloromethane, since regular latex or nitrile gloves will degrade easily! Ensure that there are no open flames or possible ignition sources when working with diethyl ether!

In a fume hood, accurately measure 400 mL of HPLC grade diethyl ether in a glass graduated cylinder and transfer to 2 L volumetric flask. Bring to volume with HPLC grade dichloromethane.

3.1.2. 80:20 Isopropyl Alcohol:Water – In a fume hood, accurately measure 400 mL of deionized water in a glass graduated cylinder and transfer to 2 L volumetric flask. Bring to volume with HPLC grade isopropyl alcohol.

3.1.3. 0.1 M Phosphate Buffer – Mass 10 g dipotassium hydrogen phosphate and 7 g potassium dihydrogen phosphate and transfer to 1 L volumetric flask. Bring almost to volume with deionised H_2O and adjust to pH 7.0 by adding phosphoric acid or potassium hydroxide before bringing to final volume with deionized H_2O.

3.2. Preparation of Stock Solutions

3.2.1. Description – This stock solution contains enough of each method compound to perform all of the experiments in this validation study, or at least 24 extracts of soil. It is necessary to prepare solutions in both acetone and in 80:20 DCM:DEE to be used in spiking and in preparation of calibration standards respectively. Read MSDS sheets before working with method compounds! Always wear protective clothing, including a clean lab coat, two layers of gloves, and a full face shield.

3.2.2. 4000 µg mL^{-1} Internal Standards – This method uses an internal standard labelled with deuterium added before the evaporation step to account for differences in solvent volumes. Accurately mass 0.02000 g of phenanthrene-d_{10} onto a piece of solvent-rinsed aluminum foil using a five decimal point balance. Quantitatively transfer to 5 mL volumetric flask by rinsing foil with 80:20 DCM:DEE. Bring to volume with 80:20 DCM:DEE.

3.2.3. 125 µg mL^{-1} Stock Solution in 80:20 DCM:DEE – Accurately mass 0.01250 g of each method compound into a piece of solvent rinsed aluminum foil using a five decimal point balance. Quantitatively transfer to 100 mL volumetric flask by rinsing with DCM:DEE. Bring to volume with DCM:DEE, replace cap and mix thoroughly.

3.2.4. 5 µg mL^{-1} Spiking Standards in Acetone – Transfer 1000 µL of stock solution to 25 mL volumetric flask, or 400 µL to 10 mL volumetric flask with 1 mL

gastight syringe depending on amount of spiking solution required. Bring to volume with acetone.

3.3. Preparation of Standards

3.3.1. Calculate required volume of stock solution and appropriate size volumetric flasks and Hamilton glass syringes for the desired calibration range.

3.3.2. In a fume hood, prepare each standard in a solvent rinsed volumetric flask. Transfer each volume using an appropriately sized Hamilton glass syringe.

3.3.3. Add 50 µL of 4000 µg mL^{-1} phenanthrene-d_{10} internal standard to each 5 mL volumetric flask using a 50 µL gastight syringe. Bring to volume with 80:20 DCM:DEE. Do not prepare standards by serial dilution.

3.3.4. Transfer approximately 1 mL of each standard to a 2 mL glass amber vial with PTFE lined cap for analysis by GC-MS by pouring from flask or using a glass disposable pipette.

3.4. Internal Standard Calculations

3.4.1. The concentration of analyte in a standard (C_A) is assumed to be proportional to the instrument signal (S_A), and likewise the concentration of an internal standard compound (C_{IS}) is proportional to the instrument signal (S_{IS}) according to the relationships:

$$S_A = K_A \times C_A$$
$$S_{IS} = K_{IS} \times C_{IS}$$

In a series of standard solutions of known concentrations, the ratio of instrument signals from the analyte and internal standard are assumed to be proportional to the ratio of concentrations as follows:

$$\left(\frac{S_A}{S_{IS}}\right)_{standard} = K \times \left(\frac{C_A}{C_{IS}}\right)$$

The equation can be used to solve for *K* by plotting S_A/S_{IS} vs. C_A/C_{IS}, which should be linear with a slope of *K* that can be estimated by regression. If the concentration of

internal standard is held constant in the calibration standards and unknown samples, C_A in the sample can be calculated from S_A in an unknown sample by the equation:

$$C_A = \left(\frac{C_{IS}}{K \times S_{IS}} \right) \times S_A$$

Using an internal standard method can correct for analyte losses if added at the beginning of extraction, or small differences in final extract volume if added before the concentration step as in this case.

3.5. Soil Preparation

3.5.1. Homogenize bulk soil sample as best as possible using a clean scoopula, spatula, or glass rod.

3.5.2. Prior to extraction, in a fume hood, solvent rinse with acetone a number of 50 mL extraction vessels and caps. Mass out 10.0 g of soil into each labelled extraction vessel. Record soil weights.

3.5.3. In a fume hood, add 1 mL of spiking solution to extraction vessels designated as "spiked" using a 1 mL gastight syringe. Avoid spraying solution on sides of vessel. Mix each sample well by agitating lightly to ensure even distribution.

3.5.4. Leave samples in fume hood for solvent to evaporate.

3.6. Ultrasonic-Assisted Extraction, 3 x 10 min

3.6.1. Prepare an ultrasonic bath by placing extraction vessels in rack and putting them in the bath, then filling to the appropriate level with water.

3.6.2. To each extraction vessel, add 10 mL of 80:20 IPA:H_2O and replace cap. Manually agitate soil by shaking to ensure complete solvent coverage then place in test tube rack.

3.6.3. Place extraction vessels in rack in ultrasonic bath. Adjust height of rack so that entire volume of extract is submerged. Sonicate on first cycle for 10 min.

3.7. Centrifugation

3.7.1. Centrifuge samples at ~2000 rpm for 5 min or until supernatant is clear of debris. Decant supernatant from each centrifuge tube into a solvent rinsed and labelled 50 mL PTFE-capped test tube.

3.7.2. Repeat steps 3.2.4.2 to 3.2.5.1 twice more.

3.8. Solid-Phase Extraction Cleanup

3.8.1. Attach 60 mL reservoir to each HLB cartridge and place cartridges into SPE manifold, closing all valves. Place collection reservoir in manifold beneath SPE cartridges.

3.8.2. Pre-condition HLB cartridges by adding 25 mL of 80:20 DCM:DEE to each reservoir. Open each valve, allowing to drain under gravity until completely saturated before applying vacuum. To ensure HLB cartridge remains covered in solvent, it may be necessary to gently slap the top of the cartridge reservoir with a gloved hand to create positive pressure which will force drops of solvent into the HLB cartridge. Adjust valves to drain at approximately 1 drop every 2 seconds. Drain solvent until just above surface of SPE sorbent and close valves. Empty manifold reservoir into DCM:DEE waste container and replace in manifold.

3.8.3. Add 20 mL of phosphate buffer to each 50 mL extraction vessel and mix. Load HLB cartridge with buffered extract by pouring into reservoirs and adjust to 2-3 drops per second. Apply vacuum until all liquid is drawn through cartridge. Continue to dry under vacuum for 10 min.

3.8.4. Condition Florisil SPE cartridge with 10 mL acetone. Add 4 g anhydrous sodium sulphate to the barrel of Florisil cartridge and attach HLB cartridge on top. Load stack onto SPE vacuum manifold, and replace waste reservoir with a rack of clean, labelled test tubes.

3.8.5. Elute HLB/Florisil stack with three 5 mL aliquots of 80:20 DCM:DEE. Drain SPE stack at a rate of 1 to 2 drops per second. Since the system is under vacuum, the solvent will continue to flow after shutting the valves. Stop the flow at about 1 cm from the top of the sorbent material, and add the next aliquot when the solvent is just above the SPE material. Remove collection tubes, this is the desired extract.

3.9. Concentration

3.9.1. Place tube containing extract in nitrogen evaporator. Add 10 µL internal standard solution to each test tube using 50 µL gastight syringe. Adjust nitrogen flow so that surface is just slightly disturbed.

3.9.2. As solvent is evaporating from tubes, rinse sides of tubes periodically with a small quantity of 80:20 DCM:DEE to reduce loss due to sorption.

3.9.3. Evaporate samples to approximately 1 mL and transfer to 2 mL amber GC vial.

3.10. Sample Storage and Handling

3.10.1. Store samples and unused standards at -10°C if not used on same day as prepared.

3.10.2. Before analysis, cool samples and standards to room temperature and mix well before use.

3.11. Analysis by GC-MS

3.11.1. These instructions are for operating the Agilent 5975 GC-MS in the Innovative Waste Management Laboratory.

3.11.2. Load sample vials into auto-sampler holes in a random order. Open GC-MS Analysis program and create a new sequence or edit an old sequence. Enter sample names according to their position in auto-sampler. Enter sample type "Sample" for extracts from soils and "Calibration" for standard curve vials. This will allow these standards to be entered into the software for automatic quantitation if desired. Enter an appropriate method, either DG_FS_BACK or DG_FS_FRONT depending on which injection port is used (FS stands for Full Scan mode).

3.11.3. Manually check GC-MS operating parameters. They are listed below:

GC Column type: 30 m HP-5, 5% phenyl methylpolysiloxane

Injection type: 2 mL in splitless mode

Injection port temperature (°C): 290

Transfer line (°C): 250

MS Source (°C): 230

Quadrupole (°C): 150

GC temperature program (°C): 50 hold 3 min, ramp 8/min to 100 hold 4.5 min, ramp 9/min to 290 hold 10 min.

Purge flow (mL/min): 6.1

Flow settings (mL/min): Constant flow of 3.0

Mass range scanned: 0 to 34 min: 40-350, 34 to 46 min: 40-450

3.11.4. Tune MS and evaluate tune performance using software.

3.11.5. Run the sequence that was created in 3.2.9.2

3.11.6. Obtain extracted ion chromatograms of the quantitation ions for each method compound to obtain analyte abundances for quantification. Quantitation ions are as follows: *p*-cresol 107, indole 117, 4-*t*-octylphenol 135, phenanthrene-d_{10} 188, phenanthrene 178, carbamazepine 193, benzo[*a*]pyrene 252. Calculate retention time of each analyte from each run using calibration standards and use the retention times, in combination with two qualitative ions, to identify analytes in unknown samples.

APPENDIX II
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