THE ROLE OF BIOCHAR AS A SOIL AMENDMENT IN REDUCING SOIL AND WATER POLLUTION BY ESTROGENS FROM BIOSOLIDS AND SLUDGE

by

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April 2015

A thesis submitted to McGill University

in partial fulfillment of the requirements of the degree of

Master of Science

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LIST OF SYMBOLS AND ABBREVIATIONS

°C	Degree Celsius
CEC	Cation exchange capacity
cm	Centimeter
cmol	Centimole
DOC	Dissolved Organic Carbon
DS	Dry weight
ECD	Endocrine Disrupting Chemical
g	Gram
GC	Gas Chromatography
ha	Hectare
HPGC	High Performance Gas Chromatography
HPLC	High Performance Liquid Chromatography
I.D.	Inside diameter
i.e.	That is
K_d	Soil-water partitioning coefficient
K_f	Freundlich adsorption coefficient
kg	Kilogram
K_{oc}	Adsorption coefficient
K_{ow}	Octanol-water distribution coefficient
L	Liter
mg	Milligram
min	Minute
mL	Milliliter
p	Statistical probability
rpm	Revolutions per minute
STP	Sewage treatment plant
WWTF	Wastewater treatment facility
µg	Microgram
µL	Microliter

ABSTRACT

Estrogens are considered as dangerous endocrine disruptors. Sludge and biosolids, which are sourced from the human waste and used in the form of as fertilizers on agricultural fields, contain estrogens. As a result of leaching, hormones may contaminate soil, ground water and surface waters. This can lead to degradation of aquatic wildlife.

Recent research has found that biochar has good potential in reducing pollution from organic and inorganic contaminants. However, there is a lack of knowledge with respect to the remediation potential of biochar in removing steroid estrogens, such as 17β-estradiol and estrone, present in sludge and biosolids.

A lysimeter study was carried out to study the fate and transport of estrogen hormones in soil. Hardwood-derived slow pyrolysis biochar was selected as a topsoil amendment (1% w/w; mixed in top 10 cm of soil) to determine its ability to provide a reliable remediation media for estrogens. Sludge and biosolids were applied to lysimeters at 28 tonnes/ha (wet basis). Lysimeters were irrigated every 15 days and the experiment ran for 45 days.

It was found that biochar is an agent-specific soil amendment. Estrogens residues in soil and water were greatly influenced by the type of applied fertilizer (in this study, sludge or biosolids). The behavior of estrogens exhibited different trends in the presence and absence of biochar. Results show that estrogens dissipated up to 94%, with losses up to 72% and leaching up to 65%. It was found that 17β-estradiol has a strong degradation ability in different treatments and fertilizers, while estrone has a greater tendency for leaching. As complicated matrices, sludge and biosolids contain multiple different pollutants which could compete for the available binding sites in biochar. In comparison to other contaminants, estrogens do not perform well in securing sorption sites, so the removal efficiency of biochar with estrogens is less when other contaminants are present. Biochar showed higher removal potential for sludge as compared to biosolids. The suggested causes of this difference lie in microbial availability and physico-chemical properties of these matrices. More studies are needed to develop methods to maximize the efficiency of biochar in reducing loss of contaminants under agricultural scenarios.

RÉSUMÉ

Les œstrogènes sont considérés comme des perturbateurs endocriniens dangereuses. Les boues et les biosolides, qui proviennent des déchets humains et utilisé sous la forme d'engrais sur les champs agricoles, contiennent des oestrogènes. À la suite de la lixiviation, des hormones peuvent contaminer le sol, les eaux souterraines et les eaux de surface. Cela peut conduire à la dégradation de la faune aquatique.

Des recherches récentes ont montré que le biochar a un bon potentiel dans la réduction de la pollution par les contaminants organiques et inorganiques. Cependant, il ya un manque de connaissances en ce qui concerne le potentiel d'assainissement du biochar dans l'élimination des oestrogènes stéroïdes, tels que 17β-estradiol et estrone, présente dans les boues et les biosolides.

Une étude de lysimètre a été réalisée pour étudier le sort et le transport des hormones œstrogènes dans le sol. Pyrolyse lente biochar de bois franc dérivé a été choisi comme une modification de la couche arable (1% p/p) pour déterminer sa capacité à fournir un support d'assainissement fiable pour les oestrogènes. Les boues et les biosolides ont été appliqués à lysimètres à 28 tonnes/ha (de base humide). Les lysimètres ont été irrigués tous les 15 jours et l'expérience encouru pour 45 jours.

Il a été constaté que le biochar est un amendement spécifique du sol-agent. Résidus d'oestrogènes dans le sol et l'eau ont été fortement influencés par le type d'engrais appliquée (dans cette étude, les boues ou les biosolides). Le comportement des oestrogènes expose différentes tendances de la présence et l'absence de biochar. Les résultats montrent que les oestrogènes ce dissipent jusqu'à 94%, avec des pertes pouvant atteindre 72% et de lixiviation jusqu'à 65%. Il a été trouvé que 17β-estradiol a une capacité de forte dégradation dans des traitements différents et des engrais, tandis que l'œstrone a une plus grande tendance à la lixiviation. Comme matrices complexes, les boues et les biosolides contiennent plusieurs polluants différents qui pourraient concourir pour les sites de liaison disponibles dans le biochar. En comparaison à d'autres contaminants, les oestrogènes ne fonctionnent pas bien dans la sécurisation des sites de sorption, donc l'efficacité d'élimination du biochar avec oestrogènes est moindre lorsque d'autres contaminants sont présents. Le biochar a montré un potentiel plus élevé d'élimination des boues par rapport aux biosolides. Les causes de cette diffirence suggérées se trouvent dans la disponibilité

microbiennes et les propriétés physico-chimiques de ces matrices. D'autres études sont nécessaires pour développer des méthodes pour maximiser l'efficacité du biochar dans la réduction de la perte de contaminants dans les scénarios agricoles.

ACKNOWLEDGEMENTS

I would firstly like to express my deepest gratitude to my supervisors, Prof. Shiv O. Prasher and Dr. Eman ElSayed for the contribution and support. I would also like to thank Barry Husk (BlueLeaf Inc.) for providing first class biochar, Gordon W. Price (Dalhousie University) for providing excellent class “A” sludge, Alexandra Furtos (Université de Montréal) for her professional analysis in LC-MS, Dušan Garić for his extremely useful advice in the laboratory work with steroidal hormones and Olga Shuvalova for her endless support and advice.

Exceptional thanks to all people that helped with field and lab experiment: Olga Shuvalova, Michael W. Saminsky, Rahul Suresh, Sanaz Alizadeh, Sukhjot S. Mann, Jennifer Rocard, Clémence Fortier-Morissette, Sedigheh Zarayan and Cassandra Rodrigue.

Funding support for this research was provided through the Canadian Water Network - Canadian Municipal Water Consortium project 'Impacts of alkaline stabilized biosolids application on fate and transport of emerging substances of concern in agricultural soils, plant biomass and drainage water.

CHAPTER 1
Introduction

1.1. Background

In recent years, the scientific world learned about the influence of sex hormones on human and animal health. There are studies that found an interesting link between effluents from wastewater treatment facilities (WWTFs) and sexually altered aquatic wildlife populations (e.g., Purdom et al., 1994; Jobling et al., 1998). Shortly after revealing findings about changes in fish sex, a book with a hypothesis about the endocrine disruption was released: Our Stolen Future (Colborn et al., 1997). Initially, only a short list of organic compounds was created, identifying sources for the observed abnormalities in fish (Harries and Britain, 1995). However, the current list of synthetic and natural compounds, which are suspected of having the potential to interfere with the normal functioning of human and animal hormonal systems, is quite impressive (Fent, 2003).

After the industrial revolution, the global population started to grow and this increased the demand for the livestock products, resulting in the intensified use of synthetic steroid hormones as growth promoters (Khan et al., 2008a). Despite the fact that some review papers have proposed only a negligible risk associated with estrogen excretions of livestock (Hanselman et al., 2003; Johnson et al., 2006), estrogens are continuously detected around the globe in waterways (e.g., D'Ascenzo et al., 2003; Isobe et al., 2003; Petrovic et al., 2004) and are associated with health abnormalities (e.g., Purdom et al., 1994; Jobling et al., 1998).

1.2. Problem statement

A great number of emerging contaminants, (ECs) such as personal care products, pharmaceuticals, estrogens, phthalate acid esters as well as organic and inorganic nanoparticles, have been detected in municipal wastewater (Clarke and Smith, 2011). Subsequently, the effluents of WWTFs are potentially contributing in the persistence of the endocrine-disrupting effects in receiving water bodies (Nakada et al., 2004). Therefore, in WWTFs, various processes are used to remove these contaminants. As hydrophobic substances, these contaminants are sorbed to some extent onto suspended solids. Eventually, they become infused in varying amounts in sludge through sedimentation, which occurs in primary and secondary treatments (Matsui et al., 2000).

It is reported that 53% of sludge in the European Union is used in agriculture directly or after composting (Kelessidis and Stasinakis, 2012), while more than 40% of produced biosolids are applied to land in the USA and Canada (Citulski and Farahbakhsh, 2010). Therefore, using sludge as a fertilizer in agricultural soils could play a major role in releasing contaminants into soils, thus polluting water bodies.

The pollution potential of sludge application on agricultural soils has been well-studied (Zuloaga et al, 2012). However, there is a lack of studies exploring remediation techniques for removal or reduction of risk from the sludge-associated contaminants. This study introduces a promising remediation method for removal of estrogens using biochar as a soil amendment. Biochar is the by-product of thermo-chemical decomposition of biomass and biological residues in the absence of oxygen in a process known as pyrolysis (Fisher et al., 2002). Due to its physical and chemical characteristics, biochar has high specific surface area, nano-scale condensed aromatic rings, micro-scale crystalline structure and macro-scale amorphous structure. Biochar is also resistant to bio-decomposition (Singh and Cowie, 2010). It has a strong sorption potential for inorganic and organic contaminants.

Biochar can be applied directly to soil as an amendment, offering additional micropore surface area for binding pollutants and reducing their bioavailability, while increasing crop yields and restoring the agricultural sustainability.

There is a lack of scientific information concerning the role of biochar in reducing estrogen hormones from agricultural use of sludge and biosolids. Therefore, laboratory and lysimeter experiments were conducted in this study to explore this area of research.

1.3. Thesis objectives

Contamination of soil, surface and ground water may potentially be caused by application of sludge from municipal STPs on agricultural fields. There is a need to find a feasible and an effective remediation method to reduce estrogen contamination. Biochar is capable of providing high micropore surface area to capture organic contaminants. The hypothesis of this study is that the addition of biochar to agricultural soils, fertilized by sludge and biosolids, could decrease the transport of hormones in the soil profile, reducing the risk of surface and ground water contamination.

In order to validate the proposed remediation capability of biochar in an agricultural setting, laboratory experiments and an outdoor lysimeter study were conducted with the following objectives:

1. To assess the sorption potential of soil and biochar towards estrogens hormones, such as17β-estradiol and estrone, at a laboratory scale;

2. To investigate the fate and transport of the estrogen hormones (17β-estradiol and estrone) originating from two different fertilizers, untreated sludge (sludge) and treated sludge (biosolids), in a sandy soil;

3. To evaluate the potential of slow-pyrolysis biochar topsoil amendments in reducing hormonal pollution from sludge and biosolids applied soils; and

4. To determine the best fertilizer in terms of reducing the hormonal contamination in soil and water.

This thesis is written in compliance with norms for academic and scholarly expression and for publication in the public domain. It is written in the monograph style, according to the standards and recommendations of the McGill University guidelines, while references are presented in the APA style.

1.4. Scope of the thesis

The results of this study were obtained for a sandy soil (92.2% sand), 1% slow pyrolysis wooden biochar, class “A” alkaline treated biosolids and raw sludge under controlled rainfall conditions. The outcomes could be different for other types of soil, biochar and sludge.

CHAPTER 2
Literature Review

2.1. Introduction

There two main directions in research of estrogenic hormones: research of fate of estrogens in STPs (Ternes et al., 1999b; Layton et al., 2000; Matsui et al., 2000; D'Ascenzo et al., 2003; Marti and Batista, 2014) and fate of estrogens in laboratory conditions (Colucci et al., 2001; Jacobsen et al., 2005; Lee et al., 2003; Sangsupan et al., 2006; Fan et al., 2007; Lorenzen et al., 2005; Sarmah et al., 2010). There are very few studies that study fate and transport of estrogens directly in agricultural lands (i.e. Yang et al., 2012) or simulate to the limit realistic conditions in soil lysimeters (Dizer et al., 2002; Casey et al., 2003; Das et al., 2004; Sangsupan et al., 2006; Casey et al., 2008).

The rising costs of landfills and incineration are leading to the popularity of land application of biosolids (EPA, 2012). In this paper, "sludge" refers to the solid, semi-solid, or liquid residue generated during the treatment of domestic sewage in a treatment works. Generally, biosolids are a form of solid, semi-solid or liquid residue (also called domestic sludge) which is generated during the treatment of domestic sewage and is able to meet land application standards equivalent to Part 503 rule (Pepper et al., 2011). In this paper, the term "biosolids" will refer to biosolids or dry stabilized biosolids or alkaline treated sludge of the class "A", which are practically the same. The applications of biosolids include their recycling for use as fertilizers to improve and maintain soil quality as well as to stimulate plant growth (EPA, 2012). Application of sludge into agricultural fields increases the concentrations of organic matter and nutrients (nitrogen and phosphorus). However, practical experience shows that in order to get full results from sludge application, about three years need to pass (No-Till Farmer Magazine, 2013).

The application of sludge or biosolids results in soil pollution. As a result, the build-up of persistent toxic compounds, chemicals, salts, radioactive materials, or disease causing agents have adverse effects on plant growth and animal health (Metcalf et al., 2010). Pollution of soil has three major consequences: direct soil pollution, pollution of ground water and surface water. Despite the wastewater treatment on the urban WWTFs, biosolids contain large amounts of pollutants. Traditional centralized WWTFs generally remove the biomass, leaving some

microorganisms, some chemical compounds, pharmaceuticals, drugs and steroidal hormones unattended. As result of rain and excessive irrigation, water moves through the soil, washes out contaminants from the applied biosolids, leaves the soil contaminated, and brings contaminants into ground water or surface water. Biochar has the potential to stop the process of contamination.

Biochar is an organic soil amendment that is known for its ability to reduce bioavailability of contaminants (Beesley et al., 2011). Adding soil amendments is one of the commonly used sustainable ways of soil remediation and restoration. Soil amendments are considered to be materials that improve physical properties of soil, such as water retention, permeability, water infiltration, drainage, aeration and structure. Biochar can serve as an absorbent for a variety of natural and synthetic organic chemicals and heavy metals, absorbing various compounds and providing enough time for biodegradation. Co-amending biosolids with biochar may decrease pharmaceutical bioavailability in soil through preferential sorption (Bair et al, 2012).

2.2. Biosolids and sludge

2.2.1. Disposal and land application of sludge and biosolids

The most common biosolids disposal methods in Canada include land application, sanitary landfill and thermal oxidation (CIELAP, 2009). According to the Hwang and Oleszkiewicz (2011), more than 660,000 metric tonnes of dry stabilized biosolids are generated each year in Canada. Land application of biosolids is recommended agricultural practice and conservative estimates of the fertilizer value of biosolids supplied free-of-charge to farmers in Ontario alone are $250 per hectare and more than $5 million dollars per year. In some other Canadian provinces (e.g., British Columbia and Quebec), considerable quantities of biosolids are used to prepare manufactured soils and/or employed for land reclamation (Hebert et al., 2008). Biosolids are particularly valuable for land reclamation (e.g., Van Ham et al., 2005) because in addition to supplying immediately available and slow-release nutrients, they supply a microbial population, organic matter and water holding capacity all of which are required to establish soil fertility and sustain plant growth. In particular, N-Viro Soil Amendment (NVSA) biosolids supply considerable liming value, which important for acid land reclamation.

The two most populated provinces, Ontario and Quebec, practice thermal oxidation (incineration) for biosolids management. Land application is the dominant method of the biosolids management in other provinces. In Ontario, 40% biosolids are applied to land and 40% are landfilled. In Quebec, 27% biosolids are applied to land and 31% are landfilled. Land application is the only option of biosolids disposal in Nova Scotia and on Prince Edward Island, whereas it is prohibited in Newfoundland and Labrador. In other provinces, 80% of biosolids are land applied (Billingsley & Anoop, 2009). Although presently biosolids are applied to less than 1% of agricultural lands in the United States, there are growing concerns about the safety of biosolids due to their rising popularity in land application (EPA, 2012).

According to the Canadian Council of Ministers of the Environment (2010), in Quebec, municipal biosolids are used to fertilize 0.5% farmland, which makes 27% of all produced sludge (Hebert et al., 2008). By 2015, the Government of Québec intends to increase that proportion considerably with the target of recycling up to soils 60% of all organic matter, including municipal sludge (MDDEP, 2011). Current application rate of biosolids in Quebec and Ontario (maximum 22 tonnes dry weight/ha/5 years or approximately 31 tonnes wet weight/ha/5 years) is mainly limited by the requirement of phosphorus by crops (Canadian Council of Ministers of the Environment, 2010).

2.2.2. Laws and regulations for land application of sludge and biosolids

Despite the established value and practice of land application of biosolids, there are no uniform policies for biosolids management in Canada as a whole. The closest guideline is provided by the regulations established in the USA by EPA in 1993 in the Code of Federal Regulations, Title 40 (Part 503), under section 405 (d) of the Clean Water Act. It governs land application of sludge with the intent to protect public health and the environment. It established management practices for land application of sewage sludge, concentration limits and loading rates for chemicals, and treatment and use requirements designed to control and reduce pathogens and attraction of disease vectors.

In Canada, the regulations for use of biosolids are at the provincial or territorial level. It is challenging to relate and compare the classes of biosolids and their qualities among the

provinces, because they use different classification/categorization schemes and adopt different nomenclature for the various classes (Canadian Council of Ministers of the Environment, 2010).

For example, most provinces define one or two classes of biosolids, whereas Quebec and Ontario define several classes based on combinations of metal, pathogen and odor properties (Canadian Council of Ministers of the Environment, 2010).

Despite differences, the regulations/guidelines for Canadian jurisdictions have much in common. They are based on Canadian Council of Ministers of Environment (2010) guidelines, which list allowable concentrations of contaminants for different solids applications. The parameters used to assess the quality of biosolids in federal and provincial regulations/guidelines include: metals, pathogens and pathogen indicators and organic chemical contaminants and odor. Hormones are not considered as quality parameters and are not treated or checked for quantity before and after the wastewater treatment processes.

2.2.3. Pollutants in sludge and biosolids

The pollutant composition of biosolids depends on the quality of wastewater entering a particular treatment facility as well as the type of treatments carried out on the influent. There are rising concerns about contamination with antibiotics, hormones and endocrine-disrupting substances that arise from pharmaceutical use and personal-care products (CIELAP, 2009). Traditional WWTFs do not perform as well in filtering out endocrine-disrupting substances as they do with traditional pollutants (CIELAP, 2009). The presence of endocrine-disrupting substance might lead to higher rates of cancer incidence in humans who drink water that has been contaminated with this pollutant as a result of biosolids leaching (CIELAP, 2009).

2.2.4. Estrogens: naturally occurring steroid hormones

The substances chosen for this experimental study are the natural estrogens: estrone and 17β-estradiol. Estrogens are hormones, which are naturally secreted in humans by the adrenal cortex, the testis, the ovary and the placenta (Fent, 2003). The amount of excreted estrogens by humans differs between the genders. The highest values of natural estrogens are excreted with the urine of pregnant women (Gallagher et al., 1937; McCue, 2014). Males also excrete estrogens, but in significantly lower values (Gallagher et al., 1937; McCue, 2014). The major sources of artificial

steroid hormones are birth control pills (BCPs) and other medical treatments. 17β-estradiol and estrone along with 17α-ethinylestradiol exhibit the highest estrogenic activity (Andersen et al., 2004). These hormones are of great importance as they are responsible for maintaining the health of reproductive tissues, breasts, skin and brain. The major functions of these steroidal hormones are in sex determination, sexual differentiation, and sexual development (Tyler et al., 1998).

Estrone and 17β-estradiol are principally excreted in hydrophobic form from human body as inactive polar soluble conjugates (D'Ascenzo et al., 2003; Andersen et al., 2004) predominantly with sulphate and glucuronide in urine or, after minor metabolism, as free hormones in feces (Lange et al., 2002; D'Ascenzo et al., 2003; Fent, 2003). Concluding this, the amounts estrogens and their conjugates in either urine or feces depend on factors such us: species, gender, age, and, for females, on the state of cycling or pregnancy (Hoffmann et al., 1997; D'Ascenzo et al., 2003).

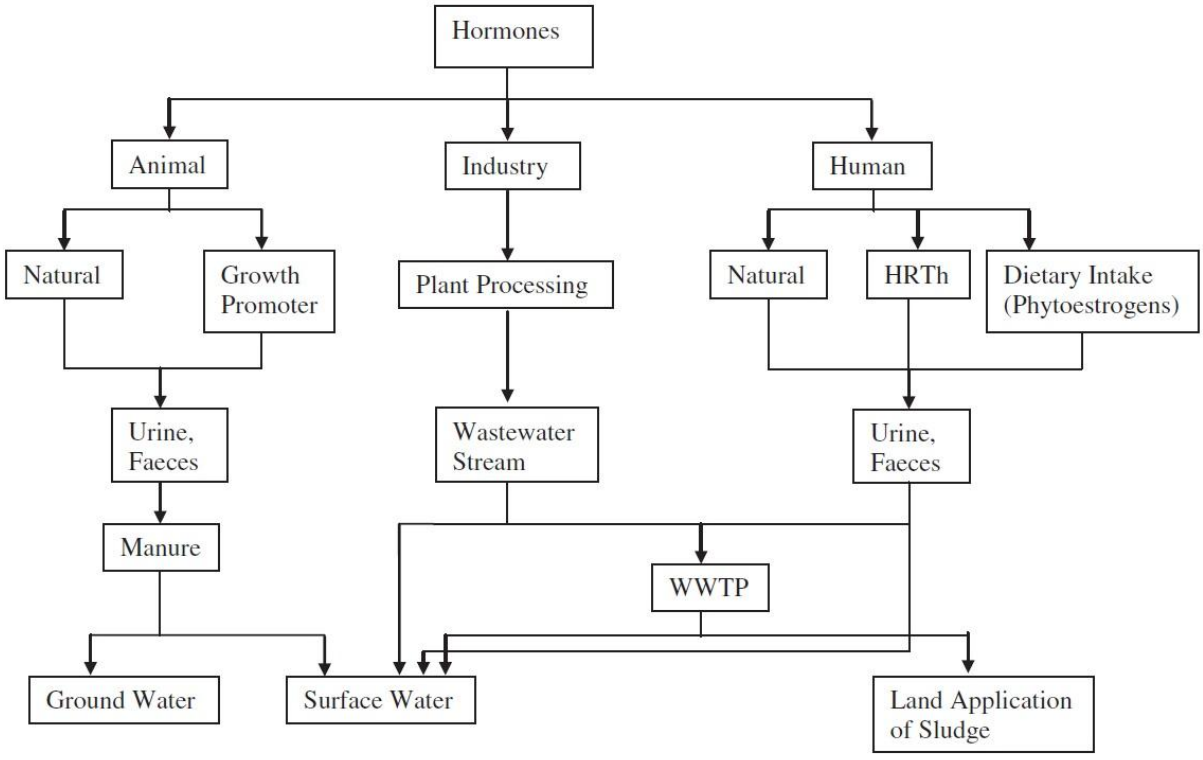


Figure 1. Sources and pathways of steroidal hormones in environment (HRTh - hormone replacement therapy, WWTP - wastewater treatment plant).

In the review study Hamid and Eskicioglu (2012) presented sources and pathways of estrogens in environment (Figure 1). The excreted free and conjugated estrogens end up in WWTFs. In general, these systems are capable of removing the majority of the estrogen loading (Khanal et al., 2006). Treatments that are commonly used in WWTFs (oxidation ditch, sequencing batch reactor, biological aeration filter, anaerobic–aerobic or anaerobic/ anoxic/aerobic process, etc.) cannot completely eliminate estrogens in the effluent (Xu et al., 2014). Depending on geographical location and climate conditions as well as site-specific flow regimes, removal capacities vary and may be unsatisfactory at certain times (Ternes et al., 1999a; Ternes et al., 1999b; Schlüsener and Bester, 2008). The same situation is observed in Canada (Servos et al., 2004; Hamid and Eskicioglu, 2012). Removal rates of WWTFs for estrogens range from as low as 34% to 100% (D'Ascenzo et al., 2003; Servos et al., 2005; Kuster et al., 2008; Schlüsener and Bester, 2008), and individual concentrations in the effluents are mainly in the lower ng L^{-1} range or below. According to the studies across the world (Komori et al., 2004; Chimchirian et al., 2007; Tan et al., 2007; Furlong et al., 2010) and Canadian studies (Servos et al., 2005; Hamid and Eskicioglu, 2012) estrone has lower dissipation rates than 17β-estradiol and is more likely to persist after treatment steps at STPs. In fact, a net increase in estrone concentration in the effluent has been reported (Baronti et al., 2000; Carballa et al., 2004; Servos et al., 2005; Hashimoto et al., 2007). In his study, Ternes et al. (1999b) observed almost complete oxidization of 17β-estradiol into estrone that occurred during a period of 1-3 hr. There is evidence that estrogens are likely to act together and in an additive manner cause observed physiological effects such as the feminization of fish (Sumpter and Johnson, 2005; Thorpe et al., 2003). Furthermore, it has been shown in Japan and Germany that concentrations of estrogen sulphates in WWTF effluents, as opposed to influents, can be elevated (Komori et al., 2004; Schlüsener and Bester, 2008), leading to the assumption that re-conjugation processes had occurred within those engineered systems. Additionally, longer sludge retention time appears to have a positive effect on the ability of the activated sludge systems to eliminate estrogens (Andersen et al., 2004), therefore it is expected that biochar can provide the necessary retention time for dissipation of estrogens.

Additional source of natural estrogens going to the environment is from livestock. Amounts of estrogens coming to the environment from the livestock are considerable (accounting that livestock is in greater population than human (The economist, 2011). Amount, type and rates of

excreted estrogens and their conjugates depend on livestock species, their reproductive stages and lactation periods (Hanselman et al., 2003). As for the prevalent form, free estrogens dominate in feces, while conjugated forms are mainly present in urine (Johnson et al., 2006). Contributions of other livestock to environmental estrogen concentrations include sheep, swine, poultry and horse breeding (Hoffmann et al., 1997; Combalbert et al., 2012). The highest estrogen concentrations have been found in fresh dairy cattle manure, accounting for up to 1,230 and 640 $\mu g\ kg^{-1}$ for 17β-estradiol and estrone respectively (Shore and Shemesh, 2003). However, manures present different type of matrix and estrogens have different fate and behavior in different matrices (Jacobsen et al., 2005; Ying and Kookana, 2007).

Concluding previously noted estrogens are naturally occurring female sex hormones. They are excreted mainly in conjugated, polar form from human or mammal organisms. Therefore the major sources for estrogens and their conjugates in the environment are on the one hand human excrement and on the other hand animal excrement originating from livestock breeding. The possible sources of natural estrogens in soil, surface and ground water bodies are in animal manure and human waste. These wastes are released to the environment either after extensive treatment in WWTFs, after minor treatment (e.g., irrigation of effluents from oxidation ponds for livestock wastes) or without treatment through direct excretion by grazing livestock. Hormones from all these sources exhibit different fate and transport due to different matrices. This study is focusing on the fate and transport of hormones originating from sludge and biosolids.

2.2.5. Adverse effects of estrogens: endocrine disruption

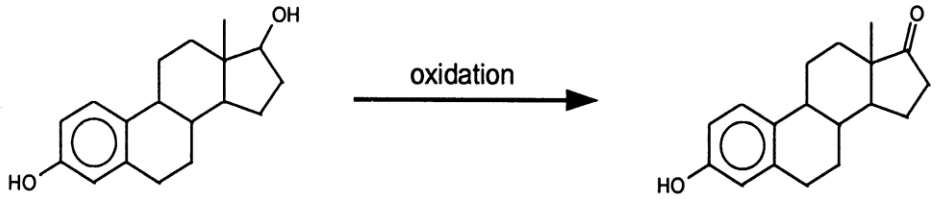
Overall, the steroid estrogens appear to be the most potent endocrine disrupters (EDCs) of sewage effluent, at least in vitro (Johnson and Sumpter, 2001). During last two decades there is an increased public and scientific concern about the potential adverse effects that may result from exposure to a group of chemicals that have the potential to alter the normal functioning of the endocrine systems in wildlife and humans. The International Programme on Chemical Safety (IPCS) defines an endocrine disruptor as "an exogenous substance or mixture that alters the function(s) of the endocrine system and consequently causes adverse health effects in an intact organism, or its progeny, or (sub)populations" (Damstra et al., 2002).

According to the IPCS report (Damstra et al., 2002) concerns regarding endocrine disrupting chemicals are primarily due to i) the adverse health and population effects observed in certain wildlife and ecosystems; ii) the increased frequency of endocrine-related human diseases; and iii) endocrine disruption demonstrated in laboratory trials in animals with exposure to some environmental chemicals. Currently, the effects of estrogens on living species predominantly resulted in disruptions of sexual development (Milnes et al., 2006; Scott et al., 2007). For example, elevated levels of vitellogenin in males and abnormal levels in females represent a widely accepted biomarker for exposure to endocrine disruptors in fish (Sumpter and Jobling, 1995; Scott et al., 2007).

According to Rodriguez-Mozaz et al. (2004a), estrogens and synthetic steroids are the most powerful compounds among the different manufactured organic chemicals with reported endocrine-disrupting properties. Estrone and 17β-estradiol were selected for this research study because they are thought to be responsible for the major part of the endocrine-disrupting effects seen in the aquatic environment (Andersen et al., 2004; Orlando et al., 2004) as well as these natural estrogens are released into the wastewater treatment process in significant amounts (Desbrow et al. 1998; Körner et al. 2001; Snyder et al. 2001, Servos et al., 2004).

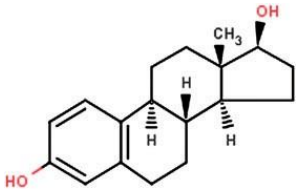
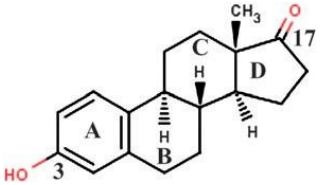
2.2.6. Molecular structure and physicochemical properties of estrogens

Primarily, physiochemical properties have major impact on the fate of chemical compounds in different environmental matrices. Differences between free and conjugated estrogens are based on their physiochemical properties (Jacobsen et al., 2005; Ying and Kookana, 2007). The common feature in the structure of steroid hormones is a tetracyclic molecular framework composed of three phenol rings (A, B and C) and one cyclopentane ring (D), which is termed the cyclo-pentano-perhydro-phenanthrene structure (Table 1.1.). Estrogens contain one condensed aromatic ring (A). Structural differences of the free hormones arise in the cyclopentane ring (D) due to the type and stereochemical arrangement of functional groups (Hanselman et al., 2003; Khanal et al., 2006). Estrone is a degradation product of 17β-estradiol (Ying et al., 2005).



17β-estradiol estrone

Table 2.1. Structures and properties of unconjugated estrogens.

Compound	17β-estradiol	estrone [d]
		
Molecular Weight	272.39	270.37
Log K_{ow} [a]	3.94	3.43
Henry's Law constant (atm m^3 mol^{-1}) [b]	$3.64 \cdot 10^{-11}$	$3.80 \cdot 10^{-10}$
Solubility in water (mg/L at 25°C) [c]	1.51±0.04	1.30±0.08

[a] Lai et al., (2000); [b] Bodzek and Dudziak, (2006); [c] Shareef et al. (2006); [d] A, B, C, and D refer to the ring nomenclature. 3 and 17 refer to the C-atom on which the functional group is placed.

By analyzing the properties of the hormones, in can be concluded that low vapor pressure of the free hormones indicates that volatilization can be excluded as significant source of environmental dissipation (Khanal et al., 2006). Additionally, the octanol-water distribution coefficients (K_{ow}) indicate that both free estrogens (17β-estradiol and estrone) have a very strong tendency to accumulate in organic matter and the low values for their aqueous solubility (Table 1.1.) suggest the same tendency (Dizer et al., 2002).

2.2.7. Fate of estrogens in the environment

Lorenzen et al., (2004) surveyed hormone activity in municipal biosolids and animal manure using YES bioasseys. They found that most samples had detectable activity levels and suggested

that further studies should be undertaken to assess the persistence and fate of these substances when applied as fertilizers to agricultural fields.

There are three main pathways of hormone dissipation in soil, which are currently noted in literature: residue formation, leaching with water and degradation.

2.2.7.1. Residue formation

The formation of non-extractable soil-bound 17β-estradiol residues has been noted by Colucci and Topp (2002) as the major pathway of hormone dissipation was formation of non-extractable residues. The studies by Fan et al. (2007a; 2007b) also demonstrated that ^{14}C-labeled 17β-estradiol will become irreversibly sorbed onto various organic matter fractions (e.g., humic acid, fulvic acid, and humin). Therefore, humic substances in soil can immobilize majority of estrogenic hormones, reducing their bioavailability and toxicity. The study by Colucci et al. (2001) supports this conclusion. They found that both 17β-estradiol and estrone formed non-extractable residues bound to soil, which were only slowly mineralized, confirming that their bioavailability was low. Jacobsen et al (2005) hypothesized non-extractable residues in soil from the fact that little of the remaining soil radioactivity was extractable in their laboratory experiment of radioactively labeled 17β-estradiol and sludge application to soil. They did not characterize the nature of the non-extractable residues but suggested that this residue is likely to present in the form of microbial biomass. Currently there are no studies which fully describe the processes and nature of this residue formation in soil. Yet, research has shown that residues of the hormones can be expected to persist in soils for time frames exceeding months (Casey et al., 2005; Lucas and Jones, 2006; Stumpe and Marschner, 2007). More research is needed to fill this knowledge gap.

2.2.7.2. Leaching

Leaching of hormones from soil has is an effective, but dangerous pathway for the removal of hormones from soil, since it does not dissipate hormones, yet makes them more available to come in contact with aquatic organisms. Preferential flow and runoff have been found to greatly affect hormone concentrations in soil and leachate, creating potential danger for surface and ground water contamination. For example, Yang et al. (2012) found that the hormone mass load correlated with antecedent rainfall amount and that a heavy rainstorm event will create a pulse of

hormones in runoff. Using undisturbed soil columns, Sangsupan et al. (2006) revealed that hormone transport is affected by both chemical and physical non- equilibrium conditions suggesting that preferential flow could lead to ground water contamination. Kjær et al., 2007 showed that pronounced macro-pore flow is also as one of the causes of estrogen leaching into the drainage of an agricultural site after manure treatment. The conclusions of the above studies are supported by findings of Arnon et al. (2008), who investigated the profile under an old (>40 years in operation) dairy farm waste lagoon for its estrogen distribution and found considerable estrogen concentrations (30–210 ng kg^{-1}) were detected down to 32 m below surface and in the ground water at 47 m below surface. They stressed that modeling cannot fully able to explain the detected estrogen concentrations, and suggested hormone-manure interactions and preferential flow paths lead to enhanced transport rates.

All studies involving lysimeters (Dizer et al., 2002; Herman and Mills, 2003; Das et al., 2004; Sangsupan et al., 2006; Casey et al., 2008) observed a significant amount of estrogen hormones in leachate. In soil containing 60 to 65% of sand, 27% of the 17β-estradiol leached through the lysimeters (Sangsupan et al., 2006). A significantly higher estrogenicity of leachate samples from shallow lysimeters compared with that of leachates from deep lysimeters, while the estrogenic effect measured for soil extracts of shallow lysimeters was lower the one from deep lysimeters (Dizer et al., 2002). This suggests that the endocrine disrupting chemicals were mostly associated with water-soluble fractions of organic matter and/or water-suspended fractions of the mineral soil matrix (Dizer et al., 2002). These results are in agreement with other studies that find that hormone degradation mainly occurs in a sorbed phase (Casey, 2003). At the same time, studies such as Carr et al. (2011) show that in drained soils degradation occurs faster than in non-drained ones. Therefore, the experiments with drained lysimeters (as the ones used in this study) can only approximate the reality of field conditions.

The application of sewage sludge to agricultural and forest fields may determine the immobilization of ECDs in soil or their movement to surface and/or ground water. One of the proposed means to reduce agricultural leaching is through the use of biochar. Biochars have been found to modify the soil through increases in surface area and porosity, which are important for sorption of estrogens, as well as cation exchange capacity (Major et al., 2009) and pH (Inyang et al., 2010; Kameyama et al., 2012; Abel et al., 2013; Angst et al., 2013). These changes have been

linked to major benefits, such as increased water retention (Abel et al., 2013; Basso et al., 2013; Ulyett et al., 2014) and enhanced microbiological activity (Steiner et al., 2009; Lehmann et al., 2011). In general, biochar benefits are most pronounced in easy- leaching sandy soils similar to the one in the current study (Jeffery et al., 2011; Abel et al., 2013).

2.2.7.3. Degradation/mineralization

The term degradation refers to any chemical alteration a compound undergoes in the environment (Klöpffer, 1996). If the degradation results in the formation of simple inorganic compounds the term mineralization is appropriate (Klöpffer, 1996).

While most lab studies involving directly spiked soil observed the immediate degradation of 17β-estradiol (Colucci et al., 2001; Lee et al., 2003; Casey et al., 2005; Jacobsen et al., 2005), field studies found long persistence of estorgens for up to 6 months after application (Casey et al., 2005; Lucas and Jones, 2006; Stumpe and Marschner, 2007; Schuh et al., 2011).

The soil environment, however, has not received a lot of attention in terms of the degradation process of estrogens. Over the last decade, researchers have started to investigate this matrix to determine the potential adverse effects of estrogen on human and wildlife health. Generally, 17β-estradiol degrades faster than estrone in soil. This is logical, given the chemical structure of these compounds. The oxidation of the 17β-estradiol molecule can be done by a broad range of enzymes in the soil. In contrast, estrone degradation likely involves ring cleavage that is energetically more demanding.

In a recent study, Stumpe and Marschner (2009) characterized mineralization of radiolabelled estrogens in 17 different soils under various conditions. Although sorption parameters in this study varied greatly for 17β-estradiol, for estrone this did not control estrogen bioavailability since it showed no effects on hormone mineralization. Fan et al. (2007) in their incubation study observed 6% of 17β-estradiol become mineralized via CO_2. Furthermore, one study reported the degradation of 17β-estradiol occurred in sterilized soils and attributed it to abiotic oxidation, which is likely facilitated by mineral surfaces, and the formation of non-extractable, slowly mineralizing soil residues was observed (Colucci et al., 2001). If this is the case biochar has great potential to enhance abiotic degradation by providing a large surface area.

2.2.8. Factors influencing the degradation of estrogens

The abovementioned removal pathways for estrogenic compounds result from the physical and chemical properties of the hormones (such as molecular structure, hydrophobicity, water solubility, dissociation constants which are described in section 2.2.6.), their matrix and surrounding environmental conditions as described below. Studies up to date (e.g., Hamid and Eskicioglu, 2012) point to sorption, abiotic degradation and biodegradation as the main processes of 17β-estradiol and estrone dissipation. Results from laboratory experiments demonstrated that 17β-estradiol rapidly degrades forming estrone as a major metabolite under aerobic conditions in soils, and degradation rates showed some dependence on soil type, pH, biological activity, temperature and moisture content (Colucci et al., 2001, Xuan et al., 2008). However, degradation of these hormones in sludge matrix is not well-studied and the processes themselves are complex with multiple co-interacting environmental factors influencing them. These processes and factors are reviewed below.

2.2.8.1. Soil water content

Soil water status and organic matter are the predominant factors effective in explaining 17β-estradiol in the lysimeter profile. Casey et al. (2003) found that 17β-estradiol concentrations were correlated to lysimeter drainage, perhaps indicating significant colloidal facilitated transport. Colucci (2001) investigated 17β-estradiol dissipation in the several soils under different moisture conditions. He found that in sandy loam soil removal of 17β-estradiol generally increased with increasing moisture content. With the silty loam soil, dissipation was also maximized with higher moisture and slowest in the air-dried soil. The total mass of 17β-estradiol remaining following the incubation was highest in the air-dried soil. The exception appears to be soil moistened to field capacity. Mineralization of 17β-estradiol was minimal in air-dried sandy loam soil, and increased with increasing moisture content except at field capacity, where a decrease in mineralization was noted. Dissipation deviated from first-order kinetics when the soil was air-dried or moistened to field capacity.

2.2.8.2. Oxygen availability

Multiple studies have shown that degradation of hormones readily occurs under aerobic but is impossible or a lot slower under anaerobic conditions (Fan et al., 2007; Joss et al., 2004). In

other words, oxygen status in soils is related to hormone persistence, where low oxygen or anaerobic conditions increase persistence (D'Ascenzo et al., 2003; Fan et al., 2007b; Hemmings and Hartel, 2006; Shappell et al., 2007; Jacobsen et al., 2005; Layton et al., 2000).

The study of removal efficiency of estrone and 17α-ethynylestradiol in municipal WWTPs under various redox conditions, demonstrated the importance of aerobic conditions for the removal of all estrogens (Joss et al., 2004). In agreement with this, Ying and Kookana (2003) showed that the biotransformation of 17β-estradiol to estrone in the loam soil under aerobic conditions is rapid and occurs within 7 days. However, under anaerobic conditions in the soil, little or no degradation of estrone was recorded despite a long study period of 70 days. The calculated half-lives for 17β-estradiol, which was found to be bio-transformed to estrone under both aerobic and anaerobic conditions, were 24 days in the soil.

A study was undertaken by Lorenzen et al. (2006) to assess the persistence of estrogenic substances in soil that could reach agricultural land via fertilization with organic amendments containing estrone, 17β-estradiol and 17α-ethynylestradiol. The compounds rapidly dissipated in soils with half-lives ranging from a few hours to a few days. The authors concluded that the risk of these chemicals to contaminate water was low. The compounds were rapidly removed from aerated soils under temperate growing conditions; this indicates that application methods that minimize preferential flow, or runoff, of animal or human wastes should protect adjacent water from contamination.

Ying and Kookana (2003) characterized the biotransformation of 17β-estradiol and estrone in the loam soil under aerobic and anaerobic conditions. This laboratory study showed that the compounds were degraded rapidly in the soil within 7 days under aerobic conditions. However, during the 70-day study under anaerobic conditions in the soil, little or no degradation of the chemicals was recorded except for 17β-estradiol. The calculated half-lives for 17β-estradiol, which was found to be bio-transformed to estrone under both aerobic and anaerobic conditions, were 24 days in the soil. In contrast, Verlicchi et al. (2012) found that estrogens are highly biodegradable compounds under aerobic and anoxic conditions.

2.2.8.3. Carbon and pH levels

Carbon plays an important role in the degradation of estrogenic hormones, yet, the mechanisms of dissolved organic carbon and estrogen interaction are far from straightforward and will depend on local soil conditions (Zimmerman et al., 2009; Beesley et al., 2011). For soil systems, a very limited numbers of studies considered DOC impacts on the mineralization of hydrophobic compounds, such as hormones (Stumpe and Marschner, 2010). Studies have found that high carbon content in soil provides for an increased degradation rate of hormones (Jacobsen et al., 2005). Controversially, other studies (Sarmah et al., 2008; Stumpe and Marschner, 2010) have demonstrated that organic waste borne dissolved organic carbon (DOC) facilitates transport of estrogens through soil and decreases estrogen mineralization and 17β-estradiol biodegradation. This was likely the result of complexes of estrogens and organic waste borne DOC masking the estrogens by making them difficult to recognize for enzymes and too big for direct plant uptake (Sarmah et al., 2008; Stumpe and Marschner, 2010). This increased persistence combined with higher mobility will increase the risk of estrogen transport to ground or surface water.

Soil with its organic matter domain is considered to be the most important sorbent for hydrophobic organic chemicals. Literature often reports high organic carbon normalized distribution coefficients (N) for estrogens (e.g. Lee et al., 2003; Sarmah et al., 2008; Yu et al., 2004) but soils with a high specific surface area have especially shown to yield low N values (Casey et al., 2003; Hildebrand et al., 2006; Sarmah et al., 2008). This suggests that mineral surfaces are important sorbents for estrogens (Sarmah et al., 2008). Stumpe and Marschner (2010) found that DOC reduced 17β-estradiol mineralization rates significantly by up to 50% during the first three days of incubation. It is hypothesized that estrogen sorption to organic waste borne DOC is significantly lower than to soil indigenous DOC due to aged carbon in soil having a more open structure, yet in the complex interaction between waste borne DOC and soil carbon, the soil carbon is replaced by a more aromatic DOC carbon, which creates more binding sites for hormones to soil (Stumpe and Marschner, 2010). While, in general, the mechanisms for estrogen sorption in presence of organic waste borne DOC were not clearly identified (Sarmah et al., 2008) and disputed, the high aromatic carbon content of biochar is a promising feature as it applies to the removal of estogens.

Biochar is known to increase carbon in soil (Zimmerman et al., 2009) but also known to provide additional surfaces for sorption, which should positively influence the degradation rates of estrogens since nearly all degradation/transformation occurred in sorbed phase (Casey, 2003). Beesley and Marmiroli (2011) found that carbon concentrations were reduced as biochar was leached in a column system, suggesting considerable outputs of carbon in solution from biochar to amended soil systems upon initial application or environmental exposure. Gomez-Eyles et al. (2011) showed a decrease in water soluble carbon after amendment of soil with hardwood-derived biochar, while Beesley et al. (2011) found that biochar increased DOC in soil pore water.

In sorption experiments with DOC and estrogens, up to 17.4% of estrogens in solution were sorbed to DOC and an increasing estrogen sorption was observed in the acidic soil (pH 4.4) (Stumpe and Marschner, 2010). It is hypothesized by Stumpe and Marschner (2010) that the acidic soil has been alkalized by the DOC solutions causing an increase to pH 5.6 in the soil. According to Marschner (1999) pH increases induce SOC swelling, resulting in larger surface areas which could make more sorption sites available for estrogens.

In general pH plays an important role in the movement of hydrophilic estrogens through soil. Harrison et al. (1999) found that high pH (such as in alkaline stabilized sludge products) can increase leaching, since the solubility of some organically complex metals is high under such conditions. In addition, low solution pH is more favorable for destruction of estrogens (Fu et al., 2006). Clara et al. (2004) found that no desorption was detected below pH 9 and a definite desorption occurred for all tested compounds at pH values above 10. About 80% of the initially adsorbed mass is released at high pH values. The optimal pH range for activated sludge is assumed to be between 6.5 and 8.5 (Gray, 1989), while in alkaline treated sludge pH ranges from 7.24 to 12.8 while the mean is 11.9 (Lopan and Harrison, 1993). From the above it is conclude that sludge has lower pH than alkaline stabilized biosolids and more efficient degradation of estrogens in sludge is expected, while applied biosolids may result in more leaching. At the same time, Furlong et al. (2010) reported the attenuation of estrogenic compounds in full-scale WWTF incorporating lime stabilization. Most of the monitored estrogenic compounds decreased in the samples. However the total estrogenic activity increased approximately four times. This shows the need for further research.

2.2.8.4. Sorption

The degree of sorption of organic contaminants to sediment has a major influence on its transport and fate in the environment (Chiou et al., 1998) and the risk of leaching and the extent of contamination of chemicals into groundwater or to surface waters (ter Laak et al., 2006). This is especially true for transport of estrogenic hormones in soil, where complex sorption processes play a major role.

Sorption is the process in which chemicals become associated with solid-phases (Schwarzenbach et al., 2003). Partitioning describes the process of distribution of a molecule between two phases (e.g., aqueous and solid) governed by equilibrium. Sorption, therefore, can be considered as one type of a partitioning process which involves the distribution of organic compounds between pore water and geo-sorbent matrices (Ehlers and Loibner, 2006). Thus, sorption refers to the reversible and irreversible uptake of a compound from an aqueous solution by sorbents such as soil, sludge, sediments or minerals.

Natural estrogen compounds are mainly removed from the aqueous phase by adsorption onto associated solid phases, such as sludge in wastewater treatment or soil in the case of land application (Khanal et al., 2006). Due to the high octanol water partitioning coefficient (Table 1.1.) estogens are expected to interact strongly with the natural organics (Filali-Meknassiet al., 2004). It is apparent that the sorption coefficient is strongly correlated to the organic content of soil and is low for sandy soils. Furthermore, sorption coefficient values are also governed by the specific surface area of the adsorbent and are correlated to clay and silt contents of the soil (Khanal et al., 2006). Clara et al. (2004) and Andersen et al. (2005) investigated sorption of estrone, 17β–estradiol, estriol and 17α-ethynylestradiol onto activated sewage sludge and found that that the sorption of estrogenic compounds can be described by linear adsorption. They calculated that the distribution coefficient (for the estrogens $log\ K_d$ [2.60; 2.84]) is within the range where sorption is a relevant removal process. Ying and Kookana (2003) Sorption test using a batch equilibrium method demonstrated that estrogens were adsorbed onto soils in the order 17α-ethynylestradiol, 17β–estradiol, estrone and estriol. Clara et al. (2004) found that despite the high initial concentration, the batch experiments did not reach saturation, which indicates very high adsorption potential of sewage for estrogenic hormones.

This adsorption potential and aromacity of chemical structure in sludge compounds govern the DOC exchange processes in sludge applied soils and seem to be one of the main processes controlling the overall estrogen desorption from soils in the presence of DOC. As described by Stumpe and Marschne (2010), the sorbed aromatic DOC may provide additional sorption sites on the soil solid phase for the hydrophobic estrogens. Since the remaining DOC in the equilibrium solution is depleted of aromatic compounds, this also reduces its sorption capacity for the estrogens (see section 2.2.8.3. on carbon and pH levels for more details).

A modeling study by Casey et al. (2003) indicated that nearly all degradation and transformation of estrone and 17β-estradiol occurred in the sorbed phase, which is consistent with the TLC tests only detecting ^{14}C sorbed to the soil and the more oxidized polar metabolites detected predominantly in the aqueous phase. Controversially, Stumpe and Marschner (2009) found that although sorption parameters in 17 different soils (under various conditions) varied greatly for 17β–estradiol and for estrone this apparently it showed no effects on hormone mineralization. From the studies above it is logical to conclude that sorption produces non-extractable estrogenic residues in soil, which are slowly released into the environment. High soil sorption affinity indicates that the hormones are unlikely to exhibit significant mobility in soil however; field studies suggest that high sorption affinity does not necessarily preclude hormone transport (Sangsupan 2006). This is consistent with findings by Hildebrand et al. (2007) who determined that at all studied concentrations from 10 to 1000 ng mL^{-1}, estrogens quickly sorb to soils, and soils have a large capacity to bind estrogens, but these endocrine-disrupting compounds can become easily desorbed and released into the aqueous phase. In their experiment >85% of the three estrogens sorbed rapidly to a sandy soil, yet the greatest degree of desorption of >80% of estrogens occurred in sandy soil with the lowest initial concentration of 10 ng mL^{-1}. Estrone sorbed more strongly to soil than 17β–estradiol or 17α-ethynylestradiol and partial oxidation of 17β–estradiol to estrone was observed. Since relative desorption from all soils was greatest with low initial concentrations, at environmentally relevant concentrations, estrogenic hormones present a risk. Sandy soils, as used in the current study, have especially high-risk of leaching since the longest half-lives for 17β-estradiol were observed on the sediment with highest sand and the lowest organic carbon content of all sorbents in the study by Lee et al., 2003. In addition, Sangsupan et al. (2006) found that sorption of 17β-estradiol decreases with soil depth. Sandy soils showed weak sorption compared to other types of soils. 27% of the 17β-estradiol leached

through the lysimeters. Approximately 50% of the remaining soil-bound 17β-estradiol was sorbed in the top 10 cm of soil. Longest half-lives for 17β-estradiol were observed on the sediment, which has the highest sand and the lowest organic carbon content of the sorbents. It is hypothesized that by addition of biochar, more sorption surface may be created and extra retention time may be acquired to enhance hormone degradation.

2.2.8.5. Temperature and pressure

Temperature is one of the physical properties that have a significant influence on the dissipation of estrogens. Degradation of hormones is facilitated under warmer temperatures (Colucci et al., 2001; Jacobsen et al., 2005; Li et al., 2005; Hemmings and Hartel, 2006; Zeng et al., 2009).

Raman et al. (2001) performed a in the laboratory experiments and studied effects of temperature on degradation of 17β–estradiol and estrone sourcing from animal manure. They found that storage of fresh samples at 5°C reduced losses of total estrogens, but did not prevent transformation of 17β–estradiol to estrone. They also found that first order decay constant is dependent on the temperature: higher the temperature, greater the constant and faster the dissipation processes.

Zeng et al., 2009 also studied the effects of temperature on the sorption and biodegradation of 17β–estradiol sourcing from sewage sludge. They observed the same results, as Raman et al., (2001) - increase in the first order decay constant as a result of increased temperature. They also confirmed that the results of their study are consistent with the results of Li et al. (2005) and Auriol et al. (2006).

Degradation of estrogens is dependent on temperature. This dependence is likely the result of changes in bioactivity of microorganisms responsible for degradation in soil, as degradation of 17β-estradiol in soil is a biological process evolving microorganisms and enzymes. Xuan et al (2008) found that the range of temperatures from 15 to 25°C is the most effective for biodegradation. They assume that bioactivity of degradation microorganisms is optimal in this range of temperatures. Results of their study are consistent with the results of Colucci et al. (2001) which found that 17β-estradiol dissipated considerably slower in cold soil.

Pressure is the other physical property that has important influence on the dissipation of estrogens. Apparently, except of ultrasonic cavitation studies that study destruction of pharmaceutical compounds, including estrogens in water, there are no research that studied effects of pressure on degradation of estrogens. While these results are not applicable in the natural conditions, it is important to note some interesting facts they found in their research. In experiments that were performed by Fu et al. (2007) it was found that increased fluid pressure has negative effect on efficiency of degradation reactions. The performed experiments were done in a closed system, meaning that increase in pressure affected the temperature. When they increased pressure from 0 to 30 psig (normal atmospheric pressure is 14.7 psi) and found that the temperature of the system increased to nearly 40°C.

2.2.8.6. Biological activity

Bioavailability also plays a large role in the rate of hormone degradation (Fan et al., 2007b; Jacobsen et al., 2005). Abiotic mechanisms, such as photodegradation, can also degrade steroidal hormones but are insignificant in soils (Fan et al., 2007a; Jurgens et al., 2002; Mansell et al., 2004). Low to non-existent degradation in sterile soils versus increased degradation and mineralization with increasing temperatures indicate that the degradation of estrogens is mainly governed by microbial processes in the soil environment. While most lab studies involving directly spiked soil observed the immediate degradation of 17β-estradiol (Colucci et al., 2001; Lee et al., 2003; Casey et al., 2005; Jacobsen et al., 2005), field studies have found that long persistence of estorgens for up to 6 months after application (Casey et al., 2005; Lucas and Jones, 2006; Stumpe and Marschner, 2007; Schuh et al., 2011).

Background concentrations of estrogens were previously found in other studies (e.g. Casey et al., 2008) and can result from extended half-lives or other reasons, described in the section about removal pathways for estrogenic compounds. According to Lee et al., (2003) half-life of estrogens is dependent on the type of matrix and, among other types of soil estrogens have longest half-life in soils with large sand content. Lucas and Jones (2006) demonstrated that natural exposure matrices could temporarily inhibit the degradation of 17β-estradiol and estrone and speculated that estrogens in soil are metabolized in the co-metabolism of microorganisms. They explained the delayed degradation pattern by the theory that the microbial population would have to adapt to the estrogens before utilizing estrogens and by the presence of antibiotics

in wastes. Antibiotics have been shown to alter 17β-estradiol degradation rates (Xuan et al., 2008; Chun et al., 2005) and are a common component of sludge.

A study by Xuan et al. (2008) supports this conclusion. In their experiment the degradation rate of 17β-estradiol was found to be directly proportional to the amount of non-sterile soil. Controversially, Colucci and Topp (2001a; b) found that 17β-estradiol compound was oxidized to estrone in three different soils, which included autoclaved and non-sterile samples. This suggests a biological transformation. In contrast, estrone was stable in autoclaved soil, suggesting that its removal was microbially mediated. Muller et al. (2008) surveyed estrone, 17β-estradiol and their conjugated forms throughout the WWTP. Primary treatment showed a weak impact on Estrogen concentrations and estrogenicity, however they observed a decrease of >90% of original concentration of both estrogen concentration and estrogenicity during biological treatment. On the base of analysis of estrogens sorbed into the sludge they suggested that biodegradation was the main vehicle for estrogen elimination.

Although most degradation processes described here are co-dependent, Stumpe and Marschner (2009) found that sorption parameters apparently do not control estrogen bioavailability for estrone (K_f ¼ 46.0-517.5 mL^{-1}), while varying greatly for 17β-estradiol (K_f ¼ 21.9-317.5 mL^{-1}). Soil enzymes such as β-glucuronidases and arylsulphatases are believed to be responsible for cleaving the conjugate structure of free estrogens (Lucas and Jones, 2006; Khanal et al., 2006).

Accordingly, bacteria also present a viable degradation method for estrogens in soil and sludge. Coombe et al. (1965) found that exposure of estrone to *Nocardia* sp. resulted in the formation of three degradative products. A plausible mechanism for their formation from estrone is presented. Yoshimoto et al. (2004) have isolated strains of *Rhodococcus,* which specifically degrade estrogens by using enrichment culture of activated sludge from WWTFs. Measurement of estrogenic activities with MVLN cells showed that these strains degrade 17β-estradiol into substances without estrogenic activity. Weber et al. (2005) found a defined mixed culture consisting of *Achromobacter xylosoxidans* and *Ralstonia* sp., which used 17β-estradiol and estrone as growth substrates.

2.2.9. Estrogen behavior in sandy soil

Physicochemical properties of estrogens are dependant of the type of matrix where they reside. The estimated half-life values vary from only a few hours to several months, depending on the type and concentration of the hormone and the water, soil or sediment type (Ternes et al. 1999b; Lucas and Jones, 2006; Schuh et al., 2011).

Generally degradation of estrogens occurs faster in aqueous environment than in soil (Kuster et al., 2004). It takes up to 9 days for estrogens to degrade in water (Williams et al., 1999; Jurgens et al., 2002). Colucci et al. (2001) found that in sandy loam soil removal of 17β-estradiol generally increased with increasing moisture content.

Estrogens are more likely to degrade in matrices saturated with microorganisms. Two studies that were performed by Ternes et al. (1999a, b) found that there is little or no degradation of estrogens in autoclaved soils in comparison with natural soils. Sandy soils show weak sorption compared to other types of soils (Sangsupan et al., 2006).

It is apparent that the sorption coefficient is strongly correlated to the organic content of soil and is low for sandy soils, yet estrogens sorb (>85%) rapidly to a sandy soil but just as quickly desorb (>80%).

Lee et al., (2003) performed a lab study where soil matrices were injected with hormones. They found that half-life for 17β-estradiol is 0.8-1.1 days for silt soil and 4.5-9.7 days for sandy soil. Longest half-lives for 17β-estradiol were observed on the sediment, which has the highest sand and the lowest organic carbon content of the sorbents. Shorter half-lives for 17β-estradiol and the range of estimated half-time values observed in aerobic soil water slurries, which is consistent with what was reported by Colucci et al. (2001) for degradation in unsaturated agricultural soil microcosms.

2.3. Biochar

Biochar is a fine-grained charcoal made by pyrolysis - the process of heating biomass (wood, manure, crop residues, solid waste, etc.) with limited to no oxygen in a specially designed furnace, which captures all emissions, gases, and oils for reuse as energy (Laired et al., 2009).

Equation 1 describes the chemical reacting of wood conversion into biochar, water, carbon dioxide, carbon monoxide and pyrolysis tar (Shoieb, 2013):

$$2C_{42}H_{60}O_{28} \rightarrow 3C_{16}H_{10}O_2 + 28H_2O + 5CO_2 + 3CO + C_{28}H_{34}O_9 \quad (1)$$

Pyrolysis temperature and time of the process define relative ratio of the main components of biochar (carbon, volatile matter, mineral matter (ash) and moisture) (Antal and Gronli, 2003; Brown, 2009; Verheijen et al., 2010).

Almost any form of organic material can be pyrolyzed to make biochar (Laird et al., 2006) Pyrolysis is the thermal decomposition of biomass occurring in the absence of oxygen. Pyrolysis will yield mainly biochar at low temperatures less than 450ºC, when the heating rate is quite slow (Fisher et al., 2002). Heat transfer is a critical attribute of pyrolysis as the pyrolysis process is endothermic and sufficient heat transfer surface has to be provided to meet process heating needs.

2.3.1. Regulations and guidelines concerning biochar application to soil

According to the review of guidelines on practical aspects of biochar application (Major, 2010) in different provinces and states biochar has different regulations. For example in Ontario, Canada, a material which has gone through a process such as pyrolysis or gasification is considered a waste and must be treated as such. However, in the province of Québec, Canada, the Conseil des appellations réservées et des termes valorisants (Council on reserved designations and value-added terms, which regulates organic certification agencies operating in the province) approves wood charcoal as an amendment in organic agriculture.

Biochar is characterized by high porosity, large surface area relative to its volume, and high alkalinity (Besley et al., 2011). The microporous structure of biochar is potentially important for water retention and absorption capacity of the soil (Day et al., 2005; Ogawa et al., 2006). Micropores are usually formed during the production of biochar (Martinez et al., 2006), and are responsible for the larger surface area of charcoal (Brown, 2009) and additional space for microbial residing (Lehmann and Joseph, 2009).

2.3.2. Advantages of using biochar

Biochar enhances plant growth and root development. It contains nutrients such as potassium, phosphorus and magnesium which increase microbial life within the soil. Biochar also promote plant growth through increasing soil levels of available nutrients and better disease and pest resistance. Biochar also reduces fertilizer requirements, it retains nitrogen that is added to the soil through chemical fertilizers, releasing the nitrogen more steadily to the plant and reducing nitrate pollution to rivers (Glasser et al., 2002). In the presence of biochar, important nutrients become more available to the plant roots in the soil. Other important functions of biochar are its ability to raise the pH of the soil, and retain water when functioning as a porous sponge. This helps provide water to plants in some drought-prone soils (Blackwell et al, 2009).

Biochar as a component of compost can have synergistic benefits; it can increase microbial activity and reduce nutrient losses during composting (Dias et al., 2010). In the process, the biochar becomes soaked with nutrients, covered with microbes, and pH-balanced, and its mobile matter content is decomposed into plant nutrient (Blackwell et al., 2009). Pure biochar can act like a sponge and when added to soils it could absorb the nutrients that the plant needs before the plant can get to them. By adding the pure biochar to the compost/ manure it absorbs the nutrients and microbes from the compost/ manure (Wang et al, 2010). In the other study it was found that the biochar-amended laboratory columns considerably improved the retardation of 17β-estradiol sourcing from animal waste (Gibson, 2012).

Biochar has a very large internal surface area, providing a suitable location for chemical reactions to occur (Mukherjee and Lal, 2013Biocharcan modify the soil environment and their benefits are most marked in sandy soils. (Abel et al., 2013; Jeffery et al., 2011). Additionally, biochar can increase the surface area, porosity, cation exchange capacity and pH in soils (Eykelbosh et al., 2015).

Biochar remediation of a contaminated soil can maintain or ideally enhance soil quality. The soil microbial community can be used as an indicator of the quality of a soil and the extent to which degradation has occurred or restoration has progressed (Harris, 2003). The soil biodiversity of contaminated land is usually poor because the contaminants are toxic to soil communities at high concentrations (Zhang et al., 2004). Since biochar is a potentially diverse niche for

microorganisms, the application of biochar to soils may assist the preservation and support of soil biodiversity and biotope for the micro and mesobiota in contaminated soils. This may be the reason for reported increases in microbial biomass and activities of biochar amended soils (Chan et al., 2008).

2.3.3. Effect of biochar on organic pollutants

There is lack of information regarding the effects of biochar on female hormones originating from human waste (sludge and biosolids) in soil and the role of wood biochar in reducing the contamination of agricultural soils by steroid estrogens. Previous studies show that presence of biochar decreases contamination from organic soil pollutants (Cao et al., 2009; Zheng et al., 2010; Jones et al., 2010; Yu et al., 2006; Spokas et al., 2009; Wang et al., 2010; Yu et al., 2006; Spokas et al., 2009; Wang et al., 2010; Yu et al., 2009; Yang et al., 2010) According to a meta-analysis on the utilization of biochar and its influence on organic pollutants, the following organic contaminants are found to be affected by the presence of the biochar as a soil amendment: polychlorinated dibenzo-p-dioxins/dibenzofurans (PCDD/Fs), PAHs, phenanthrene, diuron, atrazine and acetochlor, chlorpyrifos and carbofuran, terbuthylazine, heptachlor exo-epoxide, chlorpyrifos and fipronil, dieldrin and dichloro-diphenyl-trichloroethane (Beesley et al., 2011).

Sorption characteristics of biochar may play an important role in the degradation of hormones because previous studies have shown that sorption is crucial for the degradation of complex organic pollutants. Some studies have found that biochar is responsible for the sorption of pesticides (Cao et al., 2009; Zheng et al., 2010; Jones et al., 2010). Other studies show that biochar-amended soil has higher herbicide sorption than non-amended (Yu et al., 2006; Spokas et al., 2009; Wang et al., 2010). Yu et al. (2009) and Yang et al. (2010) found that biochar also has high sorption affinity for insecticides.

CHAPTER 3
Methodology

In order to evaluate the fate and transportof steroidal hormones in the soil amended with biochar a field experiment was conducted. It was decided to simulate the field conditions of the month of May for the Montreal area (Quebec, Canada). This research attempts to determine the fate of hormones in soil, using soil lysimeters (0.45 m I.D. × 1 m) with established preferential flow. All treatments were performed in sets of three replicas to reduce the effects of potential human and process errors. Manure, sludge, biosolids and biochar were applied in previous years on all lysimeters (top 10 cm). Therefore, the top 10 cm of soil, containing the residues from the previous year, was replaced with new soil of the same type.

3.1. Instruments and materials

3.1.1. Soil characteristics

Physical and chemical properties of soil (ElSayed and Prasher, 2013) are given in Table 3.1. The lysimeters were kept under natural field conditions. They were packed with sandy (with 92.2% sand content) ferro-humic podzol.

Table 3.1. Physical and chemical characteristics of soil.

Property	Value	Units
Soil Type	Sandy	-
Sand	92.2	(%)
Silt	4.3	(%)
Clay	3.5	(%)
Bulk density	1350	(kg m^{-3})
pH	5.5	-
Organic matter	2.97	(kg m^{-3})
Cation exchange capacity	4.9	(cmol kg^{-1})
Hydraulic conductivity	20.13 [a]	(cm Day^{-1})

[a] – Barnes et al., (2014)

Background average concentrations from all depths of hormones were found in each lysimeter by applying the same method that was used for the rest of the soil samples (as described in the section 3.2.6. further in the text).

3.1.2. Sludge and biosolids

The rate of application of 28 tonnes wet weight/ha was used in this experiment, which makes 0.445 kg sludge or biosolids per lysimeter. That is approximately 8 tonnes dry weight/ha of sludge and 20 tonnes dry weight/ha of biosolids. This rate was chosen because it corresponds to the higher estimate of the most used application rate in the most populated provinces of Canada (Ontario and Quebec) (Canadian Council of Ministers of the Environment, 2010). Sludge and biosolids were properly mixed with the top 10 cm of the soil.

The Halifax Wastewater Treatment Facility (Halifax, Nova Scotia, Canada) provided two types of sludge for this experiment: biosolids (alkaline treated sludge) and sludge (raw sludge). Sealed containers with fresh material were delivered by land with travel time of approximately one week without any additional measures for preservation. After receiving, the containers with sludge and biosolids were stored in the refrigerator at a temperature of 1.6°C. Prior to the application, sludge and biosolids were properly hand-mixed.

Biosolids provided by Halifax WWTF were processed by the N-Viro alkaline stabilization process. Biosolids that were used in this experiment were treated with cement kiln dust.

The basic physical and chemical characteristics of applied sludge and biosolids are shown in the Table 3.2.

Table 3.2. Basic properties of applied sludge and biosolids.

Properties	Sludge	Biosolids
moisture content (%)	74	29
17β-estradiol (µg/kg)	913.39±46.98	931.13±42.4
estrone (µg/kg)	457.67±8.07	416.12±7.07

The concentrations of hormones in applied on each lysimeter fertilizers are presented in the Table 3.2. This gives the amount of hormones added with fertilizers: 107.51±5.53 µg of 17β-

estradiol and 53.87±0.95 μg of estrone in sludge, 293.80±13.38 μg of 17β-estradiol and 131.30±2.23 μg of estrone in biosolids.

These amounts correspond to the maximum amounts of hormones in sludge and biosolids in the US and Canada claimed by Lorenzen et al., (2004) and US-EPA (2009) and Andaluri et al., (2012). Maximum amount of total estrogen (estrone and 17β-estradiol) presence showed in the described earlier studies is 1,806 μg/kg DS and it was reported in a survey conducted by the US Environmental Protection Agency US-EPA (2009).

It is important to note that application of sludge into agricultural fields increases concentration of organic matter and nutrients (nitrogen and phosphorus). Generally, DOC in sludge is considerably lower than in biosolids (Hsiau et al., 1997). However, practical experience shows that in order to get full results from sludge application, about three years need to pass (No-Till Farmer Magazine, 2013).

3.1.3. Biochar

Two types of pyrolysis systems are predominantly used in biochar production: fast and slow pyrolysis, where the main distinction in production relates to the heating rate and heating duration. For biochar production, slow pyrolysis is currently seen as the preferred technology as it maximizes biochar yield over production of bioenergy (Lehmann and Joseph, 2009; Sohi et al., 2010). Many studies, that use biochar, choose slow pyrolysis one produced of woody feedstocks (Bruun et al., 2012), however, there are few evidences that show benefits of slow over fast pyrolysis. As an excuse, slow pyrolysis technologies are known for the economical and sustainable production of biochar (Bridgwater, 2007; Brown, 2009; Laird et al., 2009). Slow and fast pyrolysis results in biochars with different physicochemical qualities thus providing differentiated effects in the soil environment upon application (Brewer et al., 2009; Brown, 2009). In a comprehensive research study that was performed by Brewer (2012), it was found that generally slow pyrolysis biochar demonstrate higher surface area than the fast pyrolysis one. Additionally, according to the Sarmah et al. (2010) soil amended with biochar that was produced from wood feedstock exhibits high sorptive capacity for the hormones. In this experiment slow pyrolysis wooden biochar was selected as soil amendment because of all the above reasons.

Table 3.3. Basic soil enhancement properties of biochar.

Properties	Wet Basis	Dry Basis	Units
pH value	9.35	NA	-
Electrical conductivity (1:5 w/w)	3.56	NA	S/m
Potassium (K) - Total and available	4937	4496	mg/kg
Phosphorous (P) - Total	901	820	mg/kg
Phosphorus (P) - Available	11.9	13	mg/kg
Ammonia (NH_4-N) – Mineral 12	13		mg/kg
Nitrate (NO_3-N) – Mineral	4.8	5.2	mg/kg
Moisture content	8.9	0	%
Carbon (C)	70.1	77.0	%
Hydrogen (H)	2.0	2.2	%
Sulfur (S)	0.02	0.02	%
Oxygen (O)	2.74	3.0	%
Ash (total)	15.71	17.3	%

In the current study the application rate of 1% of soil mass was used, which makes 0.215 kg biochar per lysimeter. This amount was chosen because Sohi et al. (2010) in their review of biochar found that majority of the studies apply up to 0.5% of biochar by soil mass yet 1% appears to be the upper limit used. For example, batch studies performed by Lee et al. (2003) and Sarmah et al. (2008) used the application rate of 10 tonnes/ha and the incorporation depth of 0.1 m in the soil surface. An application rate of 10 tonnes/ha makes 0.160 kg of biochar per lysimeter, therefore in this study the application rate was 1% of soil mass. Furthermore, Mukherjee and Lal (2013) reported that application rate of 1% (kg kg^{-1}) biochar can significantly improve the physical qualities of soil through water holding capacity and bulk density. Among other factors that can be improved due to the soil amendment with biochar are: surface area, porosity, aggregate stability and penetration resistance of soils.

The biochar was provided by BlueLeaf Inc., Drummondville, Quebec, Canada. This biochar is a product of the slow pyrolysis of wood lumber at 450°C. The detailed physical, chemical and elemental characterization of slow pyrolysis biochar is provided by the soil control lab of Control

Laboratories Inc., Watsonville, California, USA. Basic physical and chemical characteristics of biochar are shown in the Table 3.3.

3.1.4. Chemicals and reagents

17β-estradiol and estrone in powder form were obtained from Sigma-Aldrich Co LLC©. HPLC-grade acetonitrile, ethyl acetate and methanol were obtained from Thermo Fisher Scientific. All chemicals were of analytical grade. Double–deionized water (Milli-Q, Millipore Corp.) was used in the preparation of standard solutions and mobile phase solutions.

Stock solution (100 mg L^{-1}) was prepared weekly by dissolving the powdered 17β-estradiol and estrone hormones in acetonitrile. Working standard solutions (1 mg L^{-1} and 5 mg L^{-1}, were prepared bi-weekly by dissolving the powdered hormones in acetonitrile/water (50:50, v/v).

All glassware was hand-washed with detergent, rinsed with tap water and purified water, soaked in the HCl bath and finally rinsed with deionized water.

3.1.5. Instrumental analysis

A high performance liquid chromatography (HPLC) system with a UV detector was used in this study to perform all of the analysis and concentration quantification of free hormones in soil and water extracts. Specifically, a quaternary pump LC system from Agilent 1100 series technologies (Germany) equipped with a diode array-ultraviolet detector was used. The detection and concentration tracing of these two estrogenic hormones were carried out through a Zorbax Eclipse Plus C18 column (150 × 4.6 mm) with particle size of 5 μm (Agilent, Santa Clara, CA). The mobile phase was a volumetric mixture of ratio of 60% of purified Milli-Q water and 40% HPLC-grade acetonitrile, with flow rate of 1 mL/min and the injection volume of 100 μL. The column was at the constant room temperature during the analysis. The limit of the detection response for the HPLC-UV method was 0.32 μg mL^{-1} for 17β-estradiol, 0.25 μg mL^{-1} for estrone.

The HPLC–MS/MS analysis was performed on a XDB-C18, 2.1x100mm, 1.8 μm column from Agilent under a H_2O/CAN gradient. Mass spectra were acquired from m/z 50 to 1200 (positive electrospray ionisation) in accurate mass mode.

3.2. Experimental setup

The study of the fate and behavior of estrogens requires reliable and reproducible analytical methods, which are specifically designed for detecting trace concentrations in a variety of environmental samples.

3.2.1. Sorption-desorption experiments

In order to evaluate and characterize sorption-desorption behavior of the estrogen hormones and their mobility, batch equilibrium experiments were done.

The methods that were used are based on the methods presented by Lee et al. (2003) and Sarmah et al. (2010). There were two treatments: soil and biochar treatments. The soil treatment represented a control treatment. The biochar treatment contained 1% (w/w) of biochar based on the application rate of 10 tonnes per ha to soil.

The stock solution of each hormone was prepared in pure HPLC-grade acetonitrile. Six concentrations of estrogens (0.01, 0.05, 0.1, 0.5, 1 and 5 mg L^{-1} prepared in 0.005 M $CaCl_2$) were taken for spiking. 30 mL of each concentration were added to 2 g of each treatment. Treatments were replicated three times in the batch experiment. The treatment samples with hormones were mixed on a rotary shaker at room temperature (22 ±2 °C) in darkness to minimize photolysis and left for 24 hours to reach the equilibrium. After reaching the equilibrium time for each compound, aliquots were centrifuged at 3500 rpm for 10 minutes and the supernatants were transferred to another set of clean polyethylene centrifuge tubes. 1 mL of each replicate of each treatment's aliquot was filtered by 0.22 μm sterile filters and transferred into the amber HPLC vials and analyzed.

At the last step of the sorption test, supernatants were removed from the samples and 30 mL of purified Milli-Q water was added to each treatment sample to complete the desorption test. The centrifuge tubes were left in the darkness on a rotary shaker for 24 hours to reach the equilibrium. The samples were centrifuged, as described in the sorption test, and 1.5 mL of the supernatants were sub-sampled and filtered as described in the previous section and transferred into the amber HPLC vials to be analyzed and to determine the desorbed concentration of hormones in the treatments.

3.2.1.1. Analytical methods

3.2.1.1.1. Sorption isotherms

The sorption isotherm of each hormone was determined by fitting the equilibrium sorption results to the Freundlich sorption model (Young and Weber, 1995):

$$C = K_F C_e^{\,n} \quad (2)$$

where C is the equilibrium solid-phase solute concentration (µg/g), C_e is the aqueous-phase solute concentration (µg/L), K_F is the Freundlich capacity parameter, and n is the isotherm nonlinearity index. The parameter K_F has units of (µg/g)/(mg/L)^n and n is unitless.

The Freundlich adsorption coefficient K_F was calculated using the log transformation of the Freundlich equation.

3.2.1.1.2. Desorption isotherms

Desorption behavior of the hormones was studied by comparing the amount of desorbed to the adsorbed under equilibrium conditions.

The desorption isotherms were determined by fitting the equilibrium desorption results to the Freundlich model, relating the amount of hormones remaining adsorbed in the soil and biochar treatments to the concentration of each hormones in the solution at equilibrium (Young and Weber, 1995):

$$C = K_F C_e^{n} \quad (3)$$

where C is the content of the hormone adsorbed to each treatment at desorption equilibrium (µg/g), C_e is the concentration of the hormones in the aqueous phase at desorption equilibrium (µg/L), K_F is the Freundlich desorption coefficient, and n is the isotherm nonlinearity index. The parameter K_F has units of (µg/g)/(mg/L)^n and n is unitless.

Due to the concentration-dependency between the soil and biochar treatments, it was difficult to compare the sorption-desorption coefficient K_d and the organic carbon normalized sorption coefficient K_{oc} between the soil and the biochar treatments. Therefore, in order to compare the

sorption and desorption isotherms, the concentration-dependent effective sorption distribution coefficient K_d^{eff} was calculated using the following equation (Sarmah et al., 2010):

$$K_d^{eff} = K_F / C_e^{n-1} \quad (4)$$

were K_d^{eff} is the concentration-dependent effective sorption distribution coefficient (L/kg), C_e is the solution phase concentrations at equilibrium (µg/L) which was used at the single solution equilibrium concentration of C_e= 0.5 (mg L^{-1}), K_F is the Freundlich desorption coefficient (µg/g)/(mg/L)^n, and n is the isotherm nonlinearity index (unitless).

The organic carbon normalized partitioning coefficient K_{oc} was calculated using the following equation (Di Toro et al., 1991):

$$K_{oc} = (K_F / C_e^{n-1}) / f_{oc} \quad (5)$$

where K_{oc} is the concentration-dependent OC normalized partition coefficient (L/kg OC), C_e is the solution phase concentrations at equilibrium (µg/L) which was used at the single solution equilibrium concentration of C_e= 0.5 (mg L^{-1}), f_{oc} is the organic carbon content of the soil, K_F is the Freundlich desorption coefficient (µg/g)/(mg/L)^n, and n is the isotherm nonlinearity index (unitless).

3.2.2. Site setup

The field experiment investigating estrogens fate and transport in sandy agricultural soil was conducted in twelve outdoor PVC lysimeters set up at the Macdonald Campus of McGill University, Ste-Anne-De-Bellevue, Quebec (latitude 45°25'38.000" N, longitude 73°55'45.000" W and elevation 39.00 m (Environment Canada, 2013)). Six lysimeters were amended with biochar and other six remained non-amended. Six lysimeters were amended with (raw sludge) and other six - with biosolids (alkaline treated sludge). Therefore, there were 4 sets of three lysimeters, each with the same type of applied amendments and sludge: no biochar with sludge, biochar with sludge, no biochar with biosolids and biochar with biosolids. Random design of lysimeters was performed.

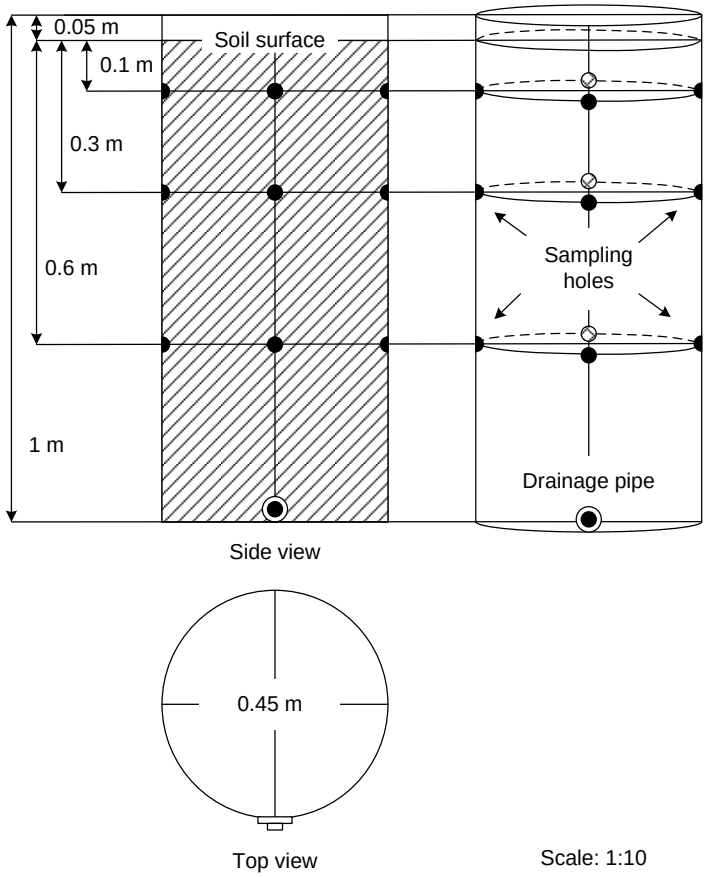


Figure 2. The schematic diagram of the lysimeter.

The lysimeters were made from (0.45 m I.D. × 1 m) PVC tubes, vertically installed, open from the top and sealed at the bottom to 0.6 m × 0.6 m PVC sheets. Each was packed in layers with a sandy soil and adjusted to a bulk density of 1350 kg m^{-3}. A drainage pipe (0.05 m I.D.) was installed at the bottom of each lysimeter. This pipe was designed to collect the leachate from the lysimeter after an irrigation event. Four soil sampling holes with the diameter of 10 mm were made in each lysimeter at depths of 0.1, 0.3 and 0.6 m from the soil surface (Figure 2). In total, there were 12 holes (0.01 m I.D.) along the side walls of the lysimeter. All lysimeters were sheltered to prevent the entry of natural precipitation and protect from the direct UV light. The column tops were kept open to prevent anaerobic conditions from building up. Estrogen hormones degrade while exposed to sunlight.

3.2.3. Rainfall simulation

The designed experiment simulated the real agricultural conditions of one of the most popular field crops in Western Quebec – corn. Natural fertilizers such as manure and biosolids are mainly applied to land outside of the frost season (Manure Management in Canada, 2004), which means that main application of biosolids is performed before corn planting. The optimum corn planting date in Western Quebec is on or before May 10 (Dow AgroSciences Mycogen Seeds, 2011). Alternatively, soil temperatures must reach a minimum of 10°C which is required for stable germination. In the province of Quebec the last date to plant corn and be eligible for crop insurance is June 1st (Dow AgroSciences Mycogen Seeds, 2011). Therefore, the main corn planting season and the main period of application for biosolids is between May 10th and June 1st. Maximum monthly total amount of rainfall for the month of May in fifty-year period 1964-2014 in Ste-Anne-De-Bellevue, Quebec (Environment Canada, 2014) was selected in order to simulate the worst-case scenario of the precipitation after the main application of biosolids. This rainfall amount is equal to 174.3 mm.

It was decided to choose artificial irrigation over the natural precipitation due to the availability of collection of samples before and after the saturation of soil in lysimeters. Total amount of rainfall was divided between three equal irrigations (58.1 mm) during one month. An additional fourth rainfall simulation event was performed. In total, the rainfall simulation was performed by four periodic irrigations on days 0, 15, 30 and 45 after the application of biosolids and biochar. 58.1 mm of water (9.24 L) was poured onto the soil surface of each lysimeter over several hours at a slow ponding rate to prevent flooding.

3.2.4. Collection of soil samples

Soil samples were collected for analysis of the initial content of hormones before the application of amendments and fertilizers (Day -1). The day when biochar, sludge and biosolids were applied on each lysimeter was considered as the beginning of the experiment (Day 0). Irrigation of the lysimeters started on the same day after the application of the treatments. Irrigation was performed on days 0, 15, 30 and 45. Nine sampling dates were selected for soil sample collection after Day 0: 1, 7, 14, 16, 23, 29, 31, 44 and 46. Samples from days 0, 14, 29 and 44 represent unsaturated soil. Samples from days 1, 16, 31 and 46 represent wet soil and samples from days 7

and 23 represent control days with dry soil samples. Here, wet soil samples represent samples of soil with moisture at field capacity. Other soil samples are considered as dry soil samples. Usually, field capacity for sandy soil is 15-25% of volumetric soil moisture content (Cornell University, 2010).

Four soil samples were collected from lysimeters from each of the four different depths of 0, 10, 30 and 60 cm from the soil surface through four sampling holes (one on each side of a lysimeter). The four samples from the same depth of the same lysimeter were mixed together to form a representative soil sample from a specific depth. All soil samples were processed during the hours following soil collection and did not go through any freezing and thawing processes.

3.2.5. Collection of water samples

Leachate samples were collected using drainage pipes, installed at the bottom of each lysimeter at the depth of 95 cm. During each irrigation event, approximately 8 liters of leachate was collected from each lysimeter, while the amount of water applied was 9.24 L (58.1 mm). One liter of homogenized and representative liquid from each lysimeter was obtained for further hormonal content analysis. Water samples were collected at days 0, 15, 30 and 45 and transported to the lab. All water samples were processed in the lab during the next two hours.

3.2.6. Sample extraction methods

All soil samples were oven-dried at 105^{o}C for 24 hours in order to measure the soil moister content. The water extraction was conducted as described in Liu et al. (2011) with minor modifications of the method using solid phase extraction (SPE). The extraction of hormones from soil samples was performed using the method developed by Liu et al. (2011) with some modifications. The modifications are as follows: different HPLC column, different HPLC solvent, different temperature of HPLC column, different injection amount, no freeze-drying for soil samples and no additional cleanup via silica gel cartridge. This method was chosen due to the high recovery results of the extracted hormones.

3.2.6.1. Soil sample extraction

In order to perform the extraction of soil samples, the following steps were done. 0.5 g sample was added to a 50 mL polyethylene centrifuge tube. The sample was extracted with 10 mL of

ethyl acetate in an ultrasonic bath for 15 min and then centrifuged at 3500 rpm for 10 min. The supernatant was transferred into another clean 50 mL polyethylene centrifuge tube. The extraction process was repeated twice using 10 mL and 5 mL of ethyl acetate. Then all approximately 25 mL of combined extract was centrifuged again and resultant supernatant was transferred into a new clean 50 mL centrifuge tube. This supernatant was evaporated to dryness under the gentle nitrogen stream at the room temperature. The residue was dissolved in 1 mL of 50/50 (v/v) acetonitrile-water solution and set for the ultrasonic bath for 5 minutes. The final extract was filtered through 0.22 μm membrane filter into the amber HPLC vial and analyzed as described.

3.2.6.2. Water sample extraction

One liter of each water sample was filtered through a 45 mm filter (Advantec, Japan). Each SPE cartridge (Oasis Co. Ltd, NY, 200 mg/3 cc) was preconditioned with 4 mL of methanol followed by 4 mL of HPLC grade water. The water samples were passed through the SPE cartridges with a flow rate of 5-10 mL min^{-1}. After eluting the compound with 6 mL of ethyl acetate, the extract was evaporated to dryness under the gentle nitrogen stream at the room temperature. The residue were dissolved in 1 mL of 50/50 (v/v) acetonitrile-water solution and set for the ultrasonic bath for 5 minutes. The final extracts were filtered through 0.22 μm membrane filters into the amber HPLC vials and analyzed as described.

3.2.7. Sample storage

It was found that estrogens can get lost in between sampling and further processing of the sample before quantification (Kuster et al., 2005). For laboratory-based studies this implies either demanding preservation techniques or the instantaneous treatment of samples.

Based on the study conducted by Comstock et al. (2001), it is apparent that freeze-thaw cycles have influence on hormone concentrations. Therefore, it was decided to avoid any freeze-thaw cycles and perform an instantaneous extraction of samples. After complete preparation for HPLC analysis, samples were stored up to 5 days at a temperature of at -20 °C in a standard laboratory freezer without light. Such storage does not pose a risk for loss of 17β-estradiol and estrone. Studies such as Colucci et al. (2001), Kuster et al. (2004), Kjær et al., (2007) and Casey et al. (2014) successfully stored samples the same way.

3.2.8. Instrumental analysis

High performance liquid chromatography (HPLC) analysis was carried out to detect and quantify free estrogen hormones. To identify the retention time (RT) of each type of hormones, the HPLC analysis of pure standards with a range of concentrations from 0.1 to 10 mg L^{-1} was performed. Pure standards were prepared by dissolving the stock solution in 50/50 (v/v) purified Milli-Q water and HPLC-grade acetonitrile. Based on the chromatogram peak areas of pure standards and linear regression, five-point calibration curve equations for each hormone were found.

The best detector response for identification of both 17β-estradiol and estrone was at 200 nm with the retention of 8 min (17β-estradiol) and 13 min (estrone). The optimal detection wavelength was determined by preparing a combined solution of 17β-estradiol and estrone at 10 μg mL^{-1} in mobile phase. The UV absorbance of the solution was then measured on a Shimadzu UV 160A UV-Visible Recording Spectrophotometer (Shimadzu, Japan) from 200-300 nm in a full-scan mode. Additional reverse-phase liquid chromatography tandem mass spectrometry (HPLC-MS/MS) analysis was performed for verification of the results from the HPLC analysis.

Calibration curve equations were used to determine the present concentrations of hormones in samples. The limit of the detection response for the HPLC-UV method was 0.32 μg mL^{-1} for 17β-estradiol, 0.25 μg mL^{-1} for estrone.

3.2.9. Data analysis

3.2.9.1. Recovery test

The recovery test methodology that was used in this study was developed by Liu et al. (2011). They received good test results: 101.2±6.0% and 105.5±5.1% for 17β-estradiol and estrone respectively. Different laboratories showed different performance and in order to find out the effectiveness of the performed extraction tests in the current laboratory, recovery tests were performed. Replicated samples of hormones from different matrices were tested for recovery results. In order to perform the recovery tests spiked and non-spiked with hormones samples were tested in parallel. Recovery results were calculated from the difference between the two samples.

3.2.9.2. Statistical analysis

The statistical analysis of variance in concentrations of hormones over the experiment time was conducted using analysis of variance (ANOVA) as described by Wallenstein et al. (1980) with repeated measures approach. To determine if biochar has increased the holding capacity of soil and if hormones are likely to get lost faster, while leaching less to ground water, we have to determine if there is a significant difference between treatments. Specifically, the Dunnett test was used. The calculations were performed using the XLSTAT (Addinsoft, 2014).

3.2.9.3. Mass balance

In order to evaluate loss rates and amounts of the hormones, a mass balance analysis was performed. At each sampling date, the mass of hormones recovered from each lysimeter was calculated as the sum of the recovered hormones in soil samples in all depths of the soil profile. The total amount of hormones was calculated while considering the amount of hormones left in soil, leached with water and lost, while taking into account the recovery error:

$$M_{in} = \sum R \cdot M + M_d \tag{6}$$

where M_{in} – amount of hormones added to a lysimeter (µg), R – a recovery coefficient (based on the results of the recovery test, shown in the Table 3.4.) (unitless), M – amount of detected hormones (µg) and M_d - amount of lost hormones (µg).

The total amount of detected hormones was calculated using this equation:

$$M = M_{soil} + M_{water} \tag{7}$$

where M – amount of detected hormones (µg), M_{soil} – amount of detected hormones in soil (µg) and M_{water} – amount of detected hormones in water (µg).

The amount of detected hormones in soil was calculated using the following equation (8). Chadwick et al. (1990) used similar methods of calculation of chemicals in soil profile.

$$M_{soil} = a \cdot \rho \cdot \sum_i^k C_i \cdot h_i \tag{8}$$

where M_{soil} – amount of hormones detected in soil (µg), a – lysimeter surface area, which is 0.159 (m^2), ρ – soil bulk density (kg DS m^{-3}), C_i - laboratory reported analytical concentration of hormone in depth range i (µg kg^{-1} DS), h_i – depth of soil layer (m), i – depth coefficient with depth ranges (cm): 0-5, 5-25, 25-45, 45-95 (values are ranging from the soil surface to the bottom of the lysimeter) (unitless).

The amount of hormones detected in water was calculated using this equation:

$$M_{water} = C_{water} \cdot V \quad (9)$$

where M_{water} – amount of hormones detected in water (µg), C_{water} - laboratory reported analytical concentration of hormones in water (µg L^{-1}) and V - volume of leachate collected from a lysimeter (L).

In order to simplify used terms regarding the mass balance, the following terms are used: leached hormones - laboratory reported mass of hormones in water, sorbed hormones - laboratory reported analytical mass of hormones in soil and lost hormones – the rest of the hormones that left from the subtraction of leached and sorbed hormones from the initial mass of added hormones.

The half-life *t½* of the hormones was calculated using the following equation (Pirkle et al., 1989):

$$t_{1/2} = t(ln2)/[\ln(C_0/C_t)] \quad (10)$$

where *t½* is the half-life in days, *t* is time in days, ln2 is the natural logarithm of 2, C_0 is the concentration at t=0, and C_t is the concentration at t.

CHAPTER 4
Results and Discussion

The results obtained in this study on the fate and transport of estrogen hormones in soil are presented and discussed. To evaluate sorption and desorption characteristics of the biochar, sorption-desorption analysis was performed. Various comparisons have been made to determine the effect of different treatments and biochar on the hormone leaching. First, each treatment and fertilizer was analyzed for the significant changes over time in both soil (sorbed hormones) and water (leached hormones) samples. Second, an analysis was performed to determine whether or not there is a significant difference between the effects of treatments and fertilizer (in both soil and water samples). The soil sample analysis accounted for different depths in soil. Additionally, the same analysis with split data was performed: dry (before irrigation) and wet (the day after irrigation) soils samples were analyzed separately. Finally, based on the mass balance results, total amounts of hormones, which were sorbed, leached and lost, were calculated.

4.1. Sorption-desorption behavior of estrogens

4.1.1. Sorption isotherms

Sorption plays a significant role in the fate and transport of chemicals in soil. Therefore, the sorption experiments in the present and absence of biochar were carried out. The sorption and desorption isotherm parameters are presented in the Tables 4.1. and 4.2.

Table 4.1. Sorption isotherm parameters for 17β-estradiol and estrone.

Hormone	Treatment	K_F	N	R^2	K_d^{eff}	$log\ K_{oc}$
17β-estradiol	B	32. 879	0.908	0.974	36.082	4.298
17β-estradiol	S	21.909	1.552	0.954	15.879	3.301
estrone	B	22.983	0.805	0.968	25.258	4.222
estrone	S	3.980	0.952	0.977	3.879	2.327

Note: S – soil treatment, B – biochar treatment (1% of biochar added to soil).

Table 4.2. Desorption isotherm parameters for 17β-estradiol and estrone.

Hormone	Treatment	K_F	N	R^2	K_d^{eff}	$log\ K_{oc}$
17β-estradiol	B	22.352	0.954	0.958	24.279	3.232
17β-estradiol	S	0.780	1.012	0.935	1.079	1.782
estrone	B	19.020	0.953	0.954	19.279	3.064
estrone	S	0.805	1.097	0.947	0.779	1.622

Note: S – soil treatment, B – biochar treatment (1% of biochar added to soil).

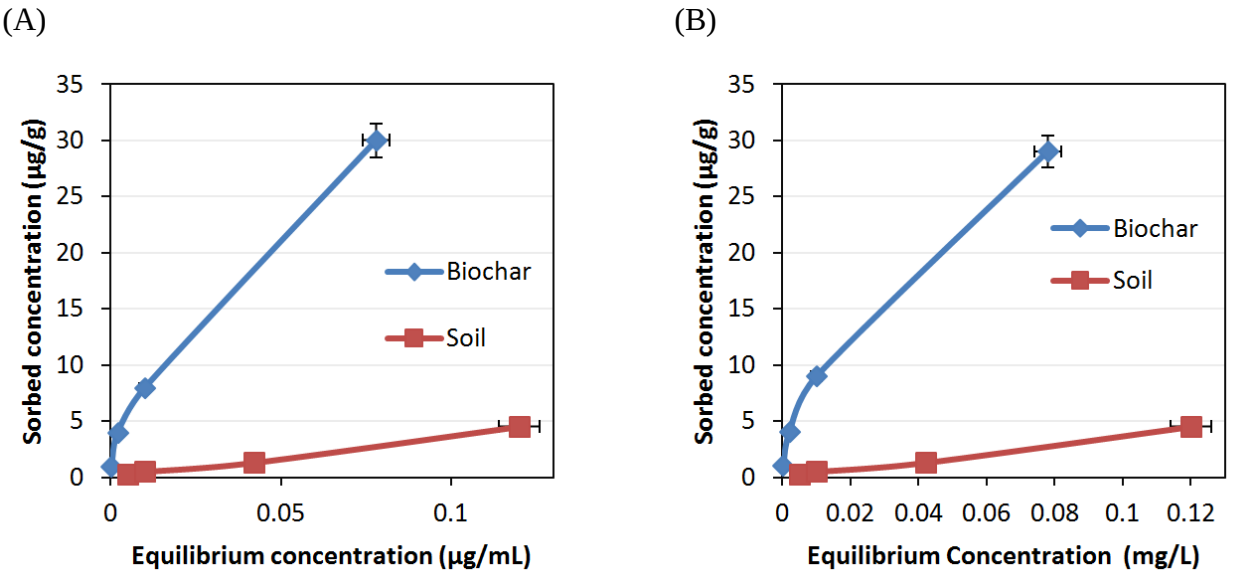


Figure 3. Sorption isotherms of 17β-estradiol (A) and estrone (B) for soil and biochar treatments.

The sorption isotherms for both hormones (17β-estradiol and estrone) in soil and biochar treatments are demonstrated in the Figure 3. The sorption isotherms of 17β-estradiol and estrone in soil and biochar treatments fit the Fruendlich model well, with R^2 values between 0.95-0.97. The 17β-estradiol sorption isotherms for the soil and biochar treatments exhibited a similar pattern. Higher K_F values are obtained by increasing the concentrations of both hormones. A significant difference (p<0.05) was observed between the effective distribution coefficient K_d^{eff} (L/kg) for each hormone in the soil and in biochar. Also, a significant difference (p<0.05) was observed between the effective distribution coefficients for soil and biochar treatments. The K_d^{eff} and the K_F values of 17β-estradiol were significantly higher (p<0.05) than those for estrone. This

indicates lower sorption affinity of 17β-estradiol compared to estrone. Additionally, K_F values for both estrogens were significantly greater (p<0.05) in the presence of biochar. This could be due to the availability of a greater number of additional sorption sites provided by the biochar. It has been reported that biochar holding capacity to estrogens are primarily depending on the micropore surface area in a given biochar (Peterson et al., 2013).

For a better evaluation of the mobility of hormones in the soil and the effect of the biochar amendment, the soil/water-organic carbon partition coefficient K_{oc} was calculated for hormones in both soil and biochar treatments. The calculated *log* K_{oc} in the soil treatment, were 1.782 and 1.622 for 17β-estradiol and estrone respectively, which indicates low sorption affinity of hormones in the sandy soil and high probability of leaching from the soil. The *log* K_{oc} value for estrone was greater than 17β-estradiol, in the presence of biochar. The results demonstrated the significant binding ability of estrogens to the surface of the tested slow pyrolysis biochar. Similar conclusions were also obtained by Sarmah et al. (2010).

4.1.2. Desorption isotherms

Freundlich model was also employed in modeling the desorption of estrogen hormones in both soil and biochar treatments. Desorption isotherms of estrone showed the lowest effective distribution coefficient at the desorption equilibrium (K_d^{eff} = 0.779 L/kg) without presence of biochar, indicating higher possibility of estrone being desorbed and leached from the sandy soil, as compared to 17β-estradiol. A statistically significant difference (p<0.05) was observed between the biochar and soil treatments in the desorption of hormones. Desorption resistance of the biochar treatment for 17β-estradiol was much stronger than that for estrone. The amount of estrogens desorbed in the biochar treatment was significantly lower than in the soil treatment. However, biochar was more resistant to the desorption of hormones at lower concentrations. In comparison to the sorption processes, desorption kinetics is slower due to diffusion processes (Hamaker and Goring, 1976). Additionally, reversible sorption of estrogens has been reported and the rate of desorption has been found to be slower, depending on the sorbent matrix (Ren et. al., 2007).

4.2. Recovery test

In order to precisely quantify the amount of hormones in soil and water samples, a better understanding of recovery processes is needed to achieve the best evaluation of extraction methods and minimize matrix interferences. Therefore, the recovery test was performed for different matrices i.e. water, soil, biochar and their combinations with sludge and biosolids. The results of the recovery test for this study showed consistent results, which are shown in the Table 4.3. They were used in the calculations of the amounts of hormones. A recovery was performed for all matrices. The final reported concentrations and amounts of hormones were respectively adjusted.

Table 4.3. Recovery test results in different matrices.

Matrix	17β-estradiol	estrone
Soil	68.32±8.2	61.41±6.7
Soil and Biochar	76.37±7.8	60.55±8.6
Sludge	60.45±5.6	64.81±3.7
Sludge and Biochar	55.41±3.2	66.46±12.8
Soil and Sludge	65.82±12.5	69.48±7.4
Soil, Sludge and Biochar	67.59±10.4	66.53±5.2
Biosolids	70.56±7.5	75.64±8.1
Biosolids and Biochar	75.22±10.8	69.34±5.6
Soil and Biosolids	69.41±3.5	61.27±7.3
Soil, Biosolids and Biochar	68.03±6.4	64.38±6.4
Water	80.56±3.2	85.81±4.5

Note: the showed results are mean (%)±standard deviation (%) (n = 3, replicate samples at the same time).

The recovery test results are variable (Table 4.3); however, they meet the norms which range from 56 to 125%, as per previous studies (Takigami et al., 2000; Farre et al., 2006; Hintemann et al., 2006; Sangsupan et al., 2006; Suzuki and Maruyama, 2006; Miege et al., 2009). Low recovery values could be the result of irreversible adsorption of the estrogen to sludge or other

extraction materials (i.e., alumina, hydromatrix, HLB cartridge) (Sangsupan et al., 2006). Loss of hormones during the various steps of extraction analysis may also play a role in lowering recovery. Additionally, the lower recoveries of estrogens in the sludge samples could be due to the presence of certain matrix interference effects. The average recoveries of estrone and 17β-estradiol in activated sludge and sediment samples were 94 and 88%, respectively (Chen et al., 2012). There is a need to obtain a balance between the removal of interfering compounds and loss of hormones during clean-up steps (Gomes et al., 2004). The amount of hormones recovered in the recovery tests should be considered as the maximum extractable quantity (Ternes et al., 2002). As demonstrated by Gomes et al. (2004) and Ternes et al. (2002), recovery experiments using the solid phase of sludge are more challenging than the aqueous phase.

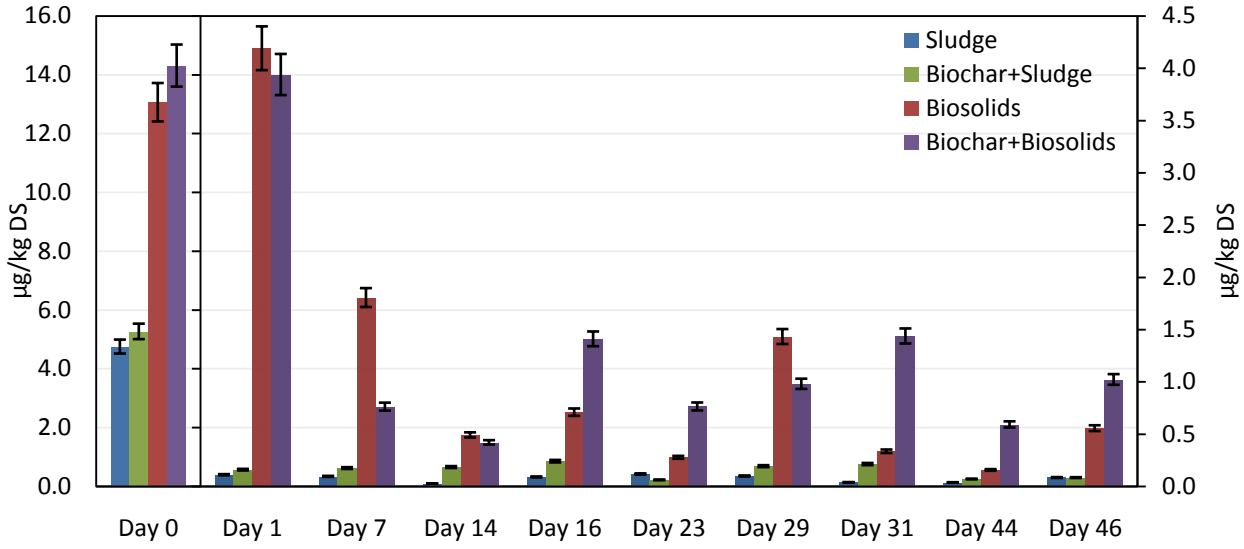
Background average concentrations from all depths of hormones were found in each lysimeter. Across all lysimeters, they averaged to 3.5±2.4 and 2.7±2.1 μg/kg DS for 17β-estradiol and estrone, respectively. Such background concentrations of hormones persisting in soil were previously found in other studies (e.g. Casey et al., 2008). It results from extended half-life (up to several months) (Casey et al., 2005; Lucas and Jones, 2006; Stumpe and Marschner, 2007; Schuh et al., 2011) or from other reasons described in the section concerning fate of estrogenens in the environment (section 2.2.7.). Additionally, according to Lee et al. (2003), half-lives of estrogens are dependent on the type of matrix and estrogens have longest half-life in soils with large sand content.

4.3. Residues of estrogens in soil

The statistical analysis of variance on measured hormone concentrations over time was done using the repeated measures ANOVA with the Dunnett test (Tables 1-6 in the appendix). The analysis showed that there are significant differences among the tested treatments over time and depth ($p<0.05$). Based on the concentrations of hormones detected in soil samples, hormone levels reduced over time. This could be due to the biological or chemical degradation involving the hormones in topsoil and lower depths.

The statistical analysis revealed the presence of significant differences ($p<0.05$) in the behavior of both hormones in the presence of biochar in the sludge and biosolids treatments. At the same time, only 17β-estradiol showed significant difference between sludge and biosolids fertilizers in

soil (Tables 1 and 5 in the appendix). Initial concentrations of 17β-estradiol, found at the soil surface on Day 0, were 5.27 and 14.31 µg/kg in the sludge and biosolids treatments, respectively (Figures 4-7). In the sludge-applied lysimeters, concentrations of 17β-estradiol dropped significantly to about 0.11 µg/kg after the first irrigation on Day 1, and then decreased to 0.08 µg/kg at Day 46. This drop in 17β-estradiol could have resulted from its downward movement in the soil profile. At depths of 10, 30 and 60 cm, 17β-estradiol concentrations ranged between 0.19-2.07 µg/kg on Day 1 and Day 7 from the first irrigation event. Then, the residues of 17β-estradiol were reduced to the lowest value at Day 46 in all depths. In the biosolids-applied lysimeters, more than 50% of 17β-estradiol concentrations decreased in the topsoil after Day 7 of irrigation (4.19 µg/kg), then decreased to 0.56 µg/kg at Day 46. A similar trend was observed for 17β-estradiol at depth 10 cm; however, at the depth of 30 cm, 17β-estradiol concentrations were almost stable at the concentration of 1 µg/kg from Day 14 to Day 31. Then a drop to 0.22 µg/kg on Day 44 was observed, followed by an increase to 0.55 µg/kg. At the depth of 60 cm, 17β-estradiol concentrations were measured at 1.38 µg/kg on Day 14 and then dropped to 0.17 µg/kg on Day 46.



Note: The figure is combined from two Y axes: Day 0 refers to the left axis and the rest to the right axis.

Figure 4. Concentrations of 17β-estradiol at the surface.

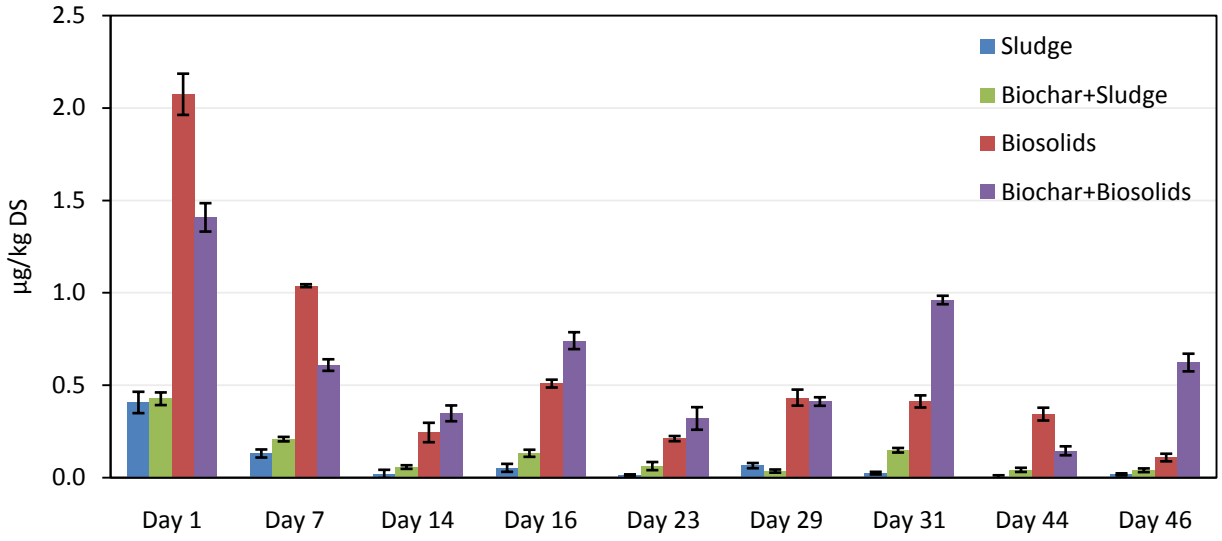


Figure 5. Concentrations of 17β-estradiol at 10 cm depth.

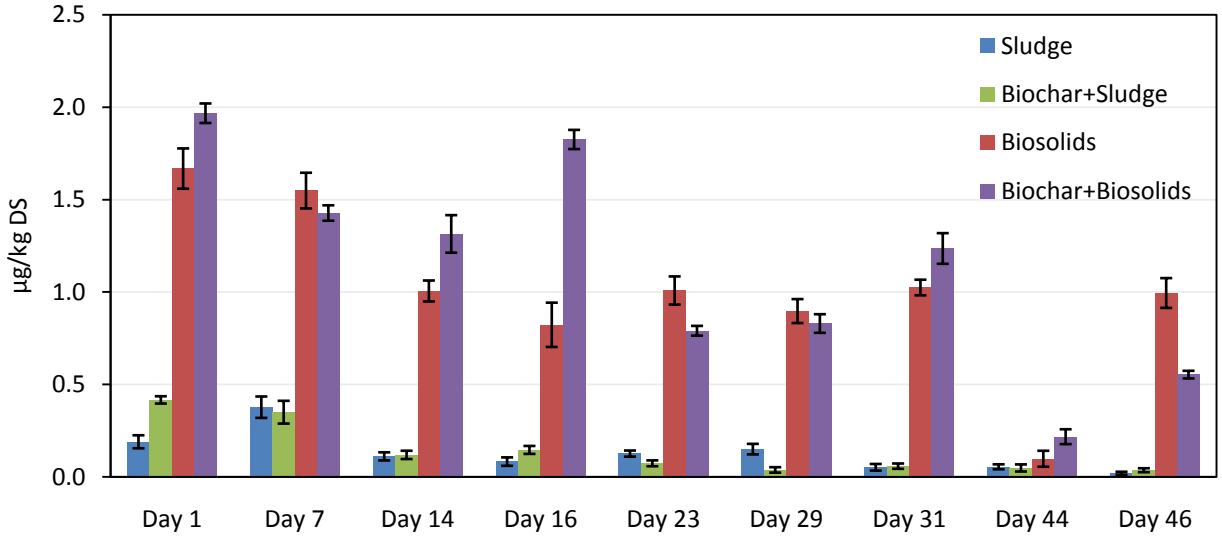


Figure 6. Concentrations of 17β-estradiol at 30 cm depth.

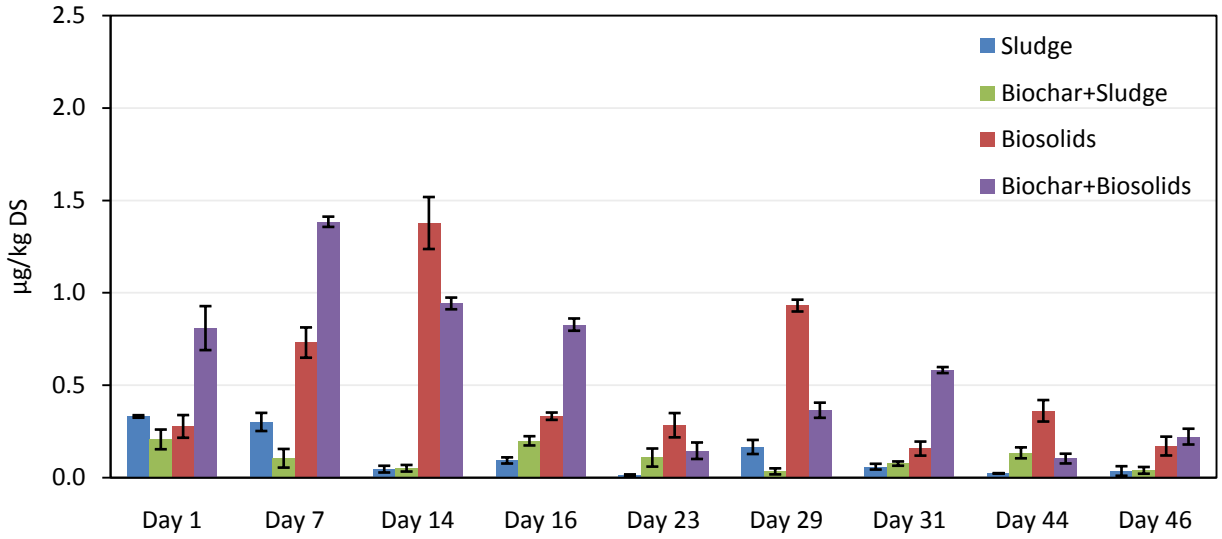


Figure 7. Concentrations of 17β-estradiol at 60 cm depth.

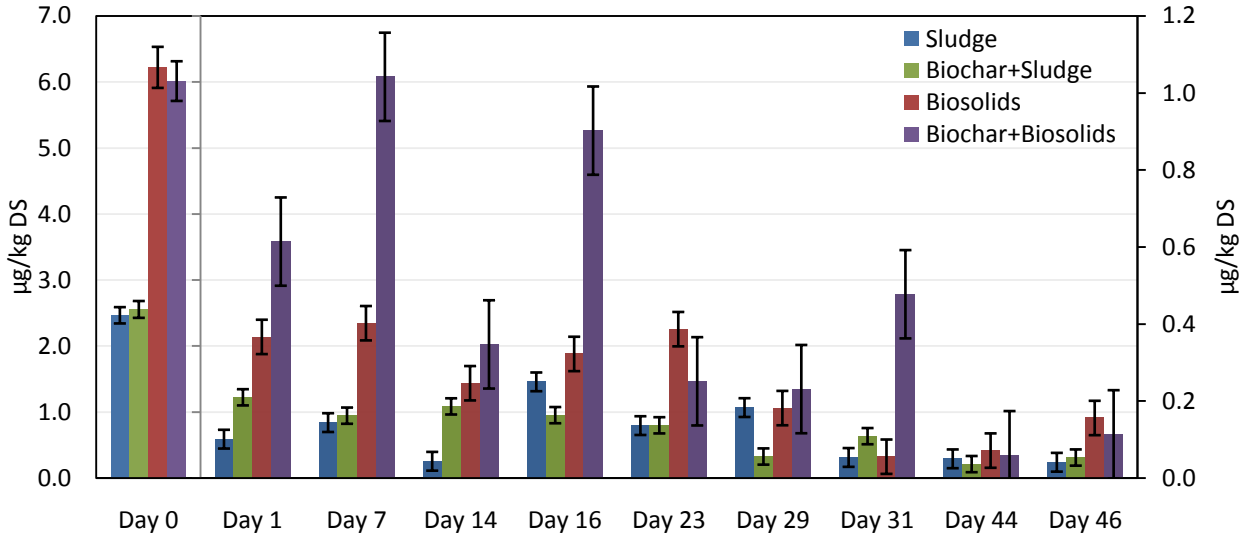
In the case of estrone, the maximum concentrations were measured on Day 0 at the values of 2.55 and 6.22 µg/kg at the soil surface in sludge and biosolids treatments, respectively (Figures 7-10). In the sludge-applied lysimeters, 40% of the initial concentrations of estrone were detected on Day 1. Then estrone concentrations were increased to 1.04 µg/kg after the second irrigation from Day 16 to 29, followed by sharp decrease on Day 44 and Day 46. At 10 cm depth, measurable concentrations of estrone were detected at 0.44 µg/kg on Day 16. Then, 55% of the initial amounts of hormones were lost by Day 29, followed by a further decrease up to Day 46. Similarly, at the depth of 30 cm, concentrations of estrone were detected at 0.27 µg/kg on Day 16, and then an 80% decrease was observed until the end of the season. Estrone exhibited the same trend at the depth of 60 cm.

In the biosolids applied lysimeters, about 65% of estrone concentrations were decreased in the topsoil after one day of the first irrigation. Then, a further 50% of estrone concentrations were reduced by Day 29, followed by almost 90% increase by Day 46. At 10 cm depth, estrone concentrations increased from 0.2 to 0.58 µg/kg by day 16 then decreased considerably by Day 46. At the depth of 30 cm, estrone concentrations reduced over time form the concentration of 0.92 µg/kg at Day 1 to 0.16 µg/kg at Day 23 then was detected at concentrations from 0.09

µg/kg. At the depth of 60 cm; however, higher estrone concentrations were detected by Day 1 (0.73 µg/kg) then reduced to 0.31 µg/kg by Day 29 then reduced noticeably by Day 46.

In the lysimeters receiving 1% slow pyrolysis biochar, the behavior of 17β-estradiol and estrone was found to be fertilizer-dependant. There was no significant difference observed in the presence of biochar in the sludge-applied lysimeters. However, in the biosolids-applied lysimeters, higher concentrations in the topsoil and at depths of 10 cm and 30 cm were measured for both hormones in the last two weeks.

Statistically, biochar has increased the sorption of 17β-estradiol in sludge-applied soil at the depth of 10 cm. In biosolids-applied soil, biochar has increased the concentrations of 17β-estradiol detected at the topsoil and at 10 cm depth. For estrone (Figures 8-11), biochar has promoted the sorption in sludge-applied soil at the depth of 10 and 30 cm, while in biosolids-applied soil, the effect of biochar was pronounced (p<0.05) at the depth of 30 cm.



Note: The figure is combined from two Y axes: Day 0 refers to the left axis and the rest to the right axis.

Figure 8. Concentrations of estrone at the surface.

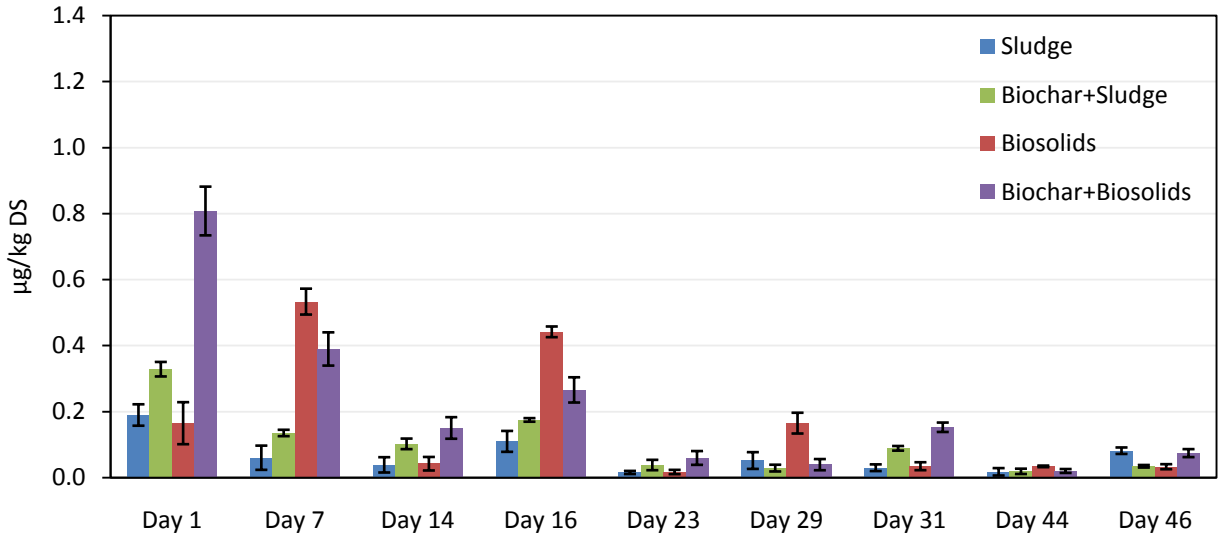


Figure 9. Concentrations of estrone at 10 cm depth.

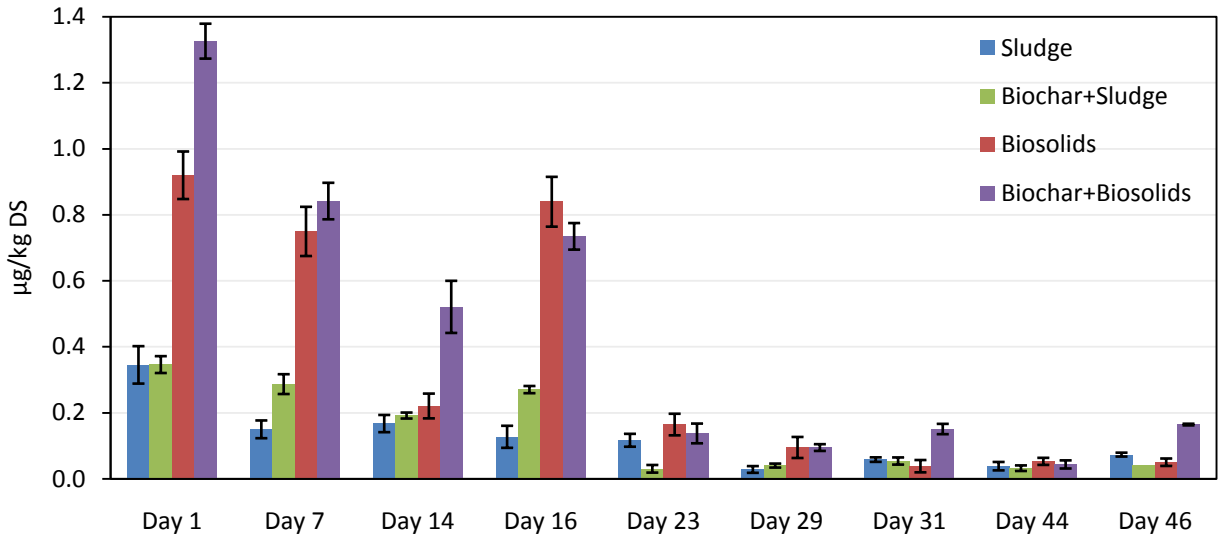


Figure 10. Concentrations of estrone at 30 cm depth.

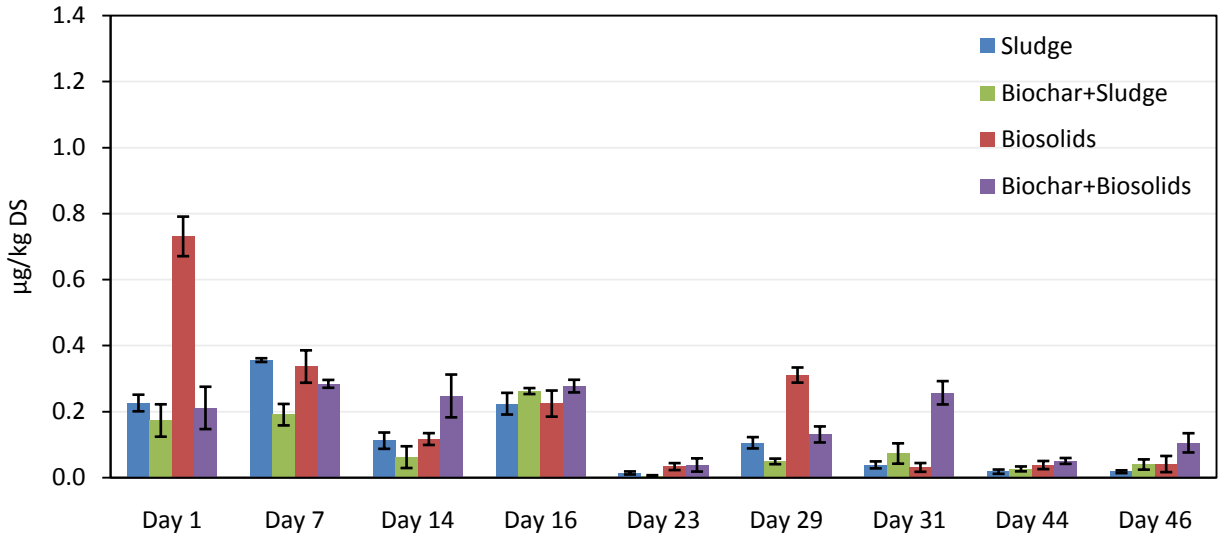


Figure 11. Concentrations of estrone at 60 cm depth.

Statistical analysis showed significant differences at the level of 5% in the behavior of both hormones in the soil profile. Results revealed that 17β-estradiol is more likely to dissipate near the soil surface, where more oxygen is available. At the same time, estrone has a tendency to dissipate in deeper soil profile where conditions are more likely to be anoxic. This observation is in agreement with the findings of Andersen et al. (2003) and Sangsupan et al. (2006); they found that steroid estrogens degraded slowly in WWTFs, as 17β-estradiol could be oxidized to estrone in a few hours followed by slower degradation rate. Similarly, Furuichi et al. (2006) and Ermawati et al. (2007) reported that the removal efficiency of estrogens in aerobic conditions was higher than in anaerobic conditions, indicating the effect of oxidation on the transformation process. The higher detected concentrations of estrone compared to 17β-estradiol in this study were due to the fact that 17β-estradiol could have bio-transformed into estrone (Johnson and Sumpter, 2001).

The results of this study confirmed the significant role of biochar in the observed retention of 17β-estradiol and estrone in the sludge and biosolids treatments. Both hormones were recovered in considerable amounts in the presence of biochar from the surface and lower depths. More influence was observed in estrone compared to 17β-estradiol. This could be explained by the fact that the obtained values of the sorption coefficient of 17β-estradiol (32.87 L/kg) were higher than

the corresponding values for estrone (22.35 L/kg). This allows greater amounts of estrone to move downward, hence increasing the mobility in the soil profile.

It was noted that 17β-estradiol is greatly influenced by the type of the applied fertilizer in the presence of biochar; while estrone exhibited a similar trend but to a lesser extent. Fundamentally, the sorption of estrogens onto sludge is governed by the physicochemical characteristics of the suspended solids and the coexisting chemicals, as well as on ambient factors such as temperature, pH, ion strength, complicated agents and the residence time in WWTFs.

The observed mobility of estrogens in this study could be explained by the findings of Ren et al. (2007) who reported that adsorption process for estrone and 17β-estradiol was reversible. This fact allows much longer time for their slow desorption; therefore, reversibility could change accordingly. Additionally, the results of thermodynamic analysis in their study showed that higher desorption ratio of both estrogens was achieved within 3 hours at 20°C compared to 24 hours at 41°C.

The retention capacity of biochar depends on the type of feedstock, pyrolysis conditions and the degree of aromatic condensation. This in turn could potentially influence the holding capacity of biochars to organic contaminants and the desired resistance to their desorption in the soil-biochar media. Further, the physical characters of the tested biochar could be of much importance, the micropore surface area is a key factor in predicting the adsorption potential of any given biochar. In the light of this point, Peterson et al. (2013) indicated that the higher micropore surface area of the biochar derived from switchgrass is responsible for the greater sorption capacity of estrone and 17β-estradiol compared to the biochar derived from corn stover. Additionally, the summary results of the Dunnett test for all hormones, treatments, fertilizers and depths were conducted on the split data (dry and wet samples). The results for dry and wet data are shown in Tables 3 and 4 in the appendix respectively.

Results from wet soil samples showed more consistency than the results from dry soil samples, showing almost the same statistical results, as all soil samples. Thus, the analysis of wet soil samples is sufficient for the analysis of the difference between treatments and fertilizers in soil amended with biochar. It can be seen in wet soil samples that biochar showed a significant effect ($p<0.05$) at 0 and 10 cm depths for 17β-estradiol in both fertilizers; also it showed significant

difference between sludge and biosolids amended with biochar at the depths 0, 10 and 30 cm. On the other hand, estrone showed significant differences between both treatments and fertilizers at the depths 10 and 30 cm.

4.4. Residues of estrogens in water

The fate of estrone and 17β-estradiol in the leachate of sludge and biosolids lysimeters in the presence and absence of biochar was measured. Water samples analysis shows that that the concentration of hormones in the leachate decreased over time (Figures 12 and 13).

The concentrations of 17β-estradiol decreased from 1.11 µg/L after the first irrigation to 0.19 µg/L after the fourth irrigation event. Biochar had no observed effect at this point; however, in biosolids-applied lysimeters, a significant amount of 17β-estradiol was found ($p<0.05$) in the leachate. At the end of the season, the concentration of 17β-estradiol reduced from 1.60 µg/L to 0.32 µg/L. Considering the presence of biochar, concentration of 17β-estradiol was higher after the first irrigation (2.29 µg/L), and then reduced to 0.51 µg/L by the end of the season. Estrone exhibited a similar trend in the sludge-applied lysimeters, with no effect of biochar's presence after the first irrigation. However, biochar showed a significant effect ($p<0.05$) in reducing the leachate from the second irrigation event to the end of the season. This confirms the binding potential of biochar in reducing leaching of estrone from the sludge applied soil. On the contrary, the leached amount of estrone in biosolids-applied lysimeters was higher. Estrone concentration reduced from 2.56 µg/L to 1.17 µg/L at the end of the season. In the presence of biochar, estrone concentration was 25% higher after the first irrigation (3.05 µg/L), and then reduced to 1.32 µg/L after the last irrigation event. Based on the results obtained from the sorption experiments, estrone showed lower ability to sorb to biochar, compared to 17β-estradiol, and this observation was confirmed by the lower sorption coefficient values of estrone (22.98 L/kg) as compared to 17β-estradiol (32.87 L/kg). Furthermore, calculated desorption coefficients were 22.35 and 19.02 L/kg for 17β-estradiol and estrone, respectively. Evidentially, the amount of estrogens, measured in the leachate from the biosolids-amended lysimeters, indicated slightly higher mobility in the presence of biochar. Considering the higher initial amount in the biosolids, this observation is vital and raises the concern in the selection process of the best fertilizer type and the potential role played by biochar in reducing groundwater contamination.

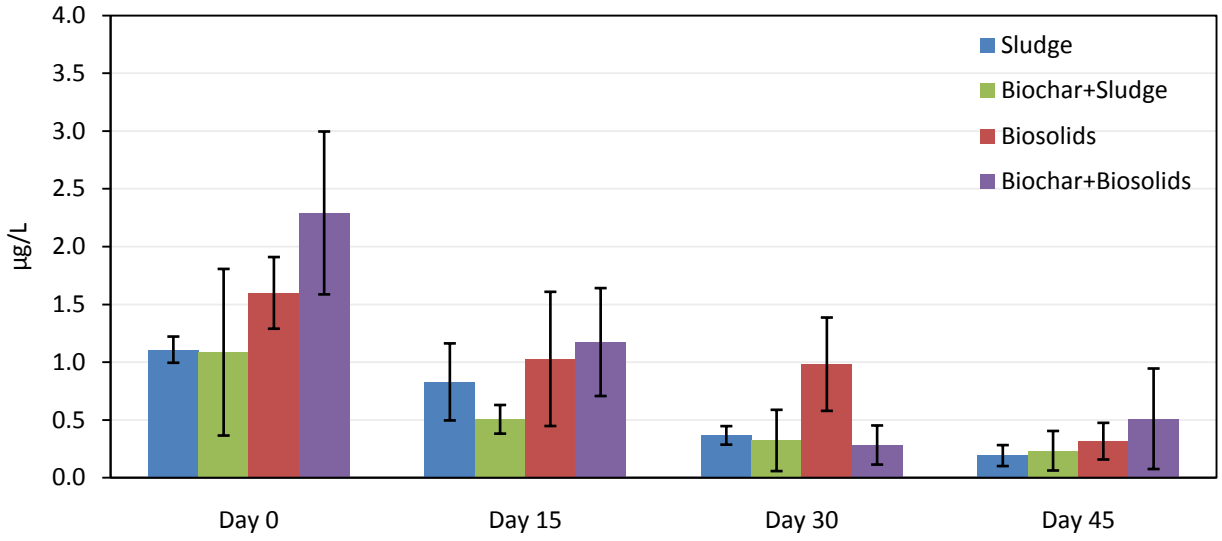


Figure 12. Concentrations of 17β-estradiol in leachate.

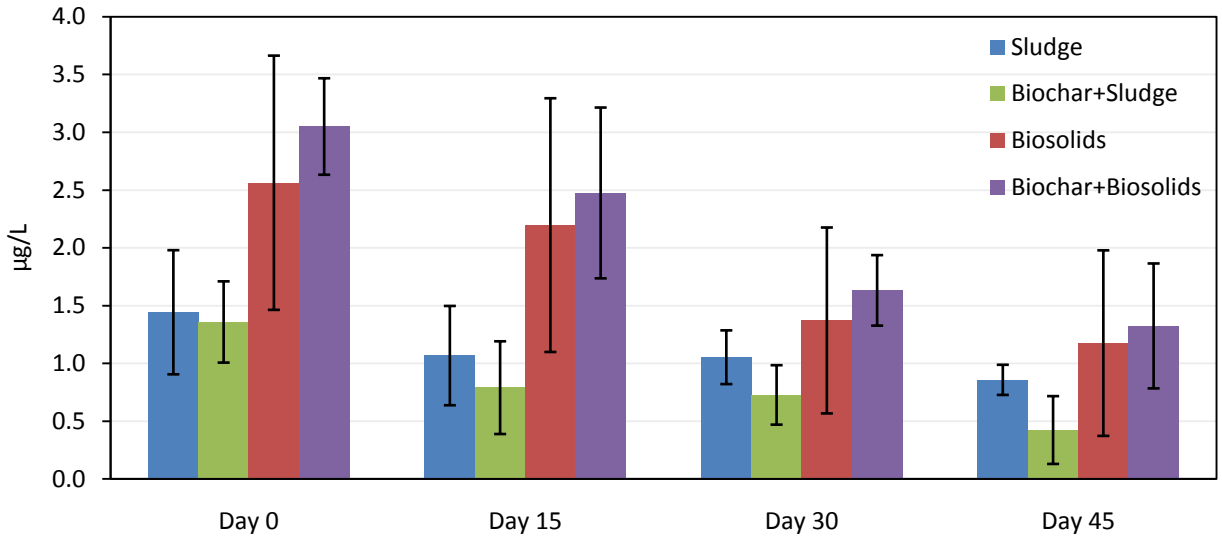


Figure 13. Concentrations of estrone in leachate.

The recovered concentrations of both hormones in this study were higher than those detected in the effluents from WWTFs. Estrogens have been detected in the effluents of WWTFs in different countries at concentrations ranging up to 158 ng/L for 17β-estradiol and 147 ng/L for estrone (Desbrow et al., 1998; Ying et al., 2002; Fernandez et al., 2007). Furtheremore, the lowest

concentration of 17β-estradiol in the study (0.32 µg/L) is found to be much higher than the effective estrogenic concentrion (16 ng/L) for fish and aquatic organisms (Zha et al., 2008; Jin et al., 2013).

The results of Dunnett test (Table 2 in the appendix) show that the selection of fertilizer made a significant difference for concentrations of 17β-estradiol. More 17β-estradiol leached in sludge than in biosolids without biochar treatment. Leachate of estrone showed significant improvement (with a lower percentage in mass balance) with biochar in sludge-amended soil. Contrary to this, leached estrone in biosolids-amended soil was significantly greater with the addition of biochar.

Hydrophobicity of sludge, combined with the low polarity of the estrogenic compounds, makes sorption onto sludge the most suggested process for their removal from wastewater (Martin et. al, 2012). However, it is reported that physical adsorption is the dominant binding mechanism for estrogens due to their weak binding energy. Therefore, they are more likely to be desorbed from sludge into the effluents. This was confirmed by the reversible sorption isotherms of estrogens from sludge (Ren et al., 2007).

Estrogens are moderately hydrophobic compounds they do not ionize at normal environmental pH. They could potentially be sorbed to sludge particles, organic matter and humic acid. The unconjugated steroidal estrogens, estrone and 17β-estradiol, are less soluble in water; therefore, they are relatively hydrophobic, as confirmed by their $log\ K_{ow}$ values, 3.13 and 4.01, respectively. Estrogens are weak acids (pKa, 10.77 and 10.71). It is reported that at high pH, they lose phenolic hydrogen. Moreover, they become increasingly less soluble with increasing ionic strength, at neutral pH (Shareef et al., 2006). This could explain the higher leachate in the biosolids-applied lysimeters, considering the alkaline treatment of the sludge to produce biosolids. At higher pH, biosolids surface is negatively charged. Under this condition, estrogens molecules will be negatively charged as well. Thereby, the expected electrostatic interaction will be repulsive, leading to the partitioning of estrogens in the aqueous phase. Similarly, biochar particles will be negative at high pH, resulting in low adsorption capacity. The presence of other compounds in the sludge could negatively affect the sorption potential of biochar. Zhang and Zhu (2005) indicated that adsorption capacity of the activated carbon was reduced with

increasing estrogens concentrations and by the presence of other compounds, such as surfactants and humic acid.

4.5. Mass balance

The mass balances of hormones in lysimeters and in leachate samples were calculated based on the mass balance equations presented in equations 6 to 9. The results are presented in Tables 4.4.-4.7.

Hormone, initially applied to soil, significantly declined from high concentrations after 46 days and four irrigations. Detailed final amounts of hormones left in the lysimeters are shown in the Table 4.4-4.7. As for sludge fertilizer, biochar showed slightly better performance in preventing leaching of 17β-estradiol, in comparison with estrone. In biosolids-amended soil, biochar actually increased the leaching of 17β-estradiol and estrone. With biosolids, there was more leaching, in general, but biosolids-applied soil, without biochar, showed the lowest (best) result for the amount of hormones in leachate as a percentage of the original amount. Sludge-applied soil showed the worst result.

Half-life of the hormones were calculated and presented in the Table 4.8. Half-life of 17β-estradiol show consistent values (7.66 and 7.88 days) under the sludge fertilizer, while half-life under the biosolids fertilizer show different, but also consistent values (10.10 and 12.08). Half-life of estrone shows consistent and similar values ranging between 7.78 and 8.65 days. The results are confirmed by the Colucci et al. (2001) and Lee et al. (2003), confirming extended half-life for estrogens in sandy soils and shorter half-life for 17β-estradiol in anaerobic conditions.

Table 4.4. Mass balance of 17β-estradiol in a soil profile and cumulative leachate.

Soil treatment and sludge fertilizer.

	Soil profile					
Day	0-5	5-15	15-45	45-95	Leachate	Total
0	101.98	0.23	0.23	0.88	-	103.32±12.45
1	1.18	13.10	10.15	35.56	11.08	71.28±7.64
7	1.01	4.22	20.19	32.37	-	57.79±12.45
14	0.28	0.65	5.92	4.97	-	11.82±4.28
16	0.95	1.71	4.40	10.02	8.69	25.78±6.27
23	1.25	0.47	6.72	1.38	-	9.83±2.05
29	1.05	2.10	8.02	17.84	-	23.01±12.37
31	0.40	0.79	2.75	6.40	3.99	14.33±2.80
44	0.39	0.16	2.89	2.48	-	5.92±2.91
46	0.89	0.58	1.01	3.9	1.74	8.11±4.40

Biochar treatment and sludge fertilizer.

	Soil profile					
Day	0-5	5-15	15-45	45-95	Leachate	Total
0	113.04	0.73	1.02	2.20	-	116.99±21.17
1	1.69	13.75	22.32	22.24	10.86	70.86±15.19
7	1.86	6.71	18.74	11.28	-	35.59±4.08
14	1.97	1.85	6.35	5.48	-	15.65±7.64
16	2.57	4.25	7.79	21.41	5.05	41.07±3.17
23	0.64	2.02	3.91	11.69	-	18.26±7.35
29	2.06	1.15	1.98	3.68	-	8.87±2.51
31	2.27	4.78	3.08	8.19	3.22	21.54±7.08
44	0.74	1.36	2.55	14.44	-	19.09±6.71
46	0.88	1.29	1.89	4.26	2.83	11.15±4.95

Note: soil profile demonstrates mass (µg) of a hormone in a range of depths (cm) in a representative lysimeter; leachate – mass of a hormone in cumulative leachate (µg); total – cumulative mass of a hormone in a representative lysimeter (µg).

Table 4.5. Mass balance of 17β-estradiol in a soil profile and cumulative leachate.

Soil treatment and biosolids fertilizer.

	Soil profile					
Day	0-5	5-15	15-45	45-95	Leachate	Total
0	280.42	0.35	3.40	4.78	-	288.95±34.28
1	44.97	66.79	89.47	29.77	12.66	243.66±15.57
7	19.37	33.44	83.08	78.47	-	214.36±12.28
14	5.27	7.87	53.93	47.88	-	114.95±4.25
16	7.60	16.39	44.11	35.71	10.28	114.09±7.90
23	2.97	6.81	54.07	30.47	-	94.32±5.68
29	15.37	13.95	48.10	50.93	-	128.35±15.98
31	3.59	13.28	54.94	16.89	9.82	98.52±14.34
44	1.67	11.08	5.23	38.84	-	56.82±5.37
46	5.69	3.51	53.35	18.37	3.16	84.08±4.93

Biochar treatment and biosolids fertilizer.

	Soil profile					
Day	0-5	5-15	15-45	45-95	Leachate	Total
0	307.17	1.82	3.98	7.97	-	320.94±45.25
1	42.27	45.34	105.54	86.65	24.37	304.17±30.71
7	8.17	16.62	76.57	148.59	-	249.95±20.91
14	4.50	11.21	70.50	101.19	-	187.40±18.08
16	15.53	23.87	97.91	88.89	11.74	237.94±27.28
23	8.19	10.32	42.38	15.65	-	76.54±9.12
29	10.51	13.27	44.49	39.17	-	107.44±10.85
31	15.43	30.95	66.27	62.49	2.99	178.13±15.54
44	6.34	4.69	11.63	11.10	-	33.76±4.05
46	10.96	20.06	29.66	23.85	5.10	89.63±10.45

Note: soil profile demonstrates mass (µg) of a hormone in a range of depths (cm) in a representative lysimeter; leachate – mass of a hormone in cumulative leachate (µg); total – cumulative mass of a hormone in a representative lysimeter (µg).

Table 4.6. Mass balance of estrone in a soil profile and cumulative leachate.

Soil treatment and sludge fertilizer.

	Soil profile					
Day	0-5	5-15	15-45	45-95	Leachate	Total
0	52.92	0.11	0.32	0.58	-	53.93±6.18
1	1.08	6.12	18.51	24.29	22.39	72.39±4.08
7	1.54	1.95	8.03	38.23	-	49.75±4.06
14	0.47	1.25	8.97	12.04	-	22.73±7.85
16	2.68	3.54	6.82	24.06	12.99	50.09±7.45
23	1.46	0.51	6.26	1.54	-	9.77±9.07
29	1.96	1.67	1.51	11.36	-	16.50±3.46
31	0.57	0.97	3.11	4.17	16.24	25.06±4.95
44	0.54	0.57	2.05	1.96	-	5.12±2.35
46	0.44	2.63	3.93	1.94	8.56	17.50±6.48

Biochar treatment and sludge fertilizer.

	Soil profile					
Day	0-5	5-15	15-45	45-95	Leachate	Total
0	54.82	0.37	0.61	0.96	-	56.76±21.24
1	2.25	10.58	18.56	18.60	13.57	63.56±15.31
7	1.74	4.36	15.39	20.49	-	41.98±4.37
14	2.00	3.30	10.28	6.68	-	22.26±4.91
16	1.75	5.64	14.49	27.17	7.88	56.93±5.07
23	1.47	1.24	1.62	0.40	-	4.73±1.25
29	0.60	0.94	2.14	5.30	-	8.98±3.65
31	1.17	2.87	2.88	7.87	7.25	22.04±4.64
44	0.39	0.62	1.72	2.86	-	5.59±1.99
46	0.57	1.12	2.19	4.29	4.22	12.39±4.35

Note: soil profile demonstrates mass (µg) of a hormone in a range of depths (cm) in a representative lysimeter; leachate – mass of a hormone in cumulative leachate (µg); total – cumulative mass of a hormone in a representative lysimeter (µg).

Table 4.7. Mass balance of estrone in a soil profile and cumulative leachate.

Soil treatment and biosolids fertilizer.

	Soil profile					
Day	0-5	5-15	15-45	45-95	Leachate	Total
0	133.53	0.23	0.95	0.61	-	135.32±25.13
1	3.93	5.23	49.34	78.46	28.95	165.91±20.34
7	4.32	17.18	40.21	36.15	-	97.86±12.35
14	2.64	1.36	11.83	12.56	-	28.39±5.17
16	3.46	14.23	45.03	24.10	21.95	108.77±13.05
23	4.15	0.55	8.81	3.61	-	17.12±4.02
29	1.95	5.23	5.08	33.37	-	46.53±4.91
31	0.59	1.12	2.05	3.34	15.48	22.58±5.45
44	0.77	1.10	2.83	4.11	-	8.81±2.66
46	1.67	1.07	2.69	4.43	11.74	21.6±3.24

Biochar treatment and biosolids fertilizer.

	Soil profile					
Day	0-5	5-15	15-45	45-95	Leachate	Total
0	129.07	0.51	0.88	1.65	-	132.11±22.64
1	6.59	26.02	71.14	22.69	30.48	156.92±18.24
7	11.18	12.56	45.15	30.52	-	99.41±12.23
14	3.72	4.85	27.94	26.58	-	63.09±14.07
16	9.68	8.56	39.41	29.78	24.73	112.16±9.50
23	2.70	1.93	7.37	4.13	-	16.13±4.95
29	2.48	1.27	5.07	14.06	-	22.88±7.04
31	5.12	4.92	8.09	27.61	16.30	62.04±10.54
44	0.64	0.66	2.33	5.45	-	9.08±3.85
46	1.22	2.40	8.81	11.33	13.23	36.99±7.45

Note: soil profile demonstrates mass (µg) of a hormone in a range of depths (cm) in a representative lysimeter; leachate – mass of a hormone in cumulative leachate (µg); total – cumulative mass of a hormone in a representative lysimeter (µg).

Table 4.8. Half-life of 17β-estradiol and estrone in soil.

Treatment	Fertilizer	17β-estradiol	estrone
Soil	Sludge	7.88±1.66	7.78±2.01
Biochar	Sludge	7.66±1.53	8.24±0.48
Soil	Biosolids	10.10±3.12	8.65±1.32
Biochar	Biosolids	12.08± 4.27	8.03±0.76

Note: half-life $t_{1/2}$ values measured in days.

Detailed final amounts of hormones leached from the lysimeters in leachate are shown in the Tables 4.4-4.7 and removal efficiencies in the Table 4.10. For sludge and biosolids fertilizers, biochar showed lower removal efficiency in the case of 17β-estradiol compared to estrone. Overall, efficiency of removal of estrone in the leachate was notably higher than that for 17β-estradiol.

Table 4.9. Removal efficiency of 17β-estradiol and estrone in the soil.

Treatment	Fertilizer	17β-estradiol (%)	estrone (%)
soil	sludge	93.99±21.71	82.72±21.09
biochar	sludge	92.08±28.54	84.50±13.33
soil	biosolids	71.29±18.73	92.33±27.97
biochar	biosolids	70.29±22.70	81.42±22.95

Table 4.10. Removal efficiency of 17β-estradiol and estrone in the leachate.

Treatment	Fertilizer	17β-estradiol (%)	estrone (%)
soil	sludge	23.21±6.94	81.92±26.13
biochar	sludge	22.76±13.09	61.11±25.27
soil	biosolids	13.36±5.55	55.60±29.92
biochar	biosolids	15.51±6.75	64.54±16.34

Based on the mass balance equations, removal efficiencies of hormones were calculated. Tables 4.9. and 4.10. show removal efficiencies of hormones in the soil and leachate respectively.

Removal efficiency of 17β-estradiol in both treatments and fertilizers showed considerably better results in comparison with removal efficiency of estrone. Removal efficiency of estrone in both treatments and fertilizers showed notably worse results. Persistence of estrone is a known fact and has been widely studied (Baronti et al., 2000; Carballa et al., 2004; Servos et al., 2005; Hashimoto et al., 2007; Hamid and Eskicioglu, 2012).

In order to perform full statistical analysis of the data, repeated measures ANOVA (Tables 1 and 2 in the appendix) analysis was done. The rescaled total masses of hormones in soil samples were analyzed for effectiveness of the treatments. From the statistical analysis (Tables 1, 2, 5 and 5 in the appendix), it was concluded that there is no statistically proven difference between treatments, since no significant difference was found between treatment groups. Strong changes within all groups overtime were found, showing that hormones dissipated from the soil overtime. It is important to note, that statistical significance, found in the combined results from within and between groups, only confirms absence of real difference between treatments (Tables 1, 5 and 6 in the appendix).

Partial significance was found between soil and biochar treatments in biosolids ($p<0.10$) for both hormones. Significance found in the combined results from within and between groups confirms this result (Tables 1, 5 and 6 in the Appendix).

CHAPTER 5
Summary and Conclusions

A lysimeter study was undertaken to evaluate the mobility of 17β-estradiol and estrone originating from sludge and biosolids in sandy soil. Additionally, the retention ability of 1% slow pyrolysis biochar, as it relates to estrogens in sludge and biosolids, was investigated. Twelve lysimeters packed with sandy soil were used to conduct the study. The lysimeters were covered with a rainfall shelter. To simulate a worst-case scenario, no crops were planted. Soil and water samples were taken at pre-determined time intervals and were analyzed in the laboratory. A batch equilibrium experiment was also performed to assess the sorption/desorption behavior of both hormones and quantify the partition coefficients (K_F/K_{Fd}) for the tested estrogens in the presence and absence of biochar.

The following conclusions were drawn from this study:

5.1. Residues of estrogens in soil

1. In soil, estrogens were initially detected at the soil surface in high concentrations, and then they significantly declined ($p<0.05$) after 46 days. The higher detected concentrations of estrone, compared to 17β-estradiol, in the soil profile confirmed the known fact that transformation of 17β-estradiol into estrone is more likely in the presence of oxygen (Andersen et al., 2005), which would be the case at the soil surface of the lysimeter.

2. It was evident that the addition of biochar to agricultural soils fertilized by sludge could decrease the mobility of hormones in the soil profile, thus reducing the risk of soil and groundwater contamination.

3. The sorption behavior of estrogens made a significant difference ($p<0.05$) in terms of their retention in the soil. The recovered amount of 17β-estradiol in soil profile was lower than estrone in the sludge and biosolids treatments. This conclusion was confirmed by the results of batch equilibrium experiments where the calculated effective sorption coefficient of 17β-estradiol and estrone were 36.08 and 25.05 (L/kg), respectively. In the presence of biochar, a similar trend was observed and the calculated values of the effective sorption coefficient of 17β-estradiol and

estrone were 15.87 and 3.87 (L/kg), respectively. Therefore, a greater amount of estrone could move downward into the lower depths.

5.2. Residues of estrogens in water

1. In sludge-applied lysimeters, the concentrations of 17β-estradiol decreased from 1.11 µg/L, after the first irrigation, to 0.19 µg/L at the end of the season. While, in biosolids applied lysimeters, the concentrations of 17β-estradiol reduced from 1.60 µg/L to 0.32 µg/L. This clearly showed the higher binding potential of biochar in reducing leaching of 17β-estradiol from biosolids applied soil, compared to the sludge applied soil.

2. In the case of estrone, the concentration in sludge-applied lysimeters was 1.50 µg/L after the first irrigation and then declined to 0.91 µg/L by the end of the season. However, in the presence of biochar, a significant effect ($p<0.05$) was observed in reduced concentrations in the leachate. The concentrations of estrone decreased initially from 1.40 to 0.45 µg/L at the end of the season, which is about 50% less than the treatment without biochar. In biosolids applied lysimeters, a higher amount of estrone was detected in the leachate, compared to 17β-estradiol. Interestingly, slightly higher conventions of estrone were recovered in the leachate in the presence of biochar.

3. The difference in the behavior of estrone in sludge and biosolids could be attributed to the hydrophobic nature of estrogens. Since, biosolids in this study underwent alkaline treatment; pH is expected to be high. This will result in a repulsion interaction between the negative biosolids surface and the negatively charged estrone molecule.

4. The observed mobility of estrogens could be also explained by the reversible sorption of estrogens. Based on the results, the calculated values of the effective desorption coefficients of 17β-estradiol and estrone were 24.27 and 19.27 (L/kg), respectively in soil. In the presence of biochar, the calculated values of the effective desorption coefficients of 17β-estradiol and estrone were 1.07 and 0.77 (L/kg), respectively.

5. The lowest concentration of 17β-estradiol in the study was 0.32 µg/L. This concentration is much higher than the effective estrogenic concentration for fish and aquatic organisms (16 ng/L). This raises the concern about the process of the application of sludge and biosolids in

agricultural soils and highlights the potential role biochar could play in reducing estrogen contamination in the environment.

5.3. Recommendation for further studies

Considering the sorption affinity of pollutants to biochar, further research is required for better understanding of the behavior of biochar in different environmental matrices in order to maximize its potential as a promising remediation technique in reducing soil, ground water and surface water contamination from steroidal hormones.

The following areas could be explored further in future studies:

1. In this study, the retention ability of biochar was limited to one type of biochar with one application rate. It is encouraged to use variable application rates of biochar in similar experiments.

2. It is recommended to study competitive sorption-desorption properties of various pollutants to biochar.

3. The data collected from the wet soil samples in lysimeters, when they reached field capacity, were sufficient for the analysis of the difference between the treatments and fertilizers in soil, amended with biochar. This needs to be investigated in future studies as this can considerably reduce the amount of materials and samples that need to be analyzed.

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Appendix

Table 1. Summary analysis of the difference between the different fertilizers (sludge and biosolids) and different treatments (biochar and soil) for 17β-estradiol and estrone in all soil samples.

.	17β-estradiol				estrone			
Treatment/Depth	0cm	10cm	30cm	60cm	0cm	10cm	30cm	60cm
Svs.B N	-	*	-	-	-	*	*	-
Svs.B T	*	*	-	-	-	-	*	-
Nvs.T S	-	-	*	-	-	-	*	-
Nvs.T B	*	*	*	*	-	*	-	-

Note: S – soil, B – biochar, N – sludge, T – biosolids. * = statistically significant at $p < 0.05$ level; - = not statistically significant at $p<0.05$.

Table 2. Summary analysis of the difference between the different fertilizers (sludge and biosolids) and different treatments (biochar and soil) for 17β-estradiol and estrone in leachate samples.

Treatment	17β-estradiol	estrone
Svs.B N	-	*
Svs.B T	-	-
Svs.T S	*	-
Nvs.T B	*	*

Note: S – soil treatment, B – biochar treatment, N – sludge fertilizer, T – biosolids fertilizer. * = statistically significant at $p < 0.05$ level; - = not statistically significant at $p<0.05$.

Table 3. Summary analysis of the difference between the different fertilizers (sludge and biosolids) and different treatments (biochar and soil) for 17β-estradiol and estrone in dry soil samples.

.	17β-estradiol				estrone			
Treatment/Depth	0cm	10cm	30cm	60cm	0cm	10cm	30cm	60cm
Svs.B N	-	*	-	*	-	-	-	-
Svs.B T	-	-	-	-	-	*	-	-
Nvs.T S	-	*	*	-	-	-	-	-
Nvs.T B	-	-	-	*	-	*	-	-

Note: S – soil, B – biochar, N – sludge, T – biosolids. * = statistically significant at $p < 0.05$ level; - = not statistically significant at $p<0.05$.

Table 4. Summary analysis of the difference between the different fertilizers (sludge and biosolids) and different treatments (biochar and soil) for 17β-estradiol and estrone in wet soil samples.

.	17β-estradiol				estrone			
Fertilizer/Depth	0cm	10cm	30cm	60cm	0cm	10cm	30cm	60cm
Svs.B N	-	*	-	-	-	*	*	-
Svs.B T	*	*	-	-	-	*	*	-
Nvs.T S	-	-	*	-	-	*	*	-
Nvs.T B	*	*	*	-	-	-	*	-

Note: S – soil, B – biochar, N – sludge, T – biosolids. * = statistically significant at $p < 0.05$ level; - = not statistically significant at $p<0.05$.

Table 5. Detailed statistical analysis for 17β-estradiol.

treatment	group		repetition		group x repetition	
	F	P>F	F	P>F	F	P>F
Svs.B N	1.987	0.231	9.158	<0.0001	0.485	0.858
Svs.B T	4.783	0.094	23.534	<0.0001	3.883	**0.002**
Nvs.T S	197.998	**<0.0001**	27.797	<0.0001	16.853	**<0.0001**
Nvs.T B	31.006	**0.005**	6.688	<0.0001	2.821	**0.015**

Note: S – soil treatment, B – biochar treatment, N – sludge fertilizer, T – biosolids fertilizer.

Table 6. Detailed statistical analysis for estrone.

treatment	group		repetition		group x repetition	
	F	P>F	F	P>F	F	P>F
Svs.B N	2.202	0.212	14.328	<0.0001	0.960	0.480
Svs.B T	6.920	0.058	27.985	<0.0001	5.231	**<0.0001**
Nvs.T S	4.557	0.100	12.768	<0.0001	2.436	**0.032**
Nvs.T B	45.929	**0.002**	32.800	<0.0001	8.551	**<0.0001**

Note: S – soil treatment, B – biochar treatment, N – sludge fertilizer, T – biosolids fertilizer.