Fate and Transport of Manure-borne Estrogens in Biochar Amended Agricultural Sandy Soil

by

Sukhjot Singh Mann

Master of Science

Department of Bioresource Engineering
Faculty of Agricultural and Environmental Sciences
McGill University
Montreal, Quebec

A thesis submitted to McGill University in partial fulfillment of the requirements of the degree of Master of Science

March 2015

ABSTRACT

Steroidal sex hormones, also known as estrogens, such as 17β-estradiol (E2) and its primary metabolite, estrone (E1), are being released in significant amount by animals in their urine and feces which generally ends up in manure and is typically spread on agricultural land. These hormones are important subject to study because of their potential toxic impact on soil, stream and groundwater quality. Because of their development effects in reptiles and amphibians, these hormones are labelled as Endocrine Disrupting Chemicals (EDCs). These compounds are able to produce endocrine disruption in living organisms even at nanogram-per-liter levels. Various studies have highlighted biochar's potential in adsorbing such hormones, given its structural and physiochemical properties. Two different studies were done to test remaining adsorption capacity of slow pyrolysis biochar during second year after its application. Adsorption was tested against two different type of manures (poultry manure and liquid swine manure) in sandy soil. While in the first year of biochar application, a significant spatial-temporal stratification of steroidal sex hormones had been observed in the biochar-amended soil (vs. non-amended soil). In the second year, biochar adsorbed hormones to a considerably lesser degree in both studies. When biochar was fresh, its pores were available for hydrophobic interactions so it was holding significant concentration of hormones during first year of application but with time there were several biotic and abiotic changes on surface of biochar and after some physical fragmentation, pores on surface were no longer available for hydrophobic interactions and also it started releasing dissolved organic carbons which facilitated even greater mobility of hormones from manure down to lower depths of soil profile.

RÉSUMÉ

Les hormones stéroïdiennes, ou estrogènes, tel que le 17β-œstradiol (E2) et son métabolite principal, l'estrone, sont couramment relâchés en quantités importantes dans l'urine et les excréments des animaux de ferme. Ce fumier, avec les hormones associées, est généralement réappliqué aux terres agricoles, et le potentiel de ces hormones pour causer des effets négatifs sur les sols, les cours d'eau et les eaux souterraines souligne l'importance des études à leur sujet. Ces hormones sont classifiées comme des perturbateurs endocriniens dû à leurs impacts sur le développement des reptiles et des amphibiens et peuvent causer des perturbations endocrines même à des concentrations de nanogrammes par litre.

Plusieurs études ont souligné le potentiel du biocharbon, donné ses propriétés structurelles et physicochimiques, pour l'adsorption de telles hormones. Cette thèse porte sur la capacité de rétention du biocharbon provenant de pyrolyse lente lors de son incorporation dans un sol sablonneux avec application d'estrogènes de deux différentes sources (fumier de volailles et lisier de porc). La première année suivant l'application du biocharbon au sol, une stratification spatiotemporelle significative des hormones stéroïdiennes fut observée dans le sol amendé par le biocharbon (versus le contrôle sans biocharbon). Cependant, le biocharbon montra une capacité d'adsorption considérablement moins élevée lors de la seconde année.

Ces résultats peuvent s'expliquer par le fait que le biocharbon frais contient des pores disponibles pour des interactions hydrophobiques avec les hormones, le permettant de retenir des concentrations importantes de ces dernières lors de la première année. Avec le temps, cependant, les changements biotiques et abiotiques à la surface du biocharbon, autant que la fragmentation physique, ont réduit la disponibilité des pores pour les interactions hydrophobiques. En plus, la dissolution des molécules organiques a additionnement facilité la mobilité des hormones vers les niveaux plus profonds du profil du sol.

CONTRIBUTION OF THE AUTHORS

The authorship for the two manuscripts, making up this thesis, is as follows:

(1) Sukhjot Mann, Zhiming Qi, Shiv Prasher. **Transport and fate of estrogens from swine manure in sandy soil under second year of biochar application** (Under review in Journal of Environmental Management)

(2) Sukhjot Mann, Zhiming Qi, Shiv Prasher. **Retention capacity of biochar amended sandy soil for estrogens from poultry manure in second year of biochar application** (under preparation)

All authors are from the Department of Bioresource Engineering, Macdonald Campus, McGill University, Montreal. All the fieldwork, analysis of data and preparation of above manuscripts were completed by the master degree candidate, Sukhjot Singh Mann under the direct supervision of Dr. Zhiming Qi, Assistant Professor in Department of Bioresource Engineering. Dr. Shiv Prasher provided all the required guidance to undertake laboratory work. Dr. Zhiming Qi and Dr. Shiv Prasher shared the expenses caused in laboratory and field during this experiment.

ACKNOWLEDEMENT

First and foremost, I would like to express my heartfelt gratitude and utmost appreciation for my supervisor, Dr. Zhiming Qi (Assistant Professor of the Department of Bioresource Engineering). Under his guidance and mentorship I have truly thrived. He has inspired me to challenge myself beyond what I thought were my limits, to reach new levels of scholarship and innovation.

Next, I would like to express appreciation and sincere gratitude to Dr. Shiv O. Prasher (Professor of the Department of Bioresource Engineering) for giving me the precious opportunity to work in his lab under his supervision. I am so grateful for his valuable suggestions and expert assistance.

Further, I thank Dr. Eman ElSayed for her support during my whole Master's degree, and for selflessly taking the time to assist me throughout my experiments, without which this thesis would not have been possible. Her guidance and resourcefulness has proved invaluable to the completion of my research.

A special word of thanks and appreciation goes to Mr. Azhar Inam (PhD Candidate at the Bioresource Engineering) for all help and emotional support during hard times throughout my study. I am very much grateful to Ms. Cassandra Rodrigue for her help in the field and laboratory without which this project was difficult to carry out. My gratitude is also conveyed to Ms. Sedigheh Zarayan who supported me technically and guided me throughout all laboratory experiments. I am also thankful to Julien Malard for helping me out in writing the abstract in French language.

I would like to thank the Department of Bioresource Engineering, McGill University for providing the "Liliane and David Stewart Fellowship in Water Resources" in support of this research work.

Last but most important, I would like to express my heartily appreciation to my family for their unconditional support.

TABLE OF CONTENT

LIST OF TABLES

LIST OF FIGURES

LIST OF SYMBOLS AND ABBREVIATIONS

°C	Degree celsius
cm	Centimeter (100 cm = 1 meter)
cmol	Centimole, unit for CEC (cation exchange capacity)
g	Gram
GC	Gas chromatography
ha	Hectare
HPLC	High Performance Chromatography
i.e.	That is
K*d*	Soil-water partitioning coefficient
kg	Kilogram
K*oc*	Adsorption coefficient
K*sat*	Saturated hydraulic conductivity
L	Liter
mg	Milligram
min	Minute
mL	Milliliter
O.S.	Organic solvent
Pr	Statistical probability
r	Correlation coefficient
rpm	Revolution per minute
SD	Standard deviation
µL	Microliter
µg	Microgram

Chapter 1

INTRODUCTION

1.1 General Introduction

Rapid increase in industrialization and anthropogenic activities has led to exposure of humans and wildlife to multitude of chemicals. Agricultural practices such as adding harmful pesticides to curb pests in the field, adding fertilizers to improve crop yield also contributed in increased exposure of environment towards chemicals.

Nutrients, pesticides and sediments are the classes of contaminants that are widely and extensively studied for their negative impact on water quality in agricultural environment. These types of contaminants persist at easily measurable concentrations. There is a new class of contaminants which are less well known but reported to have severe impact on ecological health. This class of contaminants are known as "emerging contaminants" mainly includes steroids, pharmaceuticals, antibiotic-resistance genes, prion proteins and endocrine-disrupting compounds (Snow et al., 2013). These emerging contaminants have been entering in environment since long time but their methods of detections were developed recently (USGS 2014).

Steroidal hormones, such as natural estrogens, are considered as "emerging contaminant" of major concern because of their severe endocrine disrupting properties. estrogens are excreted mainly by animal and are potential surface and groundwater contaminants (Gall et al., 2011). In general practice, management of animal waste is done by applying it to the agricultural field as manure to improve soil nutrients but this practise also make a pathway for hormones from manure to leach from top soil. Application of manure occurs at different times during the year, mainly from spring to fall. According to Agriculture division of Statistics Canada (2004), nationally farms apply manure mostly in the fall (35.4% of applications), followed closely by applications in the spring (33.2%). About one-quarter (25.9%) of manure applications were performed in the summer months. In comparison, 5.5% of manure applications, by far the lowest proportion, were in winter, generally considered an unfavourable time because of the potential for run-off due to frozen ground (Beaulieu, 2004). According to Statistics Canada (2006), manure production has been increased by 16%, or by approximately 25 million tonnes from 1981 to 2006. Annually, approximately 3.4 million hectares of land in Canada receives animal manure

(mainly liquid swine manure and poultry manure) as a top soil amendment to improve soil fertility and quality of crops. Average rainfall is more during spring season and also application rate of manure during this season is high. So probability of leaching of estrogens to ground water is high during this period of time.

1.2 Problem statement

Growing occurrence of trace organic compounds in environment such as pharmaceuticals, personal care products, pesticides, and hormones , and their potential adverse effects on aquatic and terrestrial life and on human health is of major concern (Gulkowska et al., 2007). The application of animal manure has been identified as one of the major gateway for the environment's exposure to these new classes of toxic and high-risk organic contaminants, also known as emerging contaminants. Major source of estrogens (hormones) in environment is animal manure applied to agricultural fields (Hanselman et al., 2003). Natural steroidal sex hormones *i.e.*, 17β-estradiol (E2) and estrone (E1) are the emerging contaminants of greatest concern because of their adverse developmental effects and their association with adverse long-term health issues like carcinogenicity, mutagenicity or teratogenicity (Choi et al., 2004). 17β-estradiol (E2) and estrone (E1) are highly potent compounds that can effects normal functioning of endocrine system and reproductive system of aquatic animals like fish species even at very low concentrations of ngL^{-1} (Goeppert et al., 2014; Jobling et al., 1998).

Despite the recognized negative effects of hormones from manure on aquatic animals, the fate and transport of hormones in ecosystem remain poorly understood. There is a need to develop potential remediation techniques to curb hormonal pollution originating by leaching of estrogens upon application of animal manure in agricultural fields.

1.3 A novel approach for reducing aquatic hormonal pollution

Carbon rich amendments like activated carbons and biochar, have a strong sorption affinity for poly-aromatic hydrocarbons and other organic contaminants including hormones (Lehmann, 2009). These Carbon rich amendments may provide a viable approach to tackle the hormonal pollution caused by land application of manure in agricultural fields. Biochar is a finely grained substance produced by heat treatment of organic biomass. It has high surface area per unit volume. Compared to other soil amendments, biochar's high surface area and porous structure

enable it to adsorb nutrients and water (Lehmann, 2009). It is proven that biochar has potential to adsorb hormones (Sarmah et al., 2010). Biochar production primarily uses waste biomass, such as green waste from municipal landscaping, forestry, or agriculture (for example, bagasse) (Hunt et al., 2010). It a sustainable approach because its production facilitates waste management and its application as top soil amendment can limit hormonal pollution and improve soil water and nutrient retention capacity (Lehmann, 2009) .

1.4 Objectives

Adsorption potential of biochar was tested for 17β-estradiol (E2) and estrone (E1) from liquid swine manure and poultry manure in sandy soil. Biochar was found to be effective in adsorbing hormones from poultry as well as liquid swine manure during first year of its application (Alizadeh, 2014) but the trend of its adsorption capacity with time was unknown. To test the capacity of biochar in adsorbing hormones from liquid swine manure and poultry manure, a further study was conducted on the same biochar applied in a previous study but with fresh application of poultry and liquid swine manure in the second year after the biochar's application. So conclusively following were the objectives of current study:

1. Investigate transport and fate of estrogens from swine manure in sandy soil under second year of 1% slow pyrolysis biochar application.

2. Retention capacity of 1% slow pyrolysis biochar amended sandy soil for estrogens from poultry manure in second year of biochar application.

Chapter 2

GENERAL REVIEW OF LITERATURE

2.1 Emerging contaminants

Over the period of last few decades, a number of classes of natural as well as synthetic chemicals have been explored by the scientists in the environment that were not detected in the environment earlier. These type of chemicals are known as emerging contaminants which can be defined as "any synthetic or naturally occurring chemical or any microorganism that is not commonly monitored in the environment but has the potential to enter the environment and cause known or suspected adverse ecological and/or human health effects" (USGS 2014). Emerging contaminants have been entering the environment since long but may not have been detected due to lack of detection methods or techniques. Emerging contaminants generally includes compounds such as steroid hormones, surfactants, antibiotics, pesticides metabolites, naturally occurring algal toxins and some other pharmaceuticals. Detection of these compounds in environment is difficult because of their complex nature, trace concentrations and hard to separate from interferences (Snow et al., 2013).

2.1.1 Environmental occurrence of Emerging contaminants and their pathway

Prime sources of this type of contaminants are agricultural, municipal and industrial wastewater. Over the last decade, expanding anthropogenic activities, including modern animal feeding operations, have generated excessive quantities of organic wastes (*i.e*., animal manure and biosolids). Management of this livestock manure is done by applying it to farmland as nutrient rich substrate. The application of animal manure has been identified as one of the major gateways for the environment's exposure to these new classes of toxic and high-risk organic compounds known as emerging contaminants. Application of manure occurs at different times during the year, mainly from spring to fall. In Canada, between the years 1981 and 2006, manure production is increased by 25 million tons (Statcan 2006). Highest amount of manure is applied in spring and fall (33.2% and 35% of application respectively). Animal manure is being applied as top soil amendment in approximately 3.4 million hectares of land in Canada. Average rainfall

is more during spring and fall season and also application rate of manure during these seasons is high as discussed above. So probability of leaching of emerging contaminants to ground water is high (Snow et al., 2013).

2.2 Endocrine Disrupting Compounds (EDC's)

Endocrine disrupting compounds are chemicals that may interfere with body's endocrine system and produce adverse reproductive, neurological, developmental and immune effects in both humans and wildlife. Research studies found that endocrine disrupters may cause that high risk during prenatal and early postnatal development when organs and neural system are developing (NIEHS 2014). Having endocrine-disrupting properties, some at concentrations as low as ngL^{-1}, the emerging contaminants represent a high chronic toxicity risk and are associated with adverse long-term health issues like carcinogenicity, mutagenicity or teratogenicity (Choi et al., 2004). Severe impact on male reproductive system was reported first in wild animals upon their exposure to estrogenic pollutants which caused changes in reproductive behavior in those animals (Vos et al., 2000). Many other reports also found adverse effects of EDC's exposure affecting reproductive system in bird, amphibian and fishes (Aravindakshan et al., 2004; Barnhoorn et al., 2004). Increasing incidence of cryptorchidism (displacement of testicles), hypospadias (abnormal urethra opening), testicular cancer and poor semen quality in men might be resulted from exposure to certain EDC's (Grandjean, 2001).

2.2.1 Occurrence and pathway of endocrine disrupting compounds in agriculture

Human and animal excreta is the prime source of hormones entering the environment. Hormones (EDC's) associated with animals enters environment when animal waste (manure) is applied to field as a nutrient source. Increasing trends of manure production and its application to agricultural field pose a serious threat aquatic life. The amount of hormones (estrogens) entering environment from animal manure is more than 200 times the amount of hormones comes from bio-solid application (Gall et al., 2011) which makes animal manure a significant source of hormones and something important to study upon. Steroidal estrogen hormones such as estradiol and estrone are of alarming concern because various reports suggested their toxicity at low nanogram per liter concentrations in water that can impact reproductive system of aqautic species specially fishes (Khanal, 2006). According to a study (Panter et al., 2000), exposure to

17-β-Estradiol or estrone at low concentration of 30 ng L^{-1} for 21 days stimulates vitellogenin (an egg yolk precursor protein that is normally produced only by adult females) synthesis and abnormal testicular growth in male fathead minnows. The excreta of swine and poultry contains 17-β-estradiol, estrone and estriol plus conjugates (Hanselman et al., 2003).

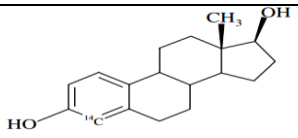
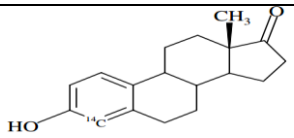
2.3 Steroid Sex Hormones

Steroid sex hormones are the hormones secreted by gonads and participates in the regulation of sexual functions of the body such as the regulation of reproductive cycle and development of accessory reproductive organs. There are three types of sex hormones named as estrogen, testosterone and gonadotrophins. These type of hormones influences secondary sex characters like body shape, mammary development and pitch of voice. The major natural estrogen are 17-β-estradiol and estrone.

2.3.1 Structure and Physicochemical Properties

Estradiol and estrone has a tetracyclic molecular framework and contains an aromatic ring as a distinctive part of that framework (Table 2.1). Structural differences can be seen at C-16 and C-17 positions where they have different type and stereo-chemical arrangement of functional groups.

Table 2.1 2-D structure of 17β-estradiol (E2) and estrone (E1) (Fan et al., 2007)

Parameter	**Steroid compound**	
	17β-estradiol (E_2)	**estrone (E_1)**
Structure		

Estrone has carbonyl group at C-17 rather than a hyroxyl group which makes it different from estradiol. Estradiol can have either a hydoxyl group which points upward from the molecule (*â* configuration) or a hydoxyl group that projects downward from the molecule (R configuration). The physicochemical properties of estradiol and estrone are given in Table 2.2.

Table 2.2 Physiochemical properties of 17β-estradiol (E2) and estrone (E1) (Z. Yu et al., 2004)

Parameter	Steroid compound	
	17β-estradiol (E_2)	**estrone (E_1)**
Empirical formula	$C_{18}H_{24}O_2$	$C_{18}H_{22}O_2$
Molecular weight (g mol^{-1})	272.3	270.4
Aqueous solubility (mgL^{-1})[a]	3.1±0.02	2.1±0.03
pK_a[b]	10.23	10.40
log KOW[c]	3.94	3.43
log KOC[d]	3.34	3.2

[a] Aqueous solubility at 23°C
[b] Deprotonation constant
[c] Octanol–water partition coefficient
[d] Adsorption coefficient

Unconjugated Estrogens are moderately hydrophobic with log K_{OW} of 3.43 for E1 and 3.94 for E2. These have low aqueous solubility between 2-3 mg L^{-1}. These conjugates are non volatile and are weak acids with pKa 10.40 for E1 and 10.23 for E2.

2.3.2 Potential impacts of environmental exposure to steroid sex hormones

Natural steroidal sex hormones *i.e.,* 17β-estradiol (E2) and estrone (E1) are the emerging contaminants of greatest concern because of their adverse developmental and carcinogenetic effects. Major source of Estrogens in environment is from animal manure applied to agricultural fields (Sarmah et al., 2008). 17β-estradiol (E2) and estrone (E1) are highly potent compounds that can affects normal functioning of endocrine system of aquatic animals like fish even at very low concentrations of ngL^{-1} (Goeppert et al., 2014). Anoxic conditions even retard the degradation of Estrogens (Ying et al., 2003) making it even more dangerous for aquatic life. Two fold risk is associated with E2 in environment. Firstly, when E2 combines with even one tenth of minimal effective dose of other estrogenic compounds makes a significant estrogenic response (Soto et al., 1995). Secondly, estrogenic chemicals are more intoxicating when are in combination rather than individual. E2 in combination with xenoestrogens, is 104 to 106 times more intoxicating than six estrogenically active alkyphenol-polyethoxylates (Sumpter & Jobling,

1993). Because E2 has been linked to formation of breast cancer due to its high estrogenic responses, it had been declared a carcinogen by United States Department of Health and Human Services in 1994.

2.3.3 Steroid hormone occurrence and pathways in the environment

The two prime source of hormones entering the environment are humans and livestock. Hormones from these sources enters into environment through discharge from wastewater treatment plants and the land application of animal waste and biosolids. As far as poultry and swine manure is concerned, 17β-E2 and the E2 metabolite estrone (E1) are the major estrogens present (Hanselman et al., 2003).

2.3.4 Environmental fate and transport of steroid sex hormones

There are some environmental concerns regarding fate of EDC's like hormones, in the water leaching to groundwater from agricultural field receiving animal manure. When concentrated in the aquatic environment then elevated levels of Estrogens can cause feminization of fish and other aquatic life (Khanal, 2006). Estrogens are moderately hydrophobic and non volatile compounds that do not ionize at average environment pH and widely sorbed by aquatic sediments and soil. Nichols et al. (1997) confirmed the contamination of aquatic environment by estrogens in manure. Risk from E1 and E2 leaching is generally considered low because their high sorption and fast dissipation in aerated top soil (Ying & Kookana, 2005). Many studies have explained the fate and transport of hormones through a soil column (Goeppert et al., 2014; Casey et al., 2005). Hormones undergo sorption to soil, transformation, degradation and/or leaching. Biodegradation has been found as the prime removal pathway that decides the fate and transport of estrogens in environment. There are two mechanisms by which microorganisms can degrade estrogens: growth linked (metabolic) and non-growth linked (co-metabolic) (C.-P. Yu et al., 2006). During growth linked degradation, microbes utilizes estrogens as energy source or carbon source for its growth. In co-metabolic reaction, microbes use their existing enzymes to degrade estrogens. Microbes undergo this mechanism because they need a primary growth substrate for sustainable bacterial growth and required for inducing the expression of degradative enzymes.

Steroid hormones also undergo sorption in soils and sediments. Some studies (Lai et al., 2000) found that sorption of hormones by different soils depends on fraction of organic matter content. However, rapid hormones sorption kinetics approaches equilibrium within a few hours (Lee et al., 2003). These finding indicates hormones to be immobile in soils but particle bound hormones may be readily transported during surface runoff and colloidal transport may regulate its transport through soils and in aquatic system (Casey et al., 2003). Surface runoff is considered as important pathway for the transport of Estrogens to the aquatic environment. However there is one more little unknown pathway called as leaching; through the soil to either shallow groundwater or through drainage water to surface water. Several studies reported the leaching of E2 and E1 through the soil profile. A constant concentration of approximately 5 ngL^{-1} of E2 was found in water resources which was believed to be there via leaching through the soil profile (Peterson et al., 2000).Jacobsen et al. (2005) analysed the estrogen degradation in aerobic environment under the presence of organic matrices including animal wastes and biosolids. This study demonstrated that the prime pathway of degradation of Estrogens in the soil is via microbial degradation and is influenced by media temperature and pH. Initial degradation rate was found to be 0.01 d^{-1}. In an another study, Colucci et al. (2001) demonstrated persistence of steroid hormones to be significantly different at different soil temperatures. There was rapid biotrasformation of Estrogens in agricultural soil with the degradation rate between 0.11 to 0.47 d^{-1} at different temperatures and moisture levels. E2 was degraded by both biotic and abiotic processes but E1 was degraded only by biotic process. Rapid oxidation of E2 to E1 was observed in both autoclaved and non-sterile loam, silt loam, and sandy loam soils, suggesting a biological transformation. Xuan et al. (2008) also reported microbial degradation of E2 in a non-sterilized soil. Das et al. (2004) estimated a degradation rate of 0.0003 to 0.075 h^{-1}.

2.4 Biochar

In simple words, When biomass such as wood, manure or leaves, is heated in a closed container with little or no available air, a carbon rich product is obtained called biochar. In other words, thermal decomposition of organic material under limited supply of oxygen. and at relatively low temperatures (<700°C) give rise to biochar. Biochar can be used as top soil amendment to improve soil productivity, carbon storage, or filtration of percolating soil water (Lehmann,

2009). The term ‘biochar’ is a relatively recent development, emerging in conjunction with soil management and C sequestration issues.

2.4.1 Trends of biochar use over time

Biochar was being studied earlier also but only during last one decade it starts getting much wider recognition. Early research on the effects of biochar on seedling growth and soil chemistry (Tryon, 1948) yielded detailed scientific information. In Japan, biochar research significantly intensified during the early 1980s (Lehmann, 2009). During 1980's, biochar was also recommended for horticultural practices. The interest in biochar was doubled in recent years. Biochar started being connected to potential soil management practices. The ability to hold nutrients more effectively than those of other organic matter in soil gives biochar advantage to be used for improving soil fertility. Recent studies suggested that biochar production and application might viewed as sustainable environmental tool in bigger picture (Figure 2.1).

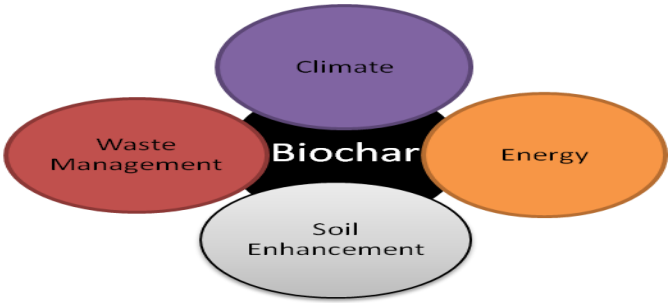


Figure 2.1 Multiple advantages of production and application of biochar (Alizadeh, 2014)

Biochar is made from agricultural and organic waste so it's production considered a sort of waste management tool. Also theproduction energy being released can be used for some other applications. After biochar application in soil, it has shown capacity to fix excessive carbon in environment and also has shown ability to hold water and nutrients in top soil which leads to better soil health and productivity (Lehmann, 2009).

2.4.2 Physical properties of biochar

The fundamental molecular structure of biochar creates both its surface area and porosity. Under X-ray diffraction, biochar is essentially amorphous in nature. It also contains some local crystalline structure of highly conjugated aromatic compounds (Qadeer et al., 1994). Crystalline

areas can be visualized as stacks of flat aromatic (graphene) sheets cross linked in a random manner. In spite of biochar small dimentions, it is a good conductor of the aromatic-aliphatic organic compounds of complex structure (including residual volatiles), and the mineral compounds (inorganic ash) are the non-conducting components that completes biochar matrix. This is complemented with the voids, formed as pores (macro-, meso- and micropores), cracks and morphologies of cellular biomass origin. The process by which biochar is made (pyrolysis) enlarges the crystallites and ordered them. Most important factor that counts for industrial application of biochar is its pore size distribution. Micropores (<2nm in diameter) makes most of the surface area and are responsible for the high adsorptive capacities for molecules of small dimensions such as gases and common solvents. Solid density is the measure of mechanical strength of biochar. The increased molecular order of pyrolysed biomass gives it a higher mechanical strength than the biomass feedstock from which it was derived.

2.4.3 Surface chemistry of biochar

Because if biochar's heterogeneous compositions, the surface chemistry of biochar is quite rich and varied. Biochar surfaces exhibit hydrophilic, hydrophobic, acidic and basic properties. The contribution of these properties on biochar behaviour largely depends upon the feedstock and on the thermal degradation process used to create the biochar. The various functional groups on the surfaces of biochar influence sorption by the nature of their surface charge and by the availability of pie electrons. The nature of the sorbate also affects its ability to sorb. Non-transition metals, for example, are sorbed strictly by electrostatic forces, whereas transition metals with their exposed pie orbital . Many organic sorbates, such as phenols, anilines and other functionalized aromatic molecules, also exhibit amphoteric behaviour and, like the amphoteric transition metals, must strike a balance between electrostatic and pie electron sorption mechanisms. In general, these molecules tend to sorb most strongly at solution pH values near their points of zero charge.

2.4.4 Nutrient content of biochar

Biochar is made from the biomass. They are expected to be high in carbon and contain a range of plant macro- and micro-nutrients. The composition of biochar largely depends upon the feedstock and the operating conditions of pyrolysis. Biochar used in this study was produced

from the slow pyrolysis of soft wood at 450°C. This slow pyrolysis biochar was bought from BlueLeaf Inc, Drummondville, Quebec, Canada.

2.4.5 Biological properties of biochar

Some studies have shown that biochar stimulates the activity of soil microbes and hence greatly influence soil microbiological properties (Pietikainen et al., 2000). The presence of pores and its size distribution helps microbes to have a suitable habitat which protects microbes from predation and desiccation. It also provides microbes with their carbon and other nutrients needs (Warnock et al., 2007). The pores of biomass upon pyrolysis increases during charring. The surface area of different biochar ranges from 10 to several hundred square metres per gram which provides a significantly increased surface area for microbial colonization. The high porosity of biochar allows it to retain moisture. A study was undertook by Pietikainen et al. (2000) who reported that biochars made from wood and humus had a higher water retaining capacity as compared to activated carbon. If added to soil, biochar may also increase the water retaining capacity of soil. Because water is universal biological solvent, its presence in pores increases the habitability of biochar for microbial growth. Quantity and quality are the two important factors that affect soil microbial population. The quality majorly depends upon the feedstock and pyrolysis parameters. Low temperature pyrolysis conditions leave residual bio-oils and other re-condensed derivatives on the biochar surfaces. They may serve as substrate for microbial growth but also they may be toxic to plants and to some microbes as well (McClellan et al., 2007).

2.4.6 Production of biochar

Depending upon the operating conditions, the complex and varying changes of biomass during pyrolysis affect both the composition and chemical structure of the resulting biochar, with significant implications for nutrient contents and, especially, nutrient availability to plants. Therefore it is very important to study the process of pyrolysis as biochar's behaviour depends largely on how it was made. Pyrolysis is the degradation of biomass by heat in the absence of oxygen (O), which results in the production of solid biochar. For same feedstock, the yield of biochar largely depends upon the factors like temperature, heating time and heating rate. Pyrolysis of cellulose at temperature more than 300°C involves reduction in molecular weight,

carbon dioxide, evaluation of water and biochar formation (Shafizadeh, 1982). Between temperature range of 300°C to 500°C, molecules are rapidly depolymerized to anhydroglucose units that further react to provide a tarry pyrolysate. At temperature more than 500°C, the anhydrosugar compounds undergo fission, dehydration, disproportionation and decarboxylation reactions to provide a mixture of low molecular weight gaseous and volatile products, as well as the residual biochar. Depending upon the operating conditions, the complex and varying changes of biomass during pyrolysis affect the composition of the resulting biochar, with significant implications for nutrient content. Bagreev et al. (2001) found the effect of temperature and holding time on C and N composition and pH of biochar (Table 2.3).

Table 2.3 Effect of temperature and holding time on C and N composition and pH of sewage sludge biochar

Temperature (°C)	Holding time (minutes)	Carbon (mg/g)	Hydrogen (mg/g)	Nitrogen (mg/g)	pH
400	30	282	20.4	38.3	7.7
600	60	271	11.4	31.9	11.5
800	60	264	4.2	16.1	11.3
950	60	249	3.5	9.4	11.0

Source: Bagreev et al. (2001)

Porosity of biochar significantly increases between 400°C and 600°C , and may be attributed to increases in water molecules released by dehydroxylation acting as pore-former and activation agent, thus creating very small (nanometre-size) pores in biochar. There is a significant increase in surface area upon increase in porosity by orders of magnitude. That is why temperature has significance consequences on differences in structural changes in terms of surface area for biochar produced under different conditions .

2.4.7 Physiochemical Changes on Biochar in Soil

There are many studies done to understand the changes in soil properties upon biochar addition. These changes includes the change in pH of the soil, cation exchange capacity, creating hydrophobic sites and increasing adsorption sites for microbes, pesticides and minerals (Hamer et al., 2004) (Brodowski et al., 2005). Biochar when added to soil also undergo several structural and physiochemical changes which further depends upon its production conditions. Upon

physical fragmentation, large biochar particles are broken into smaller pieces resulting in more exposed surface that is accessible to further chemical and biological processes. Biochar particles can also be fragmented into smaller particles when water penetrates the pores and swells during freezing, forcing the biochar particles to break (Carcaillet, 2001). Different physical compounds also may get trapped within the biochar's fine pore structure results in changed structural properties of biochar in soil. Change in structural properties leads to change in adsorption behaviour on the surface of biochar. A study conducted by Zackrisson et al. (1996) found that adsorption capacity of biochar for phenolic compounds decreased significantly with time. Over the time, biochar becomes deactivated as its pores become clogged and its sorption capacity decreases. Also, biochar undergo certain chemical changes when applied to soil. When biochar is fresh, it mainly contains an amorphous phase of aromatic structures and a crystalline phase with graphene-like sheets. There are various functional groups present on the outer surface biochar that are exposed foremost to surface oxidation (Lehmann, 2007). Cheng et al. (2006) did a wide study on biochar oxidation. This study found that chemisorption of oxygen at high temperature results in spontaneous abiotic oxidation of biochar particles. Biochar when incubated at 70°C for four months, the acid functional groups significantly increased compared to biochar incubated at 30°C. Furthermore, increasing number of acid groups makes biochar more hyrdophilic and enhance biological, chemical and physical weathering like leaching down the soil profile, fragmentation into small pieces, or releasing dissolved organic carbons (Kramer et al., 2004). However, biochar interactions with minerals results in decrease in oxidation and degradation (Brodowski et al., 2005). This study observed various biochar particles associations with minerals in a field study in Germany. They found that biochar particles in agricultural soil occurred either as discrete particles unassociated with minerals or as attached to minerals.

2.4.8 Biotic changes of biochar in soil

Microorganism are also able to bring changes in properties of biochar. A four month study by Cheng et al. (2006) was done to measure the biotic changes on biochar . It was recorded that biochar has undergone more of abiotic changes as compared to biotic changes. There was no change in pH, cation exchange capacity or other elemental compositions on biochar with microbial inoculation. Nut number of other studies confirmed that there are significant biotic

changes in biochar in the long term. The initial abiotic oxidation could actually facilitate further microbial oxidation. In an another study, Hockaday (2007) witnessed fungal hyphae growth on biochar particles in soil. Growth of fungi on biochar influenced the capacity and manner with which biochar interacts with other soil components. In a study held by Zackrisson et al. (1996) brought forward the fact that biochar particles act as centre of attraction for microbial activity , which results in decomposition of soil organic matter that are absorbed to the surface of biochar.

2.4.9 Biochar and nutrient rich substrate

In a study conducted by Hamer et al. (2002), it was found that the addition of glucose stimulates changes in properties of biochar in soil to a greater extent. Upon glucose addition, biochar mineralization and oxidation in soil was increased by 115 per cent as compared to control. There was a significant change in elemental composition of biochar when biochar was incubated with dairy manure at 30 °C for four months (Cheng et al., 2006). Further studies on application rates, mixing and stability of biochar in the soil, under different climatic conditions, are necessary to understand its long-term role in soil fertility management.

2.4.10 Environmental conditions and biochar

Many field tests suggested that decomposition is not only factor attributed to decrease in biochar's content. Many other environmental factors such as erosion, eluviation and leaching affects biochar degradation also (Leifeld et al., 2007; Rodionov et al., 2006). Biochar can be transported downwards also. Hockaday et al. (2007) provided evidences which had shown the leaching of biochar in dissolved phase identified as aromatic particles from biochar. However there is less information available about the transport of particulate biochar. Erosion is an another important mechanism by which biochar decomposes especially after initial application to soil but firm evidence is still missing. Also, the extent of mineralization of biochar during the transport in water is still unknown.

2.5 Interaction of estrogens with DOC and Soil

In a detailed study by Stumpe et al. (2010), the sorption of hormones affected by DOC is explained in the three phase system. First phase is about DOC sorption to soil, second is for

estrogens sorption to DOC and third for estrogen sorption to soils in presence or absence of DOC. This study confirmed that estrogen behaviour in soils are affected by dissolved organic carbons. Based on initial conditions, presence of dissolved organic carbon results in increasing or decreasing sorption of E2 and EE2 to the soil solid phase. Sorption of estrogens increases with pH increase in an acidic soil, which is of less importance in most agricultural fields. The decreases in estrogen sorption were associated with exchange processes between organic waste derived and soil borne DOC. However the exact mechanism by which estrogen sorption decreases in presence of dissolved organic carbon is not clear but there are enough evidence available that estrogen transport through soils would be enhanced and this aspect has to be considered in terms of risk assessments. There are profound influences of biochar on the sorption properties of soil for organic compounds. Biochar is more strong sorbent for oganic compounds as compared to organic matter present in the soil. Many studies attributed the strong sorption of soil to the presence of biochar in that soil. But upon aging, biochar may start releasing dissolved organic carbons (Lehmann, 2009) which might act as carrier for hormones to travel down the soil profile.

Preface to Chapter 3

According to the Agriculture and Agri-Food Canada and Statistics Canada (2006), manure production from swine has increased by 49 % between 1981 and 2006. Many studies confirmed the significant presence of steroid sex hormones (estrogens) in liquid swine manure which holds a possible potential threat to environment because of their adverse effect on endocrine and reproductive system of various aquatic animals, specially fish species.

Biochar have shown adsorbing potential for hormones in several studies but the duration for which it can work at same capacity is unknown. In this chapter, fate and transport of estrogens from liquid swine manure was tested in sandy soil amended with slow pyrolysis biochar during second year after biochar application. It was a 46 day period study held under the natural field conditions.

To date, this is the first field study testing the effect of slow pyrolysis biochar on fate and transport of estrogens from liquid swine manure over duration of two years.

Research papers based on the chapter

Sukhjot Mann, Zhiming Qi, Shiv Prasher. **Transport and fate of Estrogens from swine manure in sandy soil under second year of biochar application** (Under review in Journal of Environmental Management)

Chapter 3

Transport and fate of estrogens from swine manure in sandy soil under second year of biochar application

Abstract

Under growing concerns of their possible adverse effects on ecosystems, natural steroidal sex hormones, originating from liquid swine manure, have been detected at trace concentrations in a number of natural environments. Various studies have highlighted biochar's potential in adsorbing such hormones, given its structural and physiochemical properties. The remaining hormone adsorption capacity of a 1*% slow pyrolysis biochar topsoil amendment was tested in the year after its application to a sandy soil, housed in outdoor lysimeters irrigated with simulated rainfall. The fate and transport of estrogens, over a 46-day period following the incorporation of liquid swine manure (*1.6×10^3 L ha^{-1} with 4 g N L^{-1}*) into the topsoil, was monitored in biochar-amended and non-amended lysimeters. While in the first year of biochar application, a significant spatial-temporal stratification of steroidal sex hormones had been observed in the biochar-amended soil (*vs. *non-amended soil), in the second year biochar adsorbed hormones to a considerably lesser degree. Concurring with the findings of laboratory batch adsorption experiments on fresh and used biochar, the present study showed that 1% slow pyrolysis biochar`s capacity to absorb sex hormones (estrogens) released from liquid swine manure in sandy soil decreases with time. This is presumably the result of biochar surface degradation leading to the release of soluble organic carbon, thereby facilitating a heightened mobility of hormones and their resultant leaching to lower depths in the soil profile.*

Keywords. *Biochar; Swine Manure; 17-β-estradiol (E2); estrone (E1); Adsorption*

3.1 Introduction

Known for their negative impacts on water quality in agricultural environments, nutrients, pesticides and sediments constitute widely and extensively studied classes of contaminants. These types of contaminants persist at easily measurable concentrations. There are other lesser known class of so called 'emerging' contaminants (*e.g.*, steroids, pharmaceuticals, antibiotic-resistance genes, prion proteins and endocrine-disrupting compounds) those have only recently become detectable at the low concentrations at which they persist (Snow et al., 2013). However these emerging contaminants are found to be contaminating the environment and adversely affecting the health of ecosystems since long time.

Worthy of wider and more detailed study, endocrine-disrupting compounds (hormones) are capable of producing toxic effects (*i.e.*, altered reproductive, neurological or immunological responses) by acting on animal and human endocrine systems at concentrations as low as ng L^{-1} (Khanal, 2006). Significant quantities of steroidal estrogen hormones associated with livestock manure are introduced into the environment when livestock manure is applied to the topsoil of agricultural lands to improve soil fertility and crop quality.

Manure production in Canada increased by 16% between 1981 to 2006 (Hofmann, 2008). The likelihood of manure-borne steroidal estrogen hormones leaching through the soil profile to groundwater and adversely affecting water quality is high. Several studies (*e.g.*, Combalbert and Hernandez-Raquet, 2010) have reported significant steroid estrogen hormone loads in liquid swine manure, which, in Canada, accounts for close to a third of all the manure applied to the nation's 3.4 million hectares of manure-amended lands (Hofmann, 2008).

Endocrine-disrupting compounds can alter the metabolic pathways that regulate reproductive processes in aquatic animals (Arcand-Hoy and Benson, 1998). Given that exposure to hormones or estrogenic compounds can result in the population decline of aquatic fauna, it is critical, where animal manure has been applied to the topsoil of agricultural fields, to avoid the leaching of hormones through the soil profile into groundwater.

While many sorbents have been developed over the years which can remove contaminants such as metals and pesticides, some studies suggest that biochar is particularly well-suited to retain a

variety of organic and inorganic compounds in the soil (Antal et al., 2010). Biochar is the by-product of the thermo-chemical decomposition of biomass and biological residues in the absence of oxygen. Due to its high specific surface area, nano-scale condensed aromatic rings, micro-scale crystalline structure and macro-scale amorphous structure, biochar offers a strong sorption affinity for both inorganic contaminants (*e.g.* heavy metals) and hydrophobic organic contaminants (Lehmann, 2009). Applied directly to the soil, biochar provides a convenient way of disposing of organic waste, providing added value by binding pollutants and reducing their bioavailability. The present study's objectives were therefore to investigate: (i) the capacity of 1% slow pyrolysis biochar, one year after its application to a sandy soil as a topsoil amendment, to absorb liquid swine manure-borne steroid sex hormones (estrogens), and (ii) the fate and transport of steroid sex hormones (estrogens) from liquid swine manure in a sandy soil in the presence (*vs.* absence) of biochar.

3.2 Material and methods

3.2.1 Analytical chemicals

The analytical chemical standards for the female sex hormones, 17β-estradiol (a.k.a. E2; ≥98% purity) and its primary metabolite, estrone (a.k.a. E1; ≥99% purity) were purchased from Sigma Aldrich (St. Louis, MO, USA). The physiochemical properties and the chemical structure of these steroid hormones are shown in Table 2.1 and Table 2.2. High performance liquid chromatography (HPLC) grade Acetonitrile, used both as a solvent and for the mobile phase, was purchased from Fisher scientific. Double-deionized water (Milli-Q Millipore) was used in the preparation of standards solutions and mobile phase solutions.

3.2.2 Experimental setup

A field experiment investigating hormone transport through a sandy soil of the St. Amable complex (Table 3.1), in the presence or absence of biochar, was conducted in six outdoor PVC lysimeters. Located at the Macdonald Campus Farm of McGill University, in Sainte-Anne-de-Bellevue, QC (lat. 45° 24' 48.8'' N, long. 73° 56' 27.9" W), these outdoor lysimeters were maintained under natural conditions, but shaded and sheltered from rain under a canopy to allow

a controlled irrigation. To prevent any plant uptake, plant residues were removed from the soil surface and no crop was planted.

Table 3.1 Physical and chemical properties of the experimental soil

Soil type	Sand (%)	Silt (%)	pH	Bulk density ($Mg\ m^{-3}$)	OM[a] (%)	CEC[b] ($cmol\ kg^{-1}$)	K_{sat}[c] ($cm\ d^{-1}$)
Sandy	92.2	4.3	5.5	1.35	2.97	4.9	1.67±0.45

[a] Organic matter, [b] Cation exchange capacity, [c] Saturated hydraulic conductivity ± standard deviation

The present experiment was conducted between June and August 2013, during which the maximum, minimum and mean temperatures were 23.5°C, 13.1°C and 18.3°C, respectively, and the average relative humidity was 70.96%, based on meteorological data collected at the nearby Sainte-Anne-de-Bellevue weather station (WMO ID 71377, Environment Canada, lat. 45° 25' 26.9" N, long 73° 56' 15.1" W).

At their bottom, the lysimeters (0.45 m Inner Diameter× 1.00 m height) were sealed to 0.60 m × 0.60 m PVC sheets of 0.025 m of thickness. Each lysimeter was packed with sandy soil and adjusted to a bulk density of 1.35 $Mg\ m^{-3}$. A 50 mm diameter drainage pipe was installed at the bottom of each lysimeter. To take composite sample from each depth, four, 10 mm diameter, soil sampling holes were made in each lysimeter at depth of 0.10, 0.30 and 0.60 m from the top of soil surface (Figure 3.1).

Two treatments were replicated three times: soil only (control, no biochar) and top soil (0-0.05 m depth) amended with biochar. BlueLeaf biochar (BlueLeaf Inc., Drummondville, QC, Canada), which was produced by slow pyrolysis of softwood at 450°C, was applied at a dry weight basis 1:99 biochar:soil ratio (1% w/w), and mixed with top soil, thus resulting in an application rate of 10 $Mg\ ha^{1}$ (Antal et al., 2010). The structural properties and particle size distribution pattern of BlueLeaf biochar, determined by an external laboratory (Control Laboratories Inc., Watsonville, California, USA.) are given in Table 3.2 and Table 3.3 respectively.

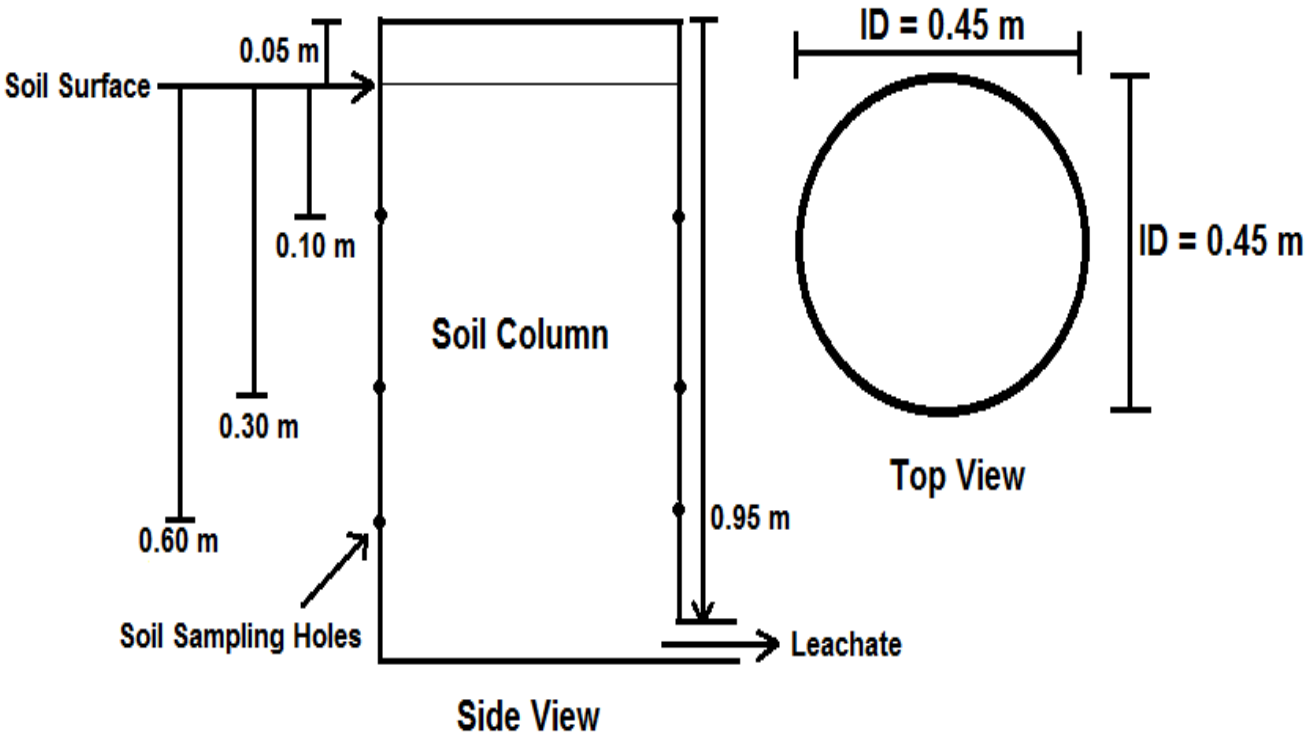


Figure 3.1 Schematic design of the lysimeter (ID = Inner Diameter).

Table 3.2 Typical properties of BlueLeaf biochar, as provided by the manufacturer

Property of biochar	Value
Total ash (g hg^{-1})	17.30
Organic carbon (g hg^{-1})	77.00
Inorganic carbon (g hg^{-1})	0.50
Hydrogen/Carbon (mol mol^{-1})[a]	0.34
Hydrogen (g hg^{-1})	2.20
Total nitrogen (g hg^{-1})	0.56

[a] The H/C ratio is indicative of the biochar's degree of carbonization.

Table 3.3 Particle size distribution analysis of slow pyrolysis biochar

Particle size	Fraction %
< 0.15 mm	2.9
0.15-0.18 mm	4.4
0.18-0.25 mm	4.9
0.25-0.425 mm	3.9
0.425-0.850 mm	9.4
0.85-2 mm	22
2-6.3 mm	13.9
6.3-9.5 mm	18.4
9.5-16 mm	6.9
16-19 mm	13.2
> 19 mm	0

Microscopic imaging of biochar samples was undertaken using a Hitachi TM3000 Scanning Electron Microscope (SEM) operating at 15 kV. Images were taken at 250× magnification. SEM images are very useful in obtaining accurate details of biochar pore structure. Structural changes in biochar after residence in the field were analysed by scanning the surface and inside — after gentle dissection — of freshly incorporated and field-aged biochar, using the SEM.

3.2.3 Manure application

Swine manure was obtained from Quinn Farm (Notre-Dame-de-l'Île-Perrot, QC). Prior to the present study, the last application of manure to the lysimeters had occurred in summer 2012. The history of manure application, nitrogen requirements for a hypothetical silage corn crop, nitrogen availability of manure and application time were among the factors considered in selecting the manure application rate. Considering a spring incorporation of manure into the soil, based on N requirements of 20 g m^{-2} to achieve a typical yield of 4.94 kg m^{-2} (Beegle, 1997), and a mean total N content of liquid swine manure of 4 g L^{-1}, the required manure application rate was

calculated to be 16 L m^{-2}. Therefore, based on the soil surface area within each lysimeter area, 2.54 L of homogenized liquid swine manure were applied to each lysimeter.

3.2.4 Soil and leachate sampling

Prior to initiating the experiment, all lysimeters (control and biochar amended) received sufficient irrigation to bring them to saturation. In order to assess the soil's residual hormone levels in light of the previous year's manure application, prior to the manure application (Day 0), composite soil samples were taken at the surface and at depths of 0.10, 0.30 and 0.60 m, and placed in sealed plastic bags. On the next day (Day 1), 2.7 L of homogenized swine manure were manually incorporated into the top soil of each lysimeter. Soil samples were taken from the manure-amended topsoil prior to the first irrigation to quantify the initial level of hormones in the top soil. A total of 9.67 L of tap water was then applied at regular intervals over a 4 h period, thereby mimicking natural rainfall of 57.8 mm. Amount of mimicking natural rainfall was calculated on the basis of worst case scenario in the month of May since last 50 years (1962-2012). On the following day (Day 2) and on Days 16, 31, and 46 after manure application (one day after Day 15, 30 and 45 irrigations), the Day 0 soil sampling protocol was repeated.

For each lysimeter, leachate exiting the drainage pipe was collected for the 24 hrs immediately following irrigation events (Days 1, 15, 30, 45). For each lysimeter, 7.5-8.0 L of leachate was collected in an amber bottle (to avoid hormone photo degradation), immediately taken to the lab, and a subsample extracted the same day, to avoid the higher rate of hormone degradation in water than in soil.

3.2.5 Mass balance calculations

Based on the mass balance principle (Eq. 1), the total mass of hormones recovered from each lysimeter at each sampling date was calculated as the sum of: (i) the hormones recovered in the soil across each depth of the soil profile (0.05-0.10 m, 0.10-0.20 m, 0.20-0.50 m, 0.50-0.75 m), and (ii) the mass of hormones recovered from leachate samples, calculated as the product of the hormone concentration by the leachate volume. This should total the initial hormone mass (µg)

present, minus any losses or unrecovered mass of hormones due to degradation or volatilization (ElSayed et al., 2013).

$$HRM_{Day1} = A_{cs}\,\rho\left(C_{0.05-0.10}\theta_{0.05-0.10}h_{0.05-0.10} + C_{0.10-0.20}\theta_{0.10-0.20}h_{0.10-0.20} + C_{0.20-0.50}\theta_{0.20-0.50}h_{0.20-0.50} + C_{0.50-0.75}\theta_{0.50-0.75}h_{0.50-0.75}\right) + C_{leach}V_{leach} + HRM_{lost} \quad (1)$$

where,

h_{x-y} is the depth of a given soil layer (cm), calculated as y-x,

A_{cs} is the soil column's cross-sectional area (1590.4 cm^2), calculated as $\pi\left(\frac{D}{2}\right)^2$, where D is the lysimeter inner diameter (45 cm),

C_{leach} is the hormone concentration in the leachate (µg L^{-1}),

C_{x-y} is the hormone concentration (µg kg^{-1}) in the soil layer ranging from x to y in depth,

V_{leach} is the volume of leachate (L),

ρ is the soil bulk density (Mg m^{-3}),

θ_{x-y} is the soil gravimetric moisture content in the soil layer ranging from x to y in depth.

3.2.6 Hormones extraction from leachate samples

Of the 7.5 to 8 L of leachate collected at the bottom of each lysimeter, a subsample (1 L) was filtered through a PreSep prefilter 47 mm (GE Water and Process Technologies, Trevose, PA), to remove suspended soil particles. Prior to hormone extraction, Oasis HLB extraction cartridges (200 mg, 6 cc; Waters Inc., Milford, MA) were conditioned with the passage of 5 ml of methanol, followed by 5 ml of high purity water (Stafiej et al., 2007). In the initial step of the solid phase extraction (SPE) process, a vacuum manifold system drew the filtered leachate subsample through the Oasis HLB extraction cartridge at a rate of 20 mL min^{-1}. The hormones were then eluted by drawing 10 mL of HPLC grade acetonitrile through the cartridge. The extract was brought to dryness under a nitrogen stream and redissolved in 1 mL of 50:50 (v/v) water-acetonitrile solution. The glass tube containing the extract was placed in a sonicator for 15 minutes to achieve homogeneous mixing. The extract was then filtered through 0.22 µm syringe-driven sterile filters (Millex-GV, Japan). The final extract was transferred into 1.5 ml amber HPLC vials for HPLC analysis.

3.2.7 Hormones extraction from soil samples

The gravimetric moisture content of a portion of each soil sample was determined and the remaining portions of the samples were stored in sealed plastic bags in the freezer at -20° C until extraction. After thawing at room temperature in their sealed bags, the remaining portions of each soil sample were thoroughly mixed. For each sample, a moist soil subsamples (5 g) was placed in a 50 mL polyethylene centrifuge tube, to which were added 10 mL of HPLC grade acetone and 5 g of anhydrous Na_2SO_4 (Xuan et al., 2008). Following thorough mixing (1 min on Vortex mixer), the mixture was vigorously shaken on a reciprocating shaker (30 min @ 250 rpm). The mixture was then centrifuged at 4000 rpm for 20 minutes. After centrifugation, the supernatant was transferred to a clean 50 mL polyethylene centrifuge tube, and a further 10 mL of acetone was added to the supernatant. The mixing, shaking and centrifugation were repeated as above, and the resultant supernatant transferred to another clean 50 ml polyethylene centrifuge tube. After another round, the final supernatant was transferred into a 50 ml pyrex glass centrifuge tube and dried completely under a gentle N_2 stream. The dried extract was redissolved homogeneously in 1 mL of 50:50 (v/v) water-acetonitrile solution by placing the glass tube containing the extract in a sonicator for 15 minutes. The extract was then filtered through sterile 0.22 µm filters (Millex-GV, Japan), then transferred into 1.5 ml amber HPLC vials for HPLC analysis.

3.2.8 HPLC analysis

An HPLC technique served to analyze the hormone content in samples. The mobile phase consisted of a 3:2 ratio of water : acetonitrile mixture, while the stationary phase (5 µm particle size) was that of a Zorbax Eclipse Plus C18 column (150 × 4.6 mm; Agilent Technologies Inc., Santa Clara, CA). Flow rate of the mobile phase was set at 1 mL min^{-1}, running on a gradient flow. Run time for each 100 µl injection was set at 15 minutes. The UV detector wavelength was set at 210 nm.

3.2.9 Data analysis

Data was analyzed with PROC MIXED in SAS v. 9.2 (SAS Institute Inc., 2010). This model used repeated measures over time and depth to determine if hormone concentration differed between treatments, over time and with varying depths.

3.2.10 Sorption Test

In a batch sorption experiment undertaken in support of the field experiments, hormone sorption efficiency of fresh *vs.* two year field-aged 1% slow pyrolysis biochar-amended soils were compared. Standard solutions of both estrone (E1) and 17β-estradiol (E2) at six different concentrations (0.01, 0.05, 0.1, 0.5, 1, and 5 ppm) were prepared in HPLC grade acetonitrile from pure standards (Sigma Aldrich, St. Louis, MO). Based on the method of Sarmah (2008), a sample of fresh or field-aged biochar-amended soil (2 g) was equilibrated with 30 mL of a given standard solution in a 50 mL aluminium-foil-wrapped glass centrifuge tube equipped with a Teflon-lined screw cap. All tubes were placed on a flat-bed shaker for 24 hr in the dark at 23±2°C.

3.3 Results and Discussion

3.3.1 Mass balance

The mass balance for E_2 for Day 0 (Table 3.4) shows the presence of a detectable mass of hormones present at different soil depths attributable to the previous year's application of swine manure. In both non-amended and biochar-amended treatments, the total quantity of E2 detectable in the system (soil + leachate) declined progressively from an initial (manure just applied) level (Day 1) through Days 2, 16, 31 and 46. The degradation rate of hormones decreased over time or at lower concentrations, which concurs with the findings of Casey et al. (2005).

Table 3.4 Mass balance for estradiol (E2) and estrone (E1) present in different soil profile ranges, leachate and overall total, prior to manure application (Day 0), immediately after manure application (Day 1, topsoil only), and 1, 15, 30 and 45 days thereafter (Days 2, 16, 31, and 46, respectively).

Day	Quantity of hormone (µg) by fraction											
	Manure-amended soil						Manure- and biochar-amended soil					
	Soil profile depth range (m)				Leachate	Total	Soil profile depth range (m)				Leachate	Total
	0.05-0.10	0.10-0.20	0.20-0.50	0.50-0.75			0.05-0.10	0.10-0.20	0.20-0.50	0.50-0.75		
	Estradiol (E2)											
0	0.00	5.66	20.59	7.89	0.00	34.14	0.00	0.00	12.88	0.00	0.00	12.88
1	229.28						192.18					
2	163.62	5.14	34.05	18.65	0.316	221.78	71.25	5.51	44.95	41.48	0.054	163.25
16	69.27	5.05	69.35	47.62	0.086	191.37	35.03	19.63	43.92	34.39	0.074	133.04
31	49.78	3.11	13.24	9.20	0.693	76.03	15.84	3.97	14.01	9.09	0.488	43.41
46	24.73	0.00	0.00	4.67	0.365	29.77	8.89	5.34	14.87	6.19	0.498	35.81
	Estrone (E1)											
0	0.12	0.00	17.98	0.00	0.00	18.10	36.13	24.96	2.98	0.00	0.00	64.08
1	487.98						462.51					
2	230.98	0.96	7.01	4.91	0.312	244.19	124.62	2.19	4.48	10.18	0.133	141.61
16	113.28	0.00	4.05	0.00	0.055	117.39	38.65	1.28	0.00	4.35	0.053	44.53
31	44.36	49.73	23.19	49.04	0.278	166.61	24.56	25.90	27.94	55.46	0.287	134.16
46	0.00	0.97	2.73	2.62	0.184	6.52	3.84	0.90	2.72	2.47	0.193	10.13

The biochar-amended treatment lost more E2 from the top soil than the non-amended control (Table 3.4). This loss might be attributed to the leaching of E2 or to its degradation. For the biochar-amended and non-amended treatments, 45% and 23%, respectively of the initial mass of hormones applied reached soil layers beneath the topsoil one day after the first irrigation event (Day 2), indicating a greater mobility of hormones under the biochar-amended treatment. This seeming anomaly might be attributable to the release of dissolved organic carbon (DOC) from biochar. The field site where we applied biochar is subject to steep temperature gradients. Biochar particles can be fragmented into smaller particles when water penetrates the pores and swells during freezing, forcing the biochar particles to break (Carcaillet, 2001). After initial physical fragmentation, the most profound changes to biochar in the soil were chemical and microbial, with the products generated including DOC (Lehmann, 2009). The DOC has a tendency to facilitate the mobility of hormones to lower depths in the soil column (Sarmah et al., 2008). The major decline in E2 mass observed in the soil profile between Day 16 and Day 31, may be attributable to biotransformation of E2 into estrone (E1) (Lee et al., 2003). This is supported by the increase in the mass of E1 between Day 16 and Day 31 (Table 3.4). While no particular trend in leachate E2 mass was evident over time (Table 3.4), the small quantities of E2 in the leachate are still of concern since even trace amount of hormones can prove to be very toxic to aquatic fauna (Khanal, 2006).

A graph (Figure 3.2) is drawn for percentage of mass retained at the surface of soil profile after each irrigation event during first and second year after biochar application. During the first year of biochar application the biochar-amended soil retained a greater mass of E2 than the non-amended soil (Figure 3.2A) throughout the experimental period (Alizadeh, 2014). Biochar has a porous structure (Lehmann, 2009), providing sites for hydrophobic interactions. The relative hydrophobicity of estrogens ($2.6 < \log K_{ow} < 4.0$) (Lai et al., 2000) is a good indication of the strong sorption affinity of these hormones, and accounts for why they were held by biochar applied at the surface of the soil (Yu & Huang, 2005). However in second year after biochar amendment, the biochar-amended soil retained less E2 than non-amended soils (Figure 3.2A). As explained above, this was attributable to the release of DOC from biochar, and its promotory effects on hormone leaching through the soil.

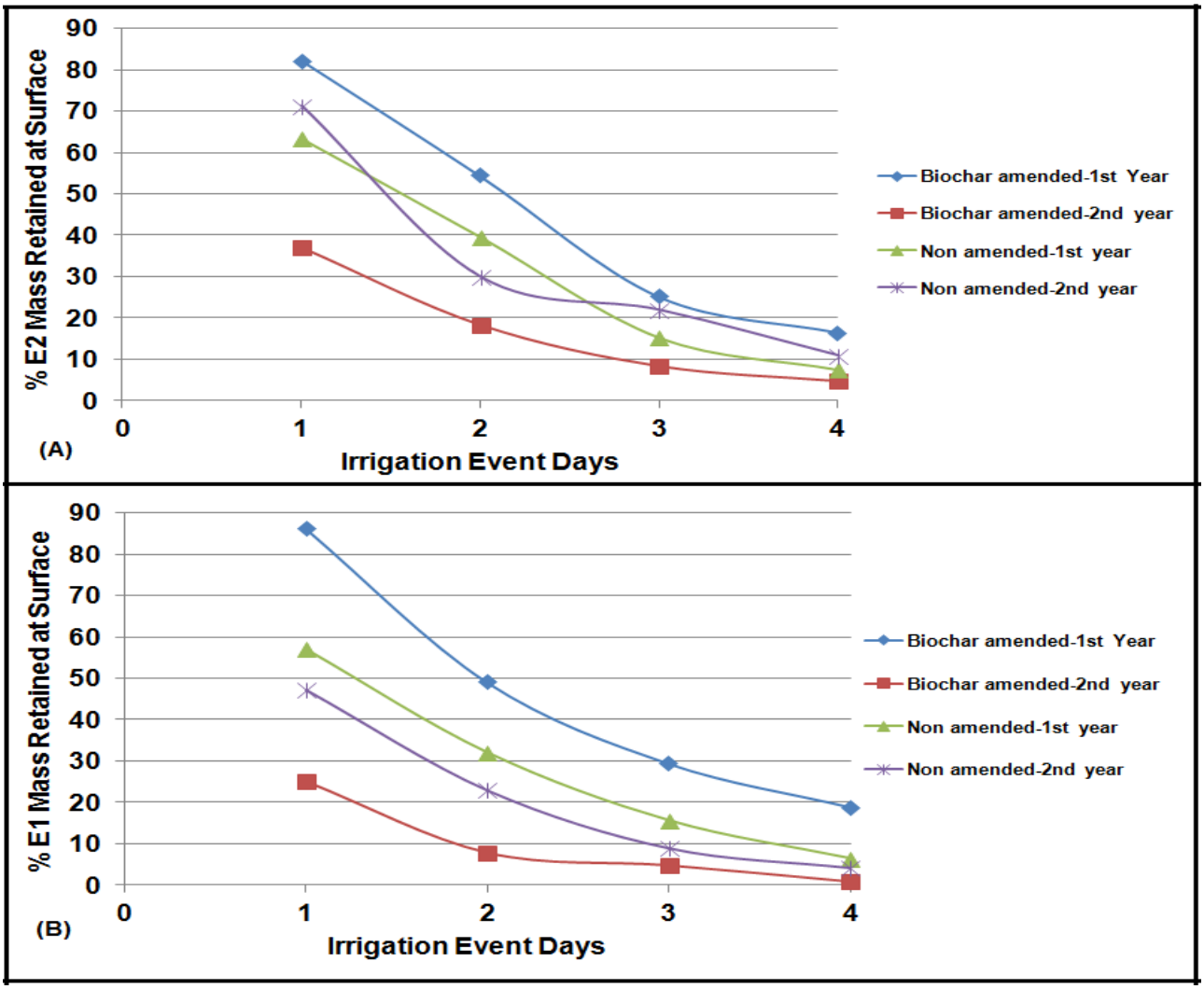


Figure 3.2 Trend of percentage mass retained at the surface of soil profile (cumulative) after each irrigation event during first and second year after biochar application: **(A)** 17β-estradiol (E_2); **(B)** estrone (E_1).

In the second year, a lesser rate of E2 degradation was observed in the biochar-amended soil than occurred in the first year. This is attributable to the abundant energy source provided to microorganisms by the release of DOC in biochar-amended treatments, leaving E2 relatively ignored (Herman & Mills, 2003).

The mass balance for estrone (E1), shows that trends for E1 were almost the same as those for E2, except that E1 moved more slowly through the soil profile than E2 (Table 3.4). The fact that the biochar-amended soil lost more E1 from the topsoil than did the non-amended soil, may be attributable to either enhanced leaching of E1 along with DOC, or to the degradation of E1. However, the chances of E1 leaching along with DOC are comparatively less than those of E2,

given the latter's greater aqueous solubility (Yu et al., 2004). This explains why a lesser quantity of hormones E1 were detected at the lower soil depths after the first two irrigation events.

Only 3.17% and 2.5% of the initial mass of hormones applied to the biochar-amended and non-amended soils, respectively, reached the lower soil layers after the first irrigation, again highlighting the lesser mobility of E1. Colucci et al., (2001) found the major losses of E1 to only occur in the top layers of the soil, where conditions were aerobic and thus favorable to E1 degradation. The increase in E1 mass at the lower depths of the soil column between day 16 and day 31 is likely attributable to biotransformation of E2 into E1 (Lee et al., 2003), as the decline in the mass of E2 in these layers between Day 16 and Day 31 attests (Table 3.4). The prominent increase in E1 under the anaerobic conditions prevailing in the deeper layers of the soil profile, compared to the lower levels under the relatively aerobic conditions of the shallower layers, is attributable to the lower microbial degradation of E1 under anaerobic conditions. As with E2, while no particular trend in leachate E1 mass was evident over the study period (Table 3.4), the small quantities of E1 in the leachate are still of concern, since even trace amount of hormones can prove toxic to aquatic fauna (Khanal, 2006).

The cumulative percentage of E1 held at the surface after each irrigation, relative to the initial amount of E1 applied in manure (Day 1) was calculated for biochar-amended and non-amended soils in the first and second years after the amendment was applied (Figure 3.2B). In the first year, biochar-amended soils held comparatively more of the total E1 mass in their top layers than did the non-amended soils, whereas in the second year the converse was true, reflecting the release of DOC from aged biochar, thereby facilitating the movement of hormones down to soil profile. Compared to E2, a lesser mass of E1 was detected in the surface layer of both treatments, since E1 is particularly prone to degradation at or near the surface, where conditions are aerobic (Colucci et al., 2001). Clearly biochar retained a comparatively greater mass of E1 in the first year after its application, when it was fresh, whereas, with time, the biochar's ability to retain hormones declined as the result of some biotic and abiotic changes in its surface structure.

3.3.2 Effect of biochar on hormone movement in soil

The downward movement of E2 and E1 over time was assessed in biochar-amended and non-amended soils (Figure 3.3 and 3.4). Clearly, the concentration of E2 was highest on the initial day of swine manure application (Day 1) and decreased thereafter over a period of 46 days

(Figure 3.3). There was rapid initial degradation of hormones even one day after manure application (Day 2). Ying et al. (2002) also reported a rapid decrease in concentration of hormones in soil. This rapid decrease could be explained by either leaching of E2 downwards immediately after the irrigation, or its rapid degradation. Estrogen degradation is two phase process. First phase is rapid degradation followed by second phase of slow degradation. Degradation rates decreased through time and with lower concentrations (Casey et al., 2005) . The high sand content of the soil's upper would result in weaker sorption of E2 than a silt or clay soil (Casey et al., 2005). The fact that, following irrigation (*i.e.*, between Day 1 and Day 2) a certain amount of E2 leached down to lower soil depths is illustrated in Figure 3.3. Degradation of E2 in the topsoil could also be the result of photo- or bio-degradation or both.

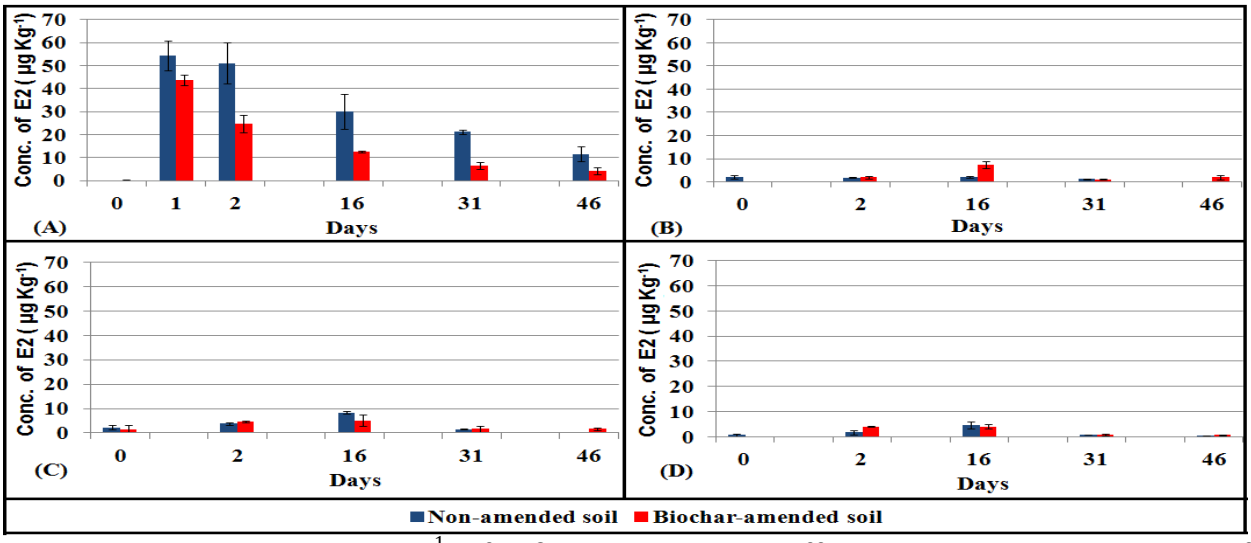


Figure 3.3 Concentration (µg kg^{-1}) of 17β-estradiol **(**E2) at different soil depths over a period of 46 days, for both biochar-amended and non-amended lysimeter soils: (A) at the soil surface; (B) at a depth of 0.10 m from the soil surface; (C) at a depth of 0.30 m from the soil surface; (D) at a depth of 0.60 m from the soil surface.

Similarly, Day 2 E1 concentrations were higher at the surface of the soil profile and then decreased over the period of 46 days (Figure 3.4A). As a comparison of parts B, C, and D of Figures 3.3 and 3.4 indicate, the leaching of E1 to lower soil depths was less than that of E2. This might be due to E1's lower aqueous solubility (2.1±0.03 mg L^{-1}) compared to that of E2 (3.1±0.02 mg L^{-1}) (Yu et al., 2004). In addition, E1 in the upper soil layer was degraded more quickly than was E2. That this degradation was greater in the biochar-amended top soil than in its non-amended counterpart may be attributable to the degradation of E1 under aerobic conditions is primarily biotic, *i.e.*, it is driven by microbes (Colucci et al., 2001). Because

biochar provides an environment for the growth of microbes (Wei et al., 2014) its presence results in a higher rate of biotic degradation of E1 in biochar-amended (*vs*. non-amended) soils.

Concentrations of E2 and E1 at a depth of 0.10 m from the soil surface are shown in Figures 3.3B and 3.4B, respectively, for both biochar-amended and non-amended soils. Given that the depth of 0.10 m is relatively close to the soil surface little difference in E2 was observed between the two treatments. However, on Day 16, greater E2 movement was apparent in the biochar-amended soil. Unlike E2, E1 showed little leaching. At this depth, levels of E1 seen on Day 31 were likely due to biotic transformation of E2 into E1 (Goeppert et al., 2014).

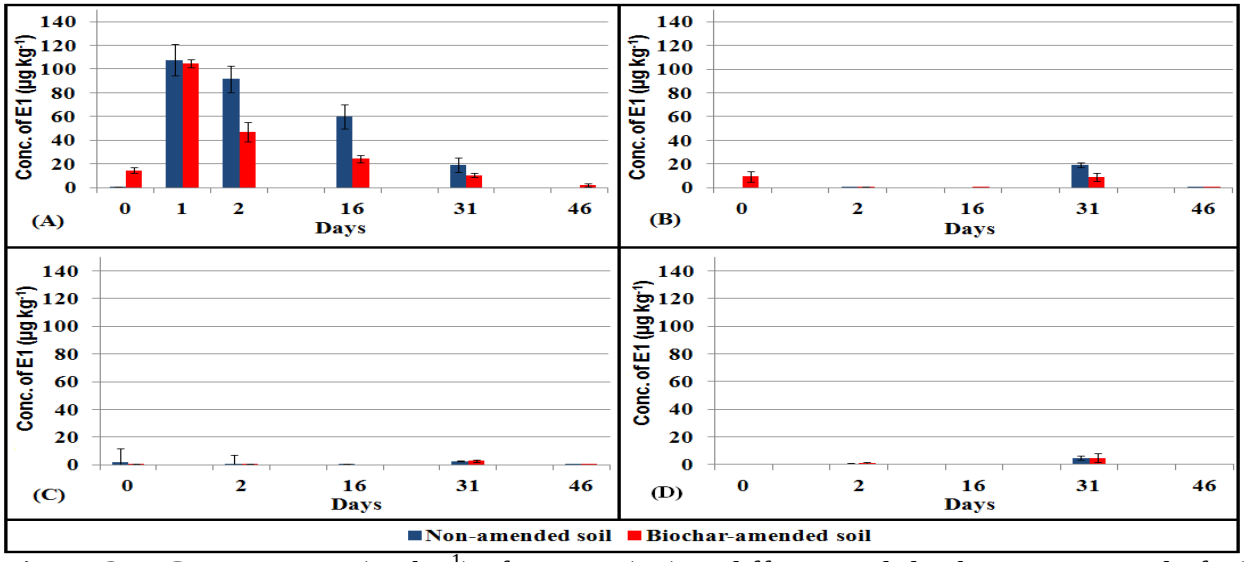


Figure 3.4 Concentration (µg kg^{-1}) of estrone **(**E1) at different soil depths over a period of 46 days, for both biochar-amended and non-amended lysimeter soils: (A) at the soil surface; (B) at a depth of 0.10 m from the soil surface; (C) at a depth of 0.30 m from the soil surface; (D) at a depth of 0.60 m from the soil surface.

Concentrations of E2 and E1 at a depth of 0.30 m from the soil surface are shown in Figure 3.3C and 3.4C, respectively, for both biochar-amended and non-amended soils. Clearly, greater quantities of E2 than E1 were leached from the topsoil to this depth.. As occurred at the 0.10 m depth, the biotic transformation of E2 into E1 was evident at the 0.30 m depth 30 between Day 16 and Day 31 (Figure 2C, 3C). Similar trends were also observed at the 0.60 m depth for both biochar-amended and non-amended treatments (Figure 3.3D and 3.4D).

While certain trends of E2 and E1 movement over time and depth were evident, no clear trend was found to indicate any difference in hormone movement between the biochar-amended and non-amended soils. This was supported by the data analysis with PROC MIXED in SAS v. 9.2

(SAS institute Inc., 2010). According to the statistical analysis (Table 3.5 for E2 and E1), difference of movement of both hormones between the treatments was insignificant**.**

Table 3.5 Repeated measures analysis of variance for estradiol (E2) and estrone (E1) residue in soil and leachate.

Effect	Probability (*P* value)*a*	
	Estradiol (E2)	Estrone (E1)
Soil		
Treatment	0.1899	0.1571
Depth	**<0.0001**	**<0.0001**
Treatment × Depth	0.0209	0.3180
Time	**<0.0001**	**<0.0001**
Treatment × Time	0.7526	0.1199
Depth × Time	**<0.0001**	**<0.0001**
Treatment × Depth × Time	0.8787	0.0359
Leachate		
Treatment	0.1967	0.4190
Day	0.0853	0.6562
Treatment × Day	0.2529	0.2740

[a] bolded values are significant ($P \leq 0.01$), others are not ($P > 0.01$)

3.3.3 Effect of biochar on hormone residues in leachate water

The leachate samples were analyzed to quantify the amount of hormones leached under both treatment. The E1 and E2 hormones are slightly soluble in water: the aqueous solubility of E2 is 3.10±0.02 mg L^{-1}, while that of E1 is 2.10±0.03 mg L^{-1} (Yu et al., 2004). This might account for the low levels of E2 and E1 in leachate even after the first irrigation event (Figure 3.5A, 3.5B). Even at levels as 5 ng L^{-1}, hormones can be toxic to aquatic species (Khanal, 2006). Statistical analysis showed no significant effect of either time or biochar amendment on either E_2 and E_1 concentrations in leachate (Table 3.5).

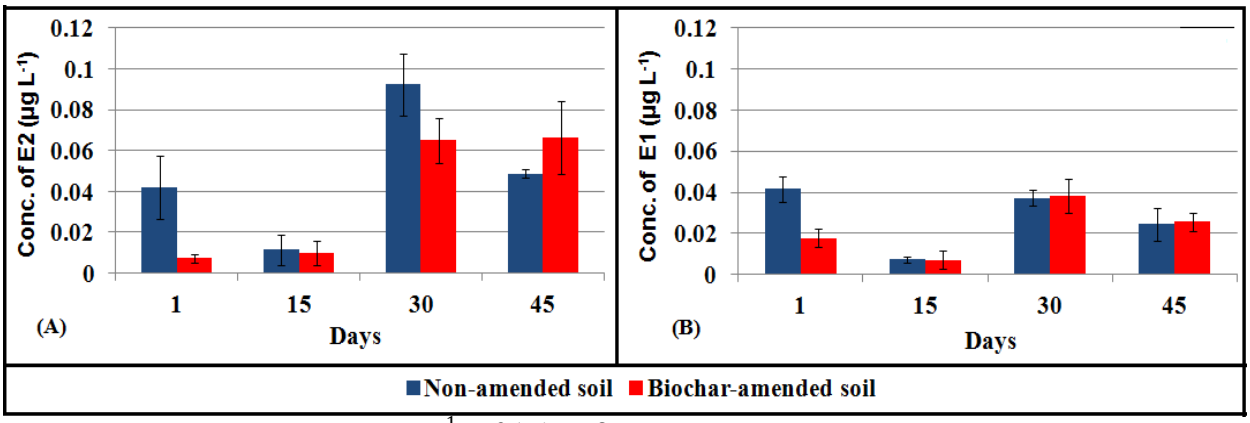


Figure 3.5 Concentrations (µg L^{-1}) of (A) 17β-estradiol (E_2) and (B) estrone (E_1) in leachate over a period of 46 days, for both biochar-amended and non-amended lysimeter soils

3.3.4 Scanning Electron Microscopy (SEM)

Clearly there were disturbed pores on surface and irregular structure inside of used biochar as compared to fresh biochar (Figure 3.6). This analysis supports our assumption of the release of DOC upon initial physical fragmentation of the biochar (Lehmann, 2009) which heightened the mobility of hormones in the biochar treatment.

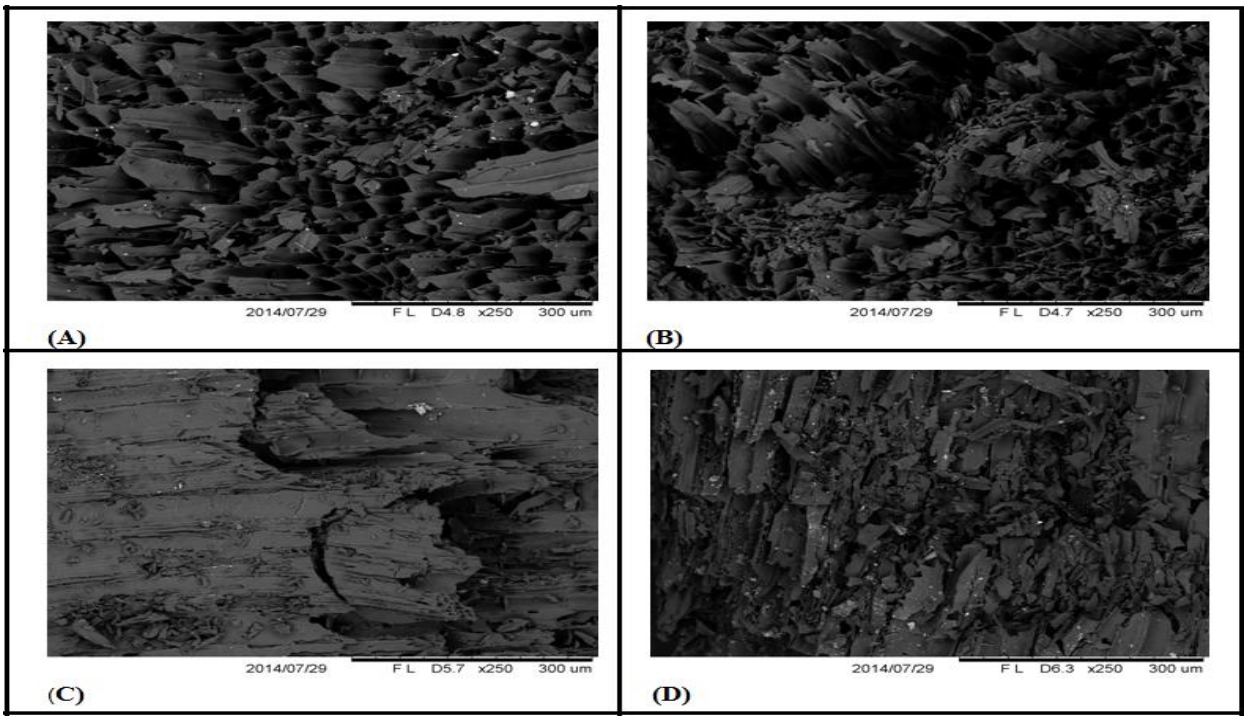


Figure 3.6 Scanning Electron Microscopy (SEM) images: (A) Surface of biochar from freshly amended soil; (B) Surface of biochar from 2-year old biochar-amended soil; (C) Inner structure of biochar from freshly amended soil; (D) Inner structure of biochar from a 2-year old biochar-amended soil.

3.3.5 Sorption efficiency of biochar

A batch sorption laboratory experiment tested the difference of sorption efficiency between soil amended with fresh biochar and biochar-amended soil having spent two years in the field. Sorption efficiency was tested on pure hormone solutions prepared in the lab (Sarmah, 2008). Aged biochar-amended soil was clearly less effective in adsorbing hormones (E2 and E1) than was fresh biochar-amended soil (Figure 3.7), which support our field results. Aged biochar-amended soil was more efficient in adsorbing E2 than E1 (Figure 7), which concurs with our field experiment findings.

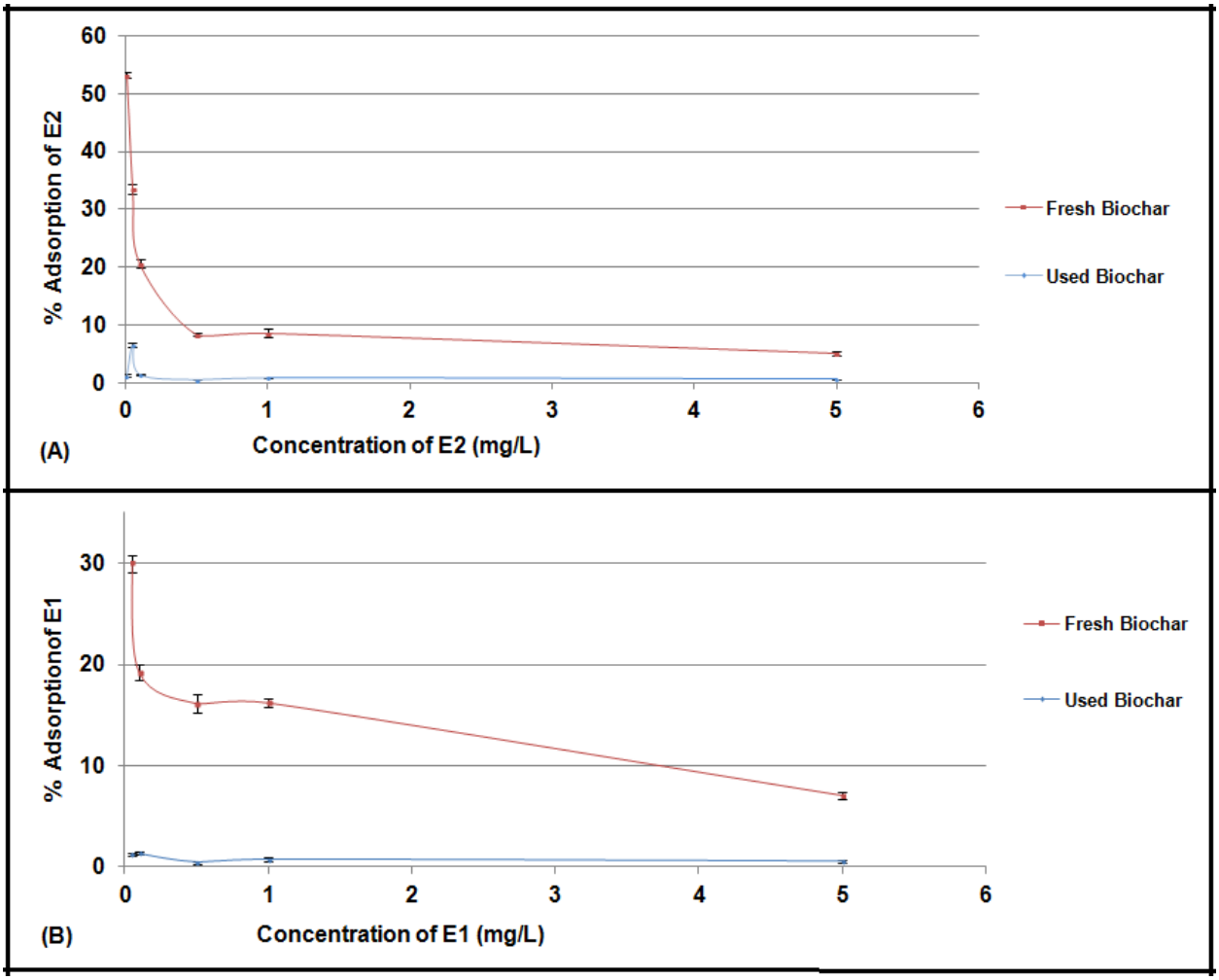


Figure 3.7 Batch sorption test of (A) 17β-estradiol (E2) and (B) estrone **(**E1) adsorbed by freshly biochar-amended soil, and field-aged (2 years) biochar-amended soil.

3.4 Conclusion

In the first year of our study on a biochar soil amendment's adsorption capacity for hormones (Alizadeh, 2014), a significant effect of soil amendment with biochar (*vs.* a non-amended control) was found with respect to the spatial-temporal stratification of steroidal sex hormones in the soil and leachate. However, in the second year of study reported here, the biochar amendment treatment had no significant effect on the spatial-temporal stratification of steroidal sex hormones in the soil and leachate. This finding was further supported by laboratory batch sorption tests comparing a 2-year aged biochar-amended soil and a newly biochar-amended soil of the same type. Many factors might have played a crucial role in the lesser adsorption capacity of biochar for hormones in the second year after its application. Certain surface structural changes in biochar might have occurred as a result of various biotic or abiotic processes. In addition, substances such as humic acid, minerals and metal oxides in the soil could alter the physical and chemical properties of the biochar's surface, thus affecting its sorption of hydrophobic organic compounds such as the hormones studied. Scanning electron Microscopy showed that the biochar, after some initial physical fragmentation began adsorbing less and might started releasing DOC to soil (Lehmann, 2009), which might act as a carrier for hormones down to soil profile, as proposed by Liu et al. (2013). These authors concurred with our results in finding that mildly-degraded biochar is less effective in improving soil water holding capacity, nutrient retention capacity and adsorption capacity for organic contaminants compared to the fresh biochar. Current study also found the mobility of 17β-estradiol (E2) to be higher than that of estrone (E1) in the presence of 1% slow pyrolysis biochar-amended soil, in the second year after of its application. So on the basis of results obtained from this study we can firmly conclude that adsorption capacity of 1% slow pyrolysis biochar for estrogens released from liquid swine manure at our specific field conditions, decreases with time. However, it should be noted that no two biochar are same, behaving differently in different conditions. Further studies are needed to explore the role of different weather conditions on different types of biochar in different type of soils. Such a wider characterization of the effects of biochar amendment of agricultural soils, is important in achieving a maximum reduction of hormonal pollution in soil and water.

Preface to Chapter 4

In last Chapter, we studied the fate and transport of estrogens from swine manure in sandy soil under second year of biochar application. Like swine manure, poultry manure is also considered as a potential threat to environment in view of growing concern towards emerging contaminants. According to the Statcan (2006), poultry livestock is among the main manure producers in southern Ontario and Quebec. Various studies confirmed appreciable concentrations of steroid sex hormones of environmental concern present in poultry manure. So it is equally important to study the effect of biochar on fate of hormones coming from poultry manure over time.

Biochar have shown adsorbing potential for hormones in several studies but the duration for which it can work at same capacity is unknown. In this chapter, remaining retention capacity of biochar for estrogens from poultry manure was tested in sandy soil during second year after biochar application. It was a 46 days period study held under the natural field conditions.

To date, this is the first field study testing the effect of slow pyrolysis biochar on fate and transport of estrogens from poultry manure over duration of two years.

Research papers based on the chapter

Sukhjot Mann, Zhiming Qi, Shiv Prasher. **Retention capacity of biochar amended sandy soil for estrogens from poultry manure in second year of biochar application** (to be submitted)

Chapter 4

Retention capacity of biochar amended sandy soil for estrogens from poultry manure in second year of biochar application

Abstract

Humans and animals produce natural sex hormones including the estrogens: estrone (E1) and 17β-estradiol (E2). These compounds are able to disrupt reproductive system of living organisms even at trace concentrations of nanogram-per-litre levels. Hypothesis of this experiment was that 1% slow pyrolysis biochar amended sandy soil can hold almost same amount of estrogens from poultry manure in second year of biochar application as it was holding in first year of its application. This experiment was undertaken for 46 days on the lysimeters packed with sandy soil with biochar as top soil amendment. Biochar held a significant concentration of hormone during first year of its application but in second year of its application (current study), concentration of hormones between biochar amended soil and control was insignificantly different. When biochar was fresh, its pores were available for hydrophobic interactions so it was holding significant concentration of hormones during first year of application but with time there were several biotic and abiotic changes on surface of biochar and after some physical fragmentation, pores on surface were no longer available for hydrophobic interactions and it started releasing dissolved organic carbons which facilitated greater mobility of hormones from poultry manure down to lower depths of soil profile.

Keywords. *Biochar; 17-β-estradiol (E2); estrone (E1); Degradation; Dissolved organic carbon*

4.1 Introduction

Over the last few decades, because of intensification of anthropogenic activities and modern animal feeding operations, excessive quantity of organic wastes (animal manure and bio-solids) have been generated. Animal manures are applied to agricultural fields as fertilizers. However, the application of animal manure has been identified as one of the major gateways for the environment's exposure to new classes of toxic and high-risk organic contaminants known as emerging contaminants. Having endocrine-disrupting properties, some at concentrations as low as ngL^{-1}, these contaminants represent a high chronic toxicity risk and are associated with adverse long-term health issues like carcinogenicity, mutagenicity or teratogenicity in aquatic animals specially fish species (Choi et al., 2004; Arcand-Hoy & Benson, 1998; Goeppert et al., 2014). Aquatic species like trouts, turtles and minnows, may be sexually reversed when exposed to estrogens even at trace concentrations of ngL^{-1} (Jobling et al., 1998). Natural steroidal sex hormones (estrogens) *i.e.,* 17β-estradiol (E2) and estrone (E1) are the emerging contaminants of greatest concern because of their adverse developmental and carcinogenetic effects. Major source of estrogens in environment is from animal manure applied to agricultural fields (Sarmah et al., 2008). There is wide recognition given to the contamination threat caused by nutrient leaching from manure amended agricultural fields but little is known about the leaching of estrogens through the soil to either shallow groundwater or (via drainage water) to surface water. Because of their endocrine disrupting properties, the estrogens, 17β-estradiol (E2) and its metabolite estrone (E1), became a potential environmental concern. Average rainfall is more during spring season and also application rate of manure during this season is high. So probability of leaching of estrogens to ground water is high during this period of time. Recent environmental studies have therefore focused on the prevalence, fate, transport pathways and ecotoxicology of these manure-borne hormones called Estrogens.

Various studies found sorbents that demonstrated the removal of contaminants that are toxic for environment (Bailey et al., 1999; Ying & Kookana, 2005). Some studies have shown significant adsorption potential of biochar for hormones (Sarmah et al., 2010). Biochar has carbon residues resulted from the pyrolysis of biomass (Schmidt & Noack, 2000). Because of biochar's 60-80% black carbon and because of its high surface area (Lehmann, 2009), this engineered sorbent has potential to be used as remediation tool to curb hormonal pollution in agricultural fields. Biochar

have shown adsorbing potential for hormones in several studies but the duration for which it can work at same capacity is unknown. To test the capacity of biochar in retaining hormones from poultry manure was done for two consecutive years. Study on adsorption potential of fresh 1% slow pyrolysis biochar for hormones from poultry manure in our specific field parameters was already done (Alizadeh, 2014). So, the objective of this study is to Investigate the transport of estrogens (hormones) from poultry manure in 1% slow pyrolysis biochar amended sandy soil and exploring the retention capacity of biochar for hormones during second year after its application.

4.2 Material and methods

4.2.1 Study Compounds

Two type of female sex hormones (estrogens) were under the study, 17β-estradiol, commonly known as E2 and its primary metabolite, estrone commonly known as E1. The analytical chemical standards for E2 (≥98% purity) and E1 (≥99% purity) were purchased from Sigma Aldrich (St. Louis, MO, USA). Double-deionised water (Milli-Q Millipore) was used in the preparation of standards solutions and mobile phase solutions. High performance liquid chromatography grade Acetonitrile, which was used both, as a solvent in extraction process and for the mobile phase in HPLC induced separation process, was purchased from Fisher scientific. The physical and chemical properties and the molecular structure of 17β-estradiol (E2) and estrone (E1) are shown in Table 2.1 and Table 2.2.

4.2.2 Soil Characteristics

Sand was used for the field experiment. Sandy soil took from Ste-Amable complex, Quebec, was packed in the lysimeters of specific dimension shown in Figure 1. Experiment site is located at the Macdonald Farm of McGill University, Sainte Anne De Bellevue, Quebec, Canada. The lysimeters were kept outdoors in natural conditions but shaded under a canopy to achieve controlled irrigation. Physical and chemical properties of soil used for experiment are shown in Table 3.1.

4.2.3 Weather parameters

All the meteorological data was collected from the Sainte Anne de Bellevue weather station WMO ID 71377 of Environment Canada. Average relative humidity during the period of experiment was 70.96 % and the maximum, minimum and mean temperatures were 23.55, 13.12 and 18.35 °C, respectively.

4.2.4 Biochar

Biochar used in this study was produced from the slow pyrolysis of soft wood at 450°C. This slow pyrolysis biochar was bought from BlueLeaf Inc, Drummondville, Quebec, Canada. Biochar was applied as top soil amendment in lysimeters in Summer 2012. One percent (w/w) of biochar was mixed with top soil with an application rate of 10t/ha (Antal et al., 2010). Some properties of this specific biochar explored by Soil control lab of Control Laboratories Inc., Watsonville, California, USA, are given in Table 3.2.

4.2.5 Experimental setup

The field experiment investigating hormones transport under the presence of biochar in a sandy soil was conducted in six outdoor PVC lysimeters. The experiment was conducted between June-August 2013. The site chosen for this experiment was located at the Macdonald farm on the Macdonald campus of McGill University in St. Anne de Bellevue, Quebec. The lysimeters (0.45 m I.D. × 1.0 m height), were sealed at the bottom to 0.60 m × 0.60 m PVC sheets. Each lysimeter was packed in layers with sandand adjusted to a bulk density of 1350 kg/m^3. A 0.05 m diameter drainage pipe was installed at the bottom of each lysimeter. To take composite sample from each depth, four horizontal soil sampling holes were made in each lysimeter at depths of 0.10, 0.30 and 0.60 m from the top of soil surface (Figure 4.1). To prevent any plant uptake, plant residues were removed from the soil surface and no crop was planted. Canopy was installed over lysimeters to avoid natural rainfall and storms. There were two type of treatments. One treatment is soil only (control) without any added biochar and other treatment is top soil amended with biochar. Each treatment lysimeter was replicated three times.

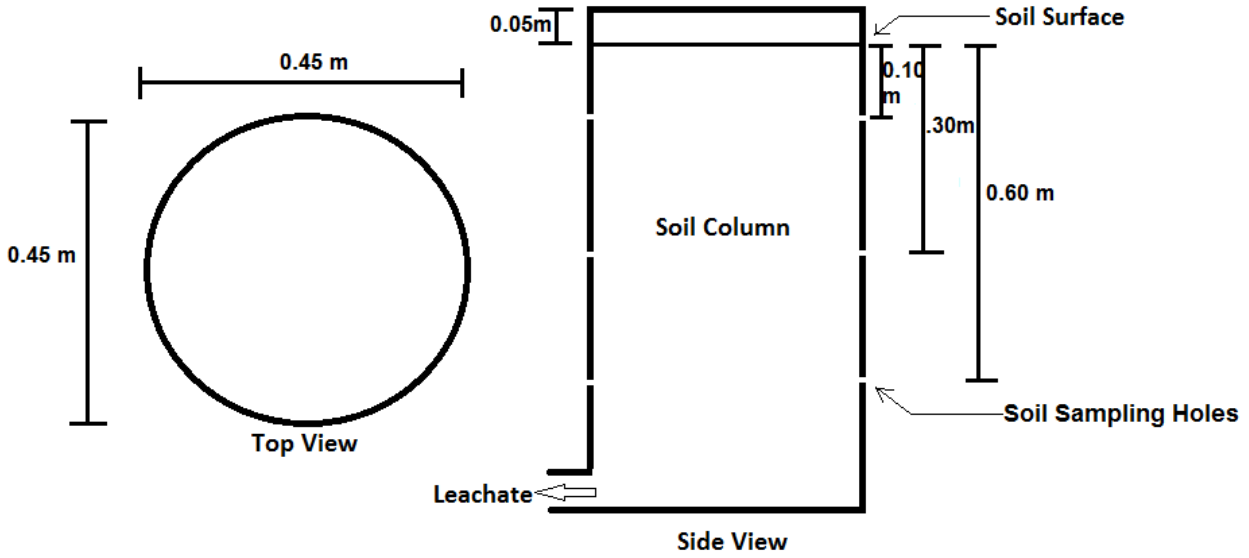


Figure 4.1 Schematic design of the lysimeter.

4.2.6 Manure application

Poultry manure was taken from Burnbrae Farms situated at 200 69th Avenue St-Zotique, Quebec. The application rate of poultry manure used in this study was calculated based on the suggestions provided by Beegle (1997). Main factors in manure application rate estimation include the crop nitrogen requirement, nitrogen availability of manure, and application time. The application rate of poultry manure was calculated for silage corn with the typical yield of 4.94 Kg/m^2 and nitrogen requirement for given yield is 0.02 kg/m^2. By considering spring as the application time, incorporation into the soil surface as application method, average total nitrogen content of poultry manure to be 0.0168 Kg/Kg and 0.50 as nitrogen availability factor when incorporation of manure was done within 1 day, the estimated application rate of poultry manure was found to be 2.47 Kg/m^2. So bringing this calculation down to lysimeter area, approximately 0.392 Kg of poultry manure was added to each lysimeter.

4.2.7 Soil and leachate sampling

Before starting of experiment, all lysimeters were irrigated and brought to saturation. Soil samples were taken before applying manure to check any previous or background hormones from last year. Next day (Day 1), 0.392 Kg of poultry manure was incorporated in the top soil of each lysimeter manually. Soil sample was taken from the top soil before first irrigation to check how much hormones were in top soil after addition of manure. Total of 9.67 litres of tap water was applied during regular intervals over four hours mimicking the natural rainfall. All the

leachate which came out of the drainage pipe was collected in amber color bottle to avoid photo degradation of collected hormones. Next day (Day 2), composite soil samples were collected at the surface as well as at depths of 10, 30 and 60 cm from the top of soil. Soil samples were taken in an air tight zip lock plastic bags. Soil samples were taken on six occasions: day 0 (for background hormone concentration) and day 1, 2, 16, 31, 46 after application of manure. Moisture of each soil sample was measured in lab using thermal method and the remaining portions of the samples were stored in sealed plastic bags in the freezer until extraction. After each of four irrigation event on Day 1, 15, 30 and 45, approximately 7.5 litres to 8 litres were collected at the bottom of each lysimeter. Leachate samples were then taken to the lab and hormones were extracted from leachate samples on the same day because hormones degradation rate is higher in water as compared to soil.

4.2.8 Mass balance calculations

For each sampling, total mass of hormones recovered was calculated by equation given by ElSayed et al. (2013) which says the sum of the hormones recovered in soil across each depth of soil profile plus mass of hormones recovered from leachate sample would total the initial hormone mass present, minus any losses or unrecovered mass because of degradation or volatilization. Mass balance equation is given below.

$$HRM_{initial} = 0.158962\rho\,[C_{(5-10)}\theta_{(5-10)}h_{(5-10)} + C_{(10-20)}\theta_{(10-20)}h_{(10-20)} + C_{(20-50)}\theta_{(20-50)}h_{(20-50)} + C_{(50-75)}\theta_{(50-75)}h_{(50-75)}] + [C_{leach}V_{leach}] + HRM_{lost}$$

where $HRM_{initial}$ = initial hormones (µg) mass applied.

HRM_{lost} = homones unrecovered or lost via degradation (µg).

ρ = soil bulk density (Kgm^{-3}).

$C_{(x-y)}\theta_{(x-y)}h_{(x-y)}$ = hormone concentration (μgkg^{-1}) and soil moisture respectively in soil layer h located between depth x to depth y (m).

C_{leach} = hormones concentration in leachate (μgL^{-1}).

V_{leach} = volume of leachate (L).

0.158962 = soil column cross-sectional area (m^2).

4.2.9 Method for hormones extraction from leachate samples

After each irrigation event, there was a total of approximately 7.5 to 8 litres of leachate collected from the bottom of each lysimeter through the drainage pipe. One litre of leachate was sub sampled from total leachate collected from each lysimeter. Hormone extraction from leachate was carried using solid phase extraction with Oasis HLB extraction cartridges (200 mg/cc, Oasis Co. Ltd, NY). An SPE vacuum manifold system was used to carry out extraction process. To remove suspended particles, each 1 litre sub sample was filtered through a PreSep prefilter 47 mm (GE Water and Process Technologies, PA, USA). Prior to extraction, Oasis HLB extraction cartridges were conditioned with 5 ml of methanol and then followed by 5 ml of high purity water (Stafiej et al., 2007). A flow rate of 20 ml min^{-1} was maintained with the help of SPE vacuum manifold system while 1 litre of sub sample was passed through conditioned cartridge followed by passage of 10 ml of HPLC grade acetonitrile. Along with the acetonitrile, hormones were also eluted from cartridges. The extract was brought to dryness under a nitrogen stream and re-dissolved in 1 ml of 50:50 (V/V) water-acetonitrile solution. The glass tube containing the extract was put on sonicator for 15 minutes to achieve homogeneous mixing. The extract was filtered via 0.22 μm syringe driven sterile filters (Millex-GV, Japan). The final extract was transferred into 1.5 ml amber color HPLC vials for HPLC analysis.

4.2.10 Method for hormones extraction from soil samples

Soil samples were kept frozen while storage after getting them from the field until hormone extraction. Soil samples were thawed at room temperature before start of extraction process. The soil in each plastic zip lock bag was thoroughly mixed. The method for extraction of hormones from soil samples were adopted from the method explained by Xuan et al. (2008). Five grams of each soil sample was taken in 50 ml polyethylene centrifuge tube. Ten millilitre of HPLC grade acetone and 5 gram of anhydrous Na_2SO_4 were added to each tube containing soil sample (Xuan et al., 2008). The materials were mixed thoroughly using a Vortex mixer for 1 minute followed by vigorous shaking for 30 minutes at 250 rpm in a reciprocating shaker. The mixture was centrifuged at 4000 rpm for 20 minutes. After centrifugation, the supernatant was transferred to another clean 50 ml polyethylene centrifuge tube. Another 10 ml of acetone was added to supernatant and all the steps repeated till centrifugation. The supernatant got from second round of centrifugation was transferred to another clean 50 ml polyethylene centrifuge tube. The

resultant supernatant centrifuged again at 4000 rpm for 20 minutes and final supernatant was transferred into 50 ml pyrex glass centrifuge tubes and dried completely under gentle N_2 stream. The dried extract was redissolved in 1 ml of 50:50 (V/V) water-acetonitrile solutions. The glass tube containing the extract was put on sonicator for 15 minutes to achieve homogeneous mixing. The extract was filtered via 0.22 μm sterile filters (Millex-GV, Japan). The final extract was transferred into 1.5 ml amber color HPLC vials for HPLC analysis.

4.2.11 HPLC analysis

Hormonal content in the leachate and soil samples were analysed by using High Pressure Liquid Chromatography (HPLC) technique. Forty percent of Millipore water and 60% of HPLC grade Acetonitrile was used as mobile phase. A Zorbax Eclipse Plus C18 column (150×4.6 mm) with particle size of 5 μm from Agilent Technologies Inc (Santa Clara, CA) was employed as stationary phase. Flow rate of mobile phase was set at 1 ml/min. It was gradient flow. Run time for each injection was set at 15 minutes. Each sample injection was set at 100 μl. The UV detector wavelength was set at 210 nm.

4.2.12 Data analysis

Data was analyzed with PROC MIXED in SAS v. 9.2 (SAS Institute Inc., 2010). This model used repeated measures over time and depth to determine if hormone concentration differed between treatments, over time and with varying depths.

4.2.13 Scanning Electron Microscopy (SEM)

Scanning Electron Microscopy (SEM) technique was used to study surface structural changes in biochar after two years of application. This analysis was carried by Hitachi TM3000 Scanning Electron Microscope operating at 15 kV. Images were taken at 250x magnification. SEM images are very useful to obtain accurate details about pore structure of biochars. Structural changes on biochar were analysed by scanning the surface and inside of fresh biochar as well as used biochar by Scanning Electron Microscope (SEM). Scanning of inside structure was achieved by gently dissecting the biochar before launching it in SEM device.

4.2.14 Sorption test

Batch sorption experiment was done to support the results got from field experimentation. Sorption efficiency for hormones were compared between fresh biochar and used biochar.

Standard solution of both estrone (E1) and estradiol (E2) at six different concentrations ranging between 0.01 mgL^{-1} to 5 mgL^{-1} were prepared in triplicates by adding calculated amounts of pure hormones bought from Sigma Aldrich (St. Louis, MO, USA) in HPLC grade acetonitrile. Method was adopted from the study conducted by Lee et al. (2003). Briefly, 2 gram of soil with 1 % slow pyrolysis fresh biochar was equilibrated with 30 ml of each standard solution in 50 ml glass centrifuge tubes with Teflon-lined screw caps. Same arrangement was done with 1% slow pyrolysis used biochar which was used in field for two consecutive years. Aluminium foil was wrapped around the tubes to avoid any photo-degradation. All tubes were placed on a flat-bed shaker for 24 hours in dark at 23±2 °C.

4.3 Results and discussion

4.3.1 Mass balance of estradiol (E2)

Mass balance calculation is shown in Table 4.1. There was a sudden decrease in mass of E2 after first irrigation event in control as well as biochar treatment at the surface (Top Soil). However, it was clear that rate of degradation at the surface reduced with time (Figure 4.3). This finding matches with outcomes of Casey et al. (2005) which reported that rate of hormone degradation decreased with time and concentration.

Table 4.1 Mass balance of estradiol (E2)

Amount of estradiol E2 (µg) in different soil profile depth ranges and in leachate, and overall, over a 46 day period

Day	Treatment											
	Soil profile depth range (cm) / leachate / Total											
	Soil + Poultry Manure Treatment (Control)						Soil + Poultry Manure + Biochar Treatment					
	5-10	10-20	20-50	50-75	Leachate	Total	5-10	10-20	20-50	50-75	Leachate	Total
0	3.88	0	71.86	61.08	0.00	136.82	6.12	12.2	54.1	25.7	0.00	98.13
1	61.99						128.2					
2	43.26	7.84	17.93	54.08	0.76	123.86	50.85	9.33	75.95	0.00	0.223	136.35
16	17.19	10.86	38.21	21.06	0.133	87.45	12.06	13.89	55.23	21.97	0.160	103.40
31	6.19	34.35	22.40	12.42	0.24	75.61	7.98	5.31	27.11	35.16	0.269	75.82
46	2.21	4.95	17.55	18.46	0.259	43.42	5.73	6.62	13.23	21.24	0.315	47.14

Based on the mass balance table, percentage of mass of hormones held at soil surface in biochar treatment and control for current year of study is calculated and compared with first year of study (Figure 4.2). During first year when biochar was freshly applied as top soil amendment, biochar was holding more mass of E2 at surface (Figure 4.2) thus allowing comparatively less mass of hormones from poultry manure in leaching down the soil profile in lysimeter as compared to control. When biochar was fresh, it had pores clear and available for hydrophobic interactions

which led to adsorption of hormones onto the biochar particles. The relative hydrophobicity of estrogens, with log Kow values ranging from 2.6 to 4.0 (Lai et al., 2000) is a good indication of the strong sorption affinity of these hormones. Due to hydrophobic properties of hormones (Yu and Huang, 2005) they were held by biochar at the surface of soil. Unlike first year, biochar has shown less retention capacity toward hormones during second year after biochar application. Biochar was holding less mass of E2 as compared to control (Figure 4.2).

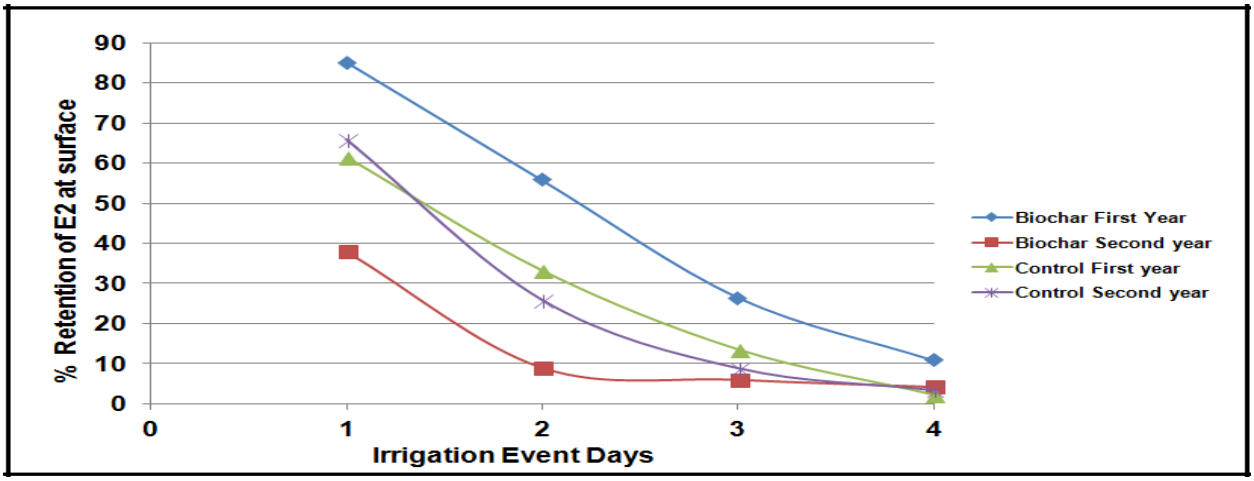


Figure 4.2 Percentage of E2 mass remaining at the surface of soil profile (cumulative) during first year and second year (Current study)

During second year after biochar application, control is holding comparatively more E2 at surface as compared to biochar treatment (Figure 4.2) during first two irrigation events but degradation was nearly same between both treatments (Figure 4.3) for this duration. It indicates that decrease of mass of E2 from surface in biochar treatment is because of leaching of E2 to lower soil profile.

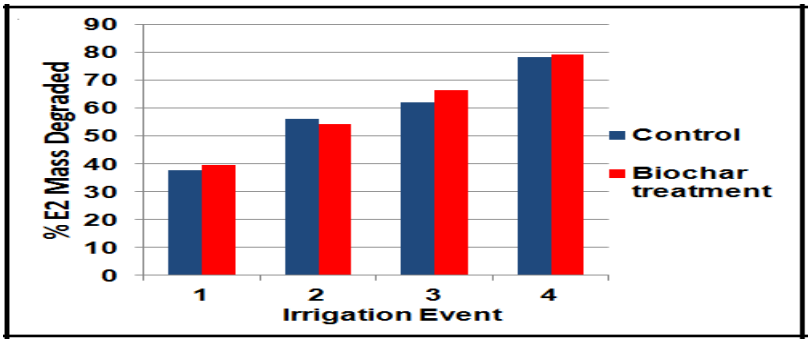


Figure 4.3 % E2 mass degraded overall (cumulative)

Comparatively more leaching in biochar treatment than control in current year (second year) was because of release of dissolved organic carbons from biochar. Because biochar was in the field

for experimentation for two years, it got some surface degradation as discussed in section 4.3.6 also. Various studies has reported surface degradation on biochar with time (Hale et al., 2011; Kwon & Pignatello, 2005; Hale et al., 2011). It was assumed in these studies that substances such as humic acid, minerals, metal oxides and native pollutants in the sediment might alter the physical or chemical properties of the biochar surface and affect the sorption of hydrophobic organic compounds. After initial fragmentation and undergoing various biotic and abiotic changes, biochar released dissolved organic carbon. Dissolved organic carbons have potential to facilitate hormones movement down to soil profile (Sarmah et al., 2008). This was the reason why biochar treatment in second year shown more mobility of hormones down the soil profile as compared to control.

4.3.2 Concentration of E2 in soil at different depth and time

Following are the bar charts indicating the concentration of E2 (μgkg^{-1}) in both treatments at different depths and time. Concentration of E2 was highest at surface (top soil) on Day 1 when poultry manure was applied and decreased afterwards with time in both treatments.

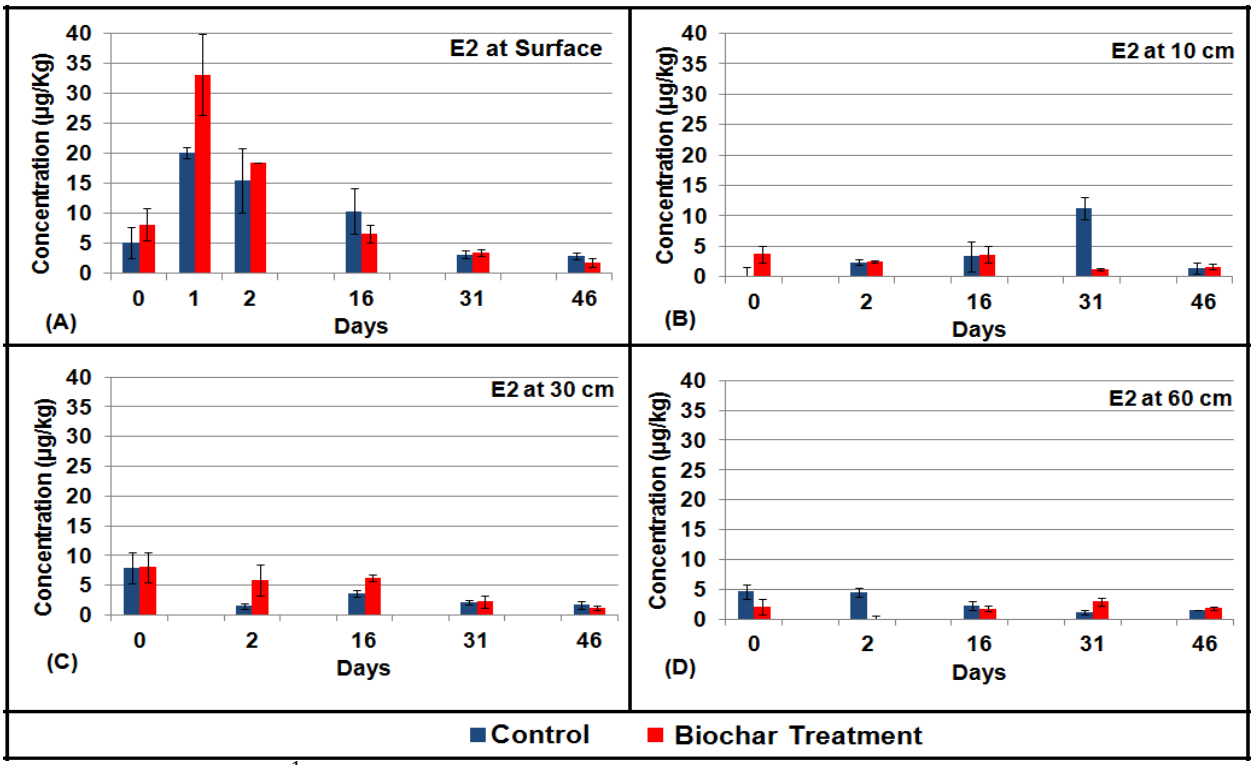


Figure 4.4. E2 (μgkg^{-1}) concentration over period of 46 days: (A) at the surface of soil profile; (B) at 10 cm from surface of soil profile; (C) at 30 cm; (D) at 60 cm.

In Figure 4.4A, although concentration of E2 in biochar treatment during day 1 and day 2 is more than control at the surface but rate of E2 movement to lower depths during following days was more in biochar treatment than control. Rate of degradation of E2 decreased with time in both treatments which matches well with findings by Casey et al. (2005) who found rate of hormone degradation decreased with time and concentration. The lower degradation rates of E2 in biochar treatment during last two irrigation events was attributed to the presence of energy source of dissolved organic carbon (Herman & Mills, 2003). In presence of this energy source, microbial degradation of E2 was ignored. At other depths, there was no significant trend in concentration because each depth was losing some mass of hormones down the soil profile and simultaneously receiving mass of hormones from the top of soil profile. For most of the days at lower depths, concentration of E2 in control and biochar treatments was almost identical. These finding has been supported by statistical analysis shown in Table 4.2 which clearly confirms that there was no statistically significant difference observed in movement of hormones between control and biochar treatment at different depth and time during the second year of biochar application.

Table 4.2 Repeated measures analysis of variance for E2 and E1 residue in soil

	Probability	
Effect	**E2**	**E1**
Treatment	0.3396[a]	0.6249[a]
Depth	0.0002[a]	0.0159[a]
Treatment × Depth	0.3537[a]	0.6785[a]
Time	< 0.0001[b]	0.0003[a]
Treatment × time	0.6217[a]	0.2882[a]
Depth × Time	< 0.0001[b]	0.0944[a]
Treatment × Depth × Time	0.4795[a]	0.1802[a]

[a] denotes statistically insignificant
[b] denotes statistically significant

4.3.3 Mass balance of Estrone (E1)

Mass balance shown in Table 4.3 was calculated based on the equation explained by Elsayed et al. (2013) as discussed in section 4.2.8. Control was losing E1 faster than Biochar treatment to lower depths of soil profile. There was no regular trend of E1 mass seen in leachate. Like E2, there was sudden decrease in mass of E1 after first irrigation in both treatments. But the rate of loosing of E1 decreased with time. In figure 4.5, there was an increase in mass of E1 between day 16 (second irrigation event) and day 31 (third irrigation event), which was because of

biotransformation of E2 into E1 (Xuan et al., 2008). This change was observed more in lower depths which had anaerobic conditions favourable for this biotransformation (Yu et al., 2013). This change was quite evident from the decreased mass of E2 between day 16 and day 31 (Table 4.1).

Table 4.3 Mass balance of estrone (E1)

Amount of estrone E1 (µg) in different soil profile depth ranges and in leachate, and overall, over a 46 day period

Day	Treatment											
	Soil profile depth range (cm) / leachate / Total											
	Soil + Poultry Manure Treatment (Control)						Soil + Poultry Manure + Biochar Treatment					
	5-10	10-20	20-50	50-75	Leachate	Total	5-10	10-20	20-50	50-75	Leachate	Total
0	0.32	29.34	0.00	0.00	0.00	29.65	0.39	15.84	4.35	21.53	0.00	42.12
1	12.51						12.46					
2	6.66	2.72	4.51	0.00	0.105	13.98	5.87	9.57	0.00	0.00	0.11	15.56
16	4.11	3.47	5.84	6.69	0.689	20.79	2.91	4.46	12.10	6.79	0.623	26.89
31	5.34	17.37	11.25	10.31	0.275	44.54	3.68	16.62	2.74	27.57	0.264	50.87
46	1.56	1.58	3.78	5.37	0.244	12.53	2.41	0.99	3.55	4.59	0.254	11.79

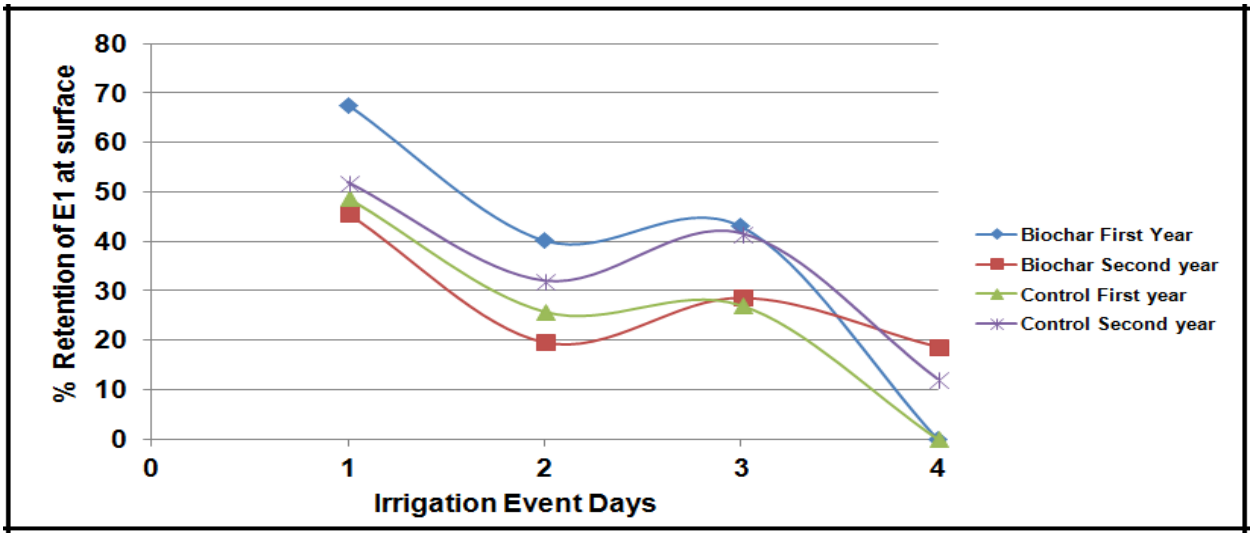


Figure 4.5 Percentage of E1 mass remaining at the surface of soil profile (cumulative) during first year and second year (Current study)

During first year of application of biochar, biochar treatment held more mass of E1 as compared to control but during second year after biochar application, biochar treatment was holding less mass of E1 (Figure 4.5). Biochar provides environment for growth of microbes (Wei et al., 2014) so there is higher rate of biotic degradation of E1 in biochar treatment as compared to control (Figure 4.6). Decrease in E1 degradation on third irrigation event (Figure 4.6) was because of the gain in E1 mass from the biotransformation of E2 which is similar to the finding of Yu et al. (2013).

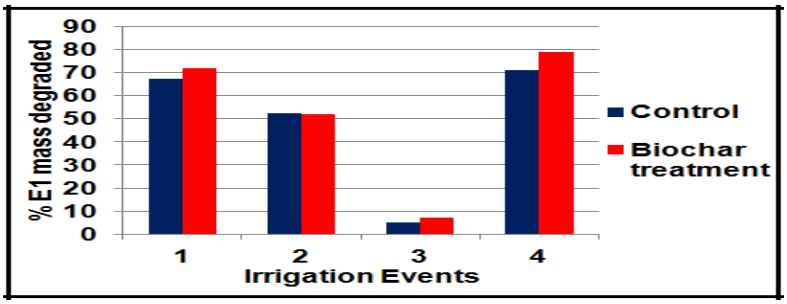


Figure 4.6 % E1 mass degraded overall (cumulative)

4.3.4 Concentration of E1 in soil at different depth and time

Bar charts in figure 4.7 indicates the concentration of E1 (µg kg^{-1}) in both treatments at different depths and time. Concentration of E1 was high on Day 1 when poultry manure was applied and decreased afterwards with time.

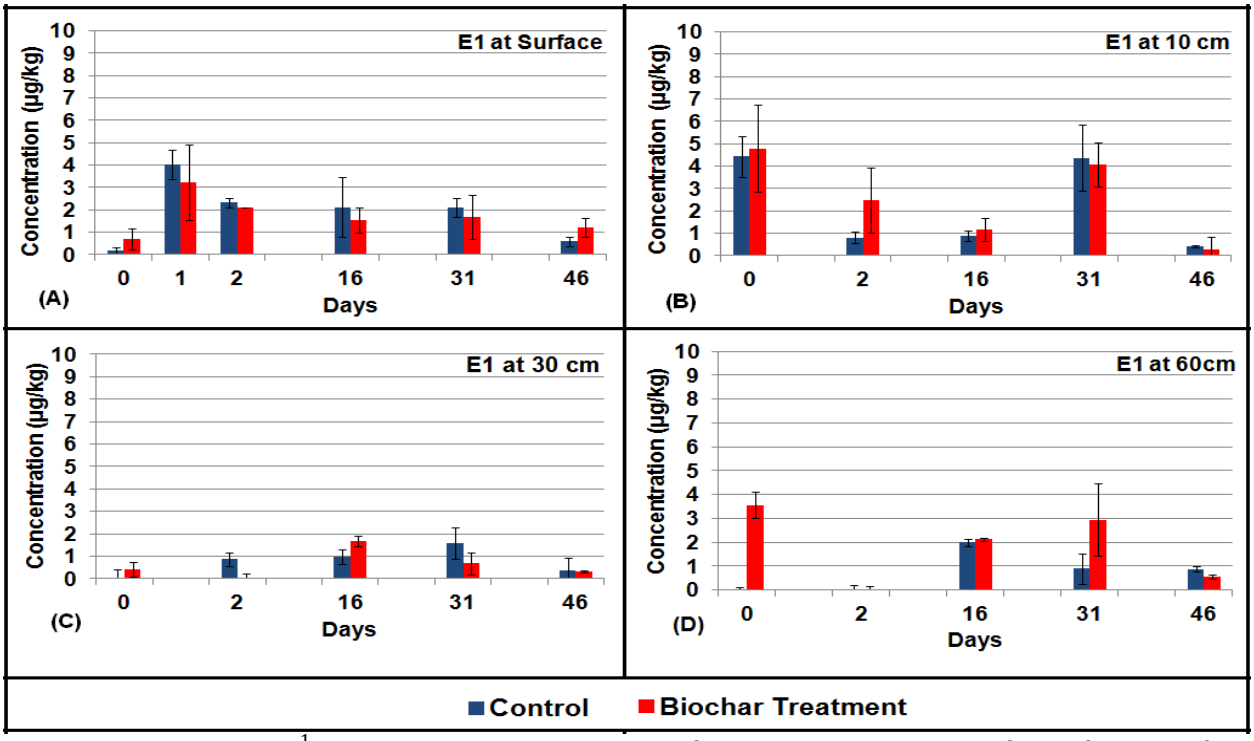


Figure 4.7 E1 (µg kg^{-1}) concentration over period of 46 days: (A) at the surface of soil profile; (B) at 10 cm from surface of soil profile; (C) at 30 cm; (D) at 60 cm.

At all depths, there was some background concentration of E1 from the manure applied during last year of study when biochar was freshly applied. There was some increase in concentration of E1 between day 16 and day 31 because of biotransformation of E2 into E1 which is witnessed by Xuan et al. (2008) also and it was quite evident from the decline of E2 concentration (Figure 4.4) between these days. Biochar treatment had higher concentration during these days which might

be because of higher mobility of hormones. Reason for higher mobility was the release of dissolved organic carbons from biochar upon physical degradation which acted as carrier of hormones down the soil profile as discussed in section 3.1.1. The increasing number of observations suggests that biochar can be degraded, by both biotic and abiotic processes leads to initial physical fragmentation discussed in section 3.3 clearly. However the trends were not much clear in lower depths and were difficult to predict as those depths were losing and gaining some mass of E1 simultaneously. According to statistical data analysis, there was no statistical significant difference observed between control and biochar treatment at different depths and time (Table 4.2).

4.3.5 Concentration of E2 and E1 in leachate

Concentration of hormones collected in the leachate after each of irrigation events is represented in Figure 4.8. In Figure 4.8A, E2 concentration in leachate from control after first irrigation is higher than E2 concentration in leachate from Biochar treatment. This might be because there was a certain amount of E2 left in lower depth of soil profile from last year of poultry application which is quite evident from mass balance also (Table 4.1). During following irrigation events, trend is regular in which biochar treatment released slightly higher E2 concentration as compared to control and concentration kept on increasing in leachate after each irrigation event. There is no clear difference in concentration of E1 found in leachate (Figure 4.8B and Table 4.4). Concentrations of hormones found in leachate is quite low but it is important to note that hormones even in trace concentrations are highly toxic to aquatic animals (Khanal, 2006).

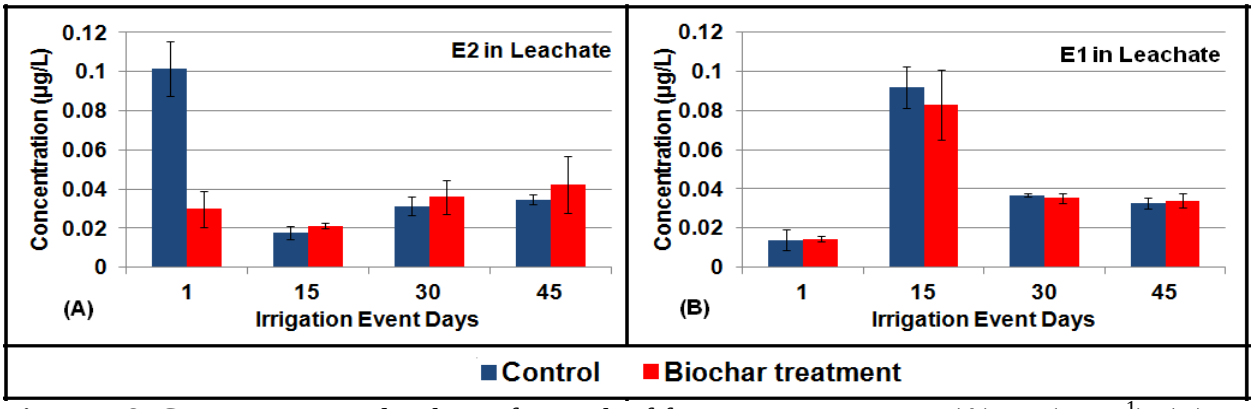


Figure 4.8 Concentration in leachate after each of four irrigation events: (A) E2 (µg L^{-1}); (B) E1 (µg L^{-1}).

Table 4.4 Repeated measures analysis of variance for E2 and E1 residue in leachate

Effect	Probability	
	E2	**E1**
Treatment	0.0719[a]	0.6768[a]
Day	0.0007[a]	< 0.0001[b]
Treatment × Day	0.0104[a]	0.3442[a]

[a] denotes statistically insignificant
[b] denotes statistically significant

4.3.6 Scanning Electron Microscopy (SEM)

Scanning Electron Microscopy helped analysing the structural differences between fresh biochar and Used biochar. Upon analysing figure 4.9, it is clear that the pore structure inside and on surface of biochar got disturbed in the biochar which was being used for experimentation in field for two consecutive years. The temperature around the field site where we applied biochar falls even till -25 degree centigrade during winter. Biochar particles can be fragmented into smaller particles when water penetrates the pores and swell during freezing, forcing the biochar particles to break (Carcaillet, 2001). Also, fragmented biochar led to release of dissolved organic carbons (Lehmann, 2009) which facilitated the mobility of hormones down the soil profile (Sarmah et al., 2008).

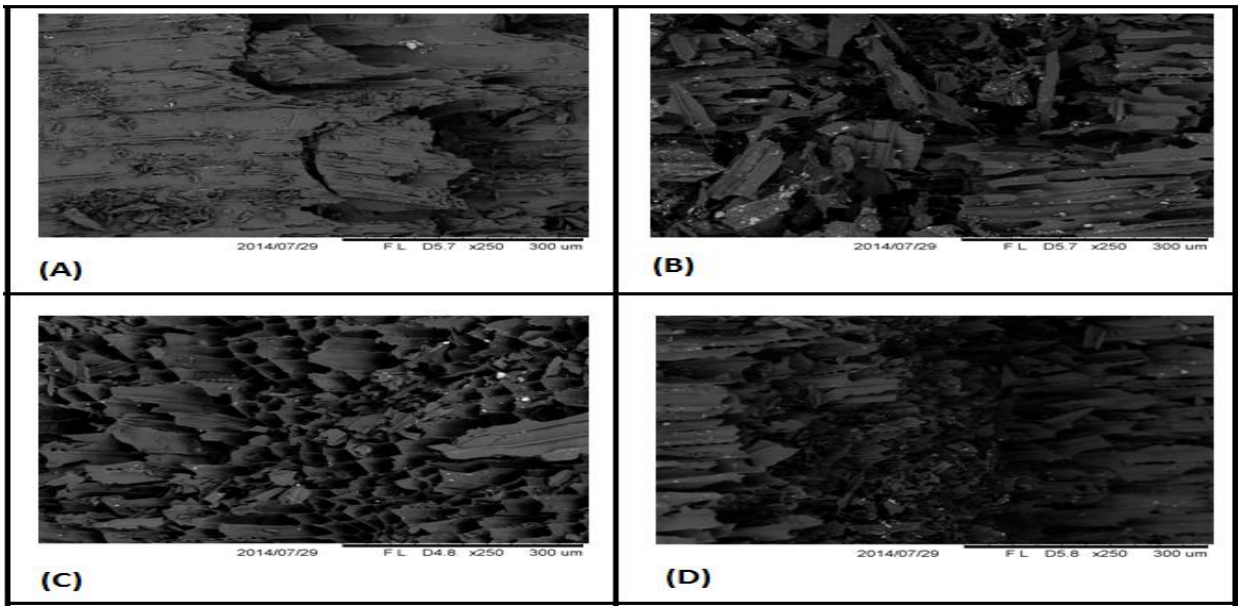


Figure 4.9 Scanning Electron Microscopy (SEM) images of: (A) Inside surface of fresh biochar; (B) Inside surface of used biochar; (C) Outer surface of fresh biochar; (D) Outer surface of used biochar.

4.3.7 Sorption test

A batch sorption experiment was conducted on fresh biochar and on biochar, which was being used in field for two years, with six different concentrations of pure E2 and E1. Methodology adopted for this test is explained in section 4.2.14. The results for the sorption of pure hormones onto biochar are shown in figure 4.10 in the form of percentage removal of hormones by biochar. Clearly, less percentage of pure hormones were removed from the solution containing used biochar which means it has less capacity to adsorb hormones as compared to fresh biochar and this supports our findings discussed in previous sections that biochar retention capacity for hormones decreases with time.

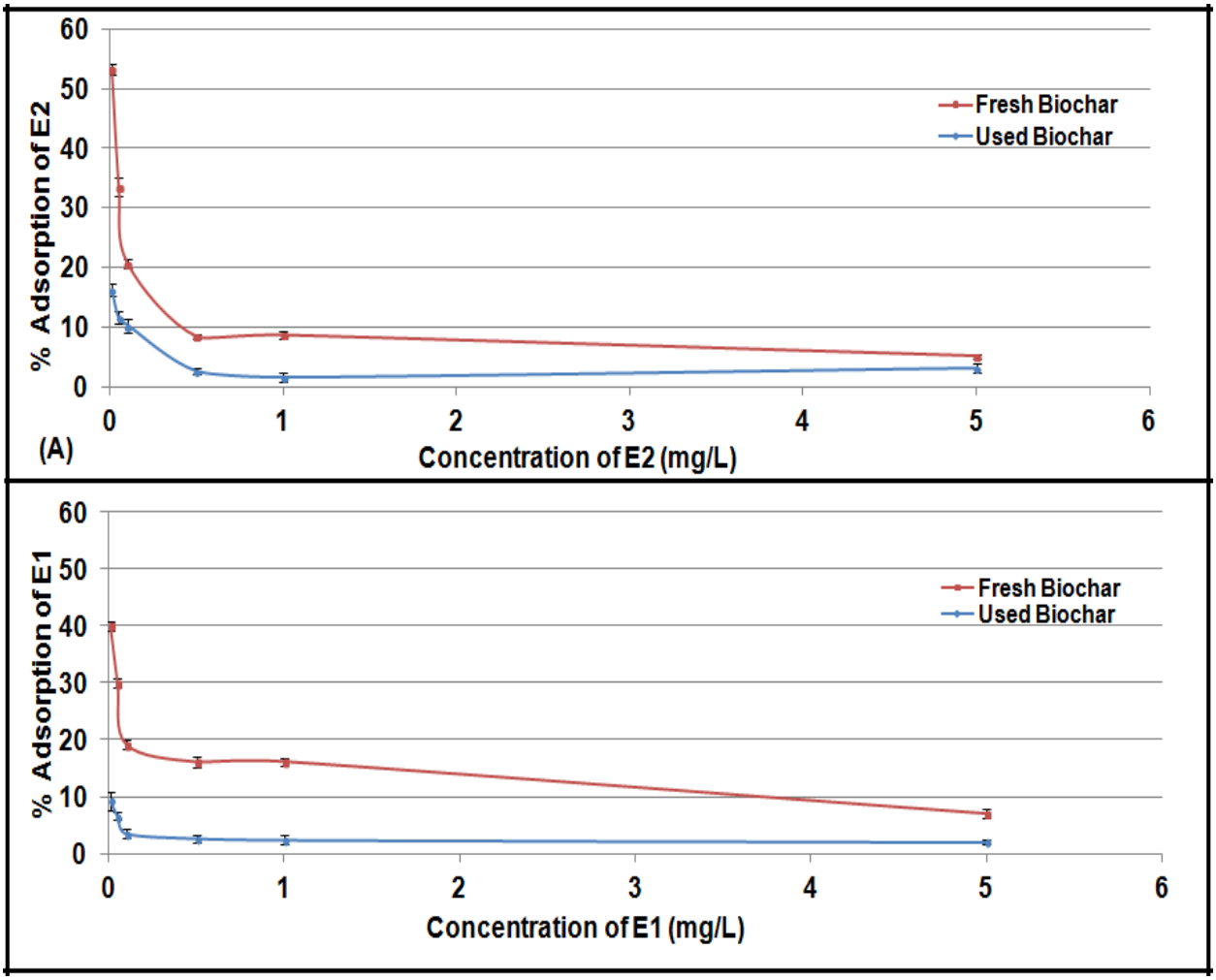


Figure 4.10 Percentage adsorption by fresh biochar and used biochar for hormones: (A) E2; (B) E1.

4.4 Conclusion

Poultry manure is a potential source of estrogens (hormones) that could impact the environment severely. Biochar have potential to adsorb hormones so in this study 1 % slow pyrolysis biochar was tested to adsorb hormones from poultry manure in sandy soil for two consecutive years at our specific field conditions. It was found that in first year of biochar application, there was a significant difference of movement of hormones between the treatments but in second year after biochar application (current study) there was no significant difference observed in movement of hormones between the treatments. Pore structure on surface of biochar underwent various biotic and abiotic changes with time which led to less hydrophobic interactions with hormones and also led to release of dissolved organic carbons which further facilitated the mobility of hormones down the soil profile instead of holding them at surface. All these findings leads to the conclusion that retention capacity of 1% slow pyrolysis biochar for hormones from poultry manure decreased with time at our specific field conditions. There are huge gaps in science regarding the study of biochar because biochar behaves different in different field conditions (Lehmann, 2009). There is possibility that the type of biochar used in this study might work for longer duration at same capacity under conditions which are different from the conditions kept in this current study.

There are not sufficient studies available on basis of which we can categorize biochar applications in different field parameters. Therefore, there is a need to do field trials on biochar under wide range of parameters so that it can be used as a potential remediation tool to curb pollution caused by emerging contaminants.

Chapter 5

Summary and Conclusion

Main objective of this study was to analyse the retention behaviour of 1 % slow pyrolysis biochar for steroidal sex hormones coming from two different sources over a period of two years. Several other studies highlighted biochar's potential in adsorbing hormones, given its structural and physiochemical properties but the capacity to retain hormones over time was unknown. So, hypothesis of our experiment was that 1 % slow pyrolysis biochar keeps on adsorbing hormones coming from manure (poultry and swine manure) during each successive year at our specific field conditions. It was a 46 day long experiment conducted under natural field conditions during spring season for two consecutive years. While in the first year of biochar application, a significant spatial-temporal stratification of steroidal sex hormones had been observed in the biochar-amended soil (vs. non-amended soil) which confirmed that 1 % slow pyrolysis biochar when applied fresh retains hormones at the top soil from both manures. But during the second year, same biochar held significantly lesser mass of hormones at the top soil and had shown no significant spatial-temporal stratification of steroidal sex hormones in the biochar-amended soil (vs. non-amended soil). When biochar was fresh, its pores were available for hydrophobic interactions so it was holding significant concentration of hormones during first year of application but with time there might be several biotic and abiotic changes on surface of biochar and after some physical fragmentation, pores on surface were no longer available for hydrophobic interactions and also it started releasing dissolved organic carbons which facilitated even greater mobility of hormones from manure down to lower depths of soil profile. Our hypothesis turned out to be untrue. Our findings confirmed that adsorption or retaining capacity of 1% slow pyrolysis biochar for estrogens released from liquid swine manure and poultry manure at our specific field conditions, decreased with time. However, it should be noted that no two biochars are same, behaves differently in different conditions. In this study, the retention ability of biochar was limited to only one type of biochar and one type of soil. There is possible probability that same biochar we used over time could work better in some different soil or different climate conditions. Also, the feedstock from which biochar is produced also plays a

major role in its behaviour in soil. It is also a possible probability that if some other type of biochar was used in our specific field conditions then our hypothesis might have turned out to be true. Further studies are needed to explore the role of different weather conditions on different types of biochar in different type of soils. Such a wider characterization of the effects of biochar amendment of agricultural soils, is important in achieving a maximum reduction of hormonal pollution in soil and water. Also, the competitive sorption ability and desorption resistance of biochar should be evaluated in presence with other micro pollutants, such as antibiotics.

References

- Alizadeh, Sanaz. (2014). Use of biochar for reducing female sex hormonal pollution in agricultural areas. from http://digitool.Library.McGill.CA/R/?func=dbin-jump-full&object_id=121578
- Antal, Michael Jerry, Jr., Downie, Adriana, Manley-Harris, Merilyn, Sarmah, Ajit K., Smernik, Ronald J., Srinivasan, Prakash, & van Zwieten, Lukas. (2010). Retention capacity of biochar-amended New Zealand dairy farm soil for an estrogenic steroid hormone and its primary metabolite. *Australian Journal of Soil Research, 48*, 648+.
- Aravindakshan, J., Paquet, V., Gregory, M., Dufresne, J., Fournier, M., Marcogliese, D. J., & Cyr, D. G. (2004). Consequences of xenoestrogen exposure on male reproductive function in spottail shiners (Notropis hudsonius). *Toxicological Sciences, 78*(1), 156-165. doi: 10.1093/toxsci/kfh042
- Arcand-Hoy, Lisa D., & Benson, William H. (1998). Fish reproduction: An ecologically relevant indicator of endocrine disruption. Environmental Toxicology and Chemistry, 17(1), 49-57. doi: 10.1002/etc.5620170108
- Bagreev, A., Bandosz, T. J., & Locke, D. C. (2001). Pore structure and surface chemistry of adsorbents obtained by pyrolysis of sewage sludge-derived fertilizer. *Carbon, 39*(13), 1971-1979. doi: 10.1016/s0008-6223(01)00026-4
- Bailey, Susan E., Olin, Trudy J., Bricka, R. Mark, & Adrian, D. Dean. (1999). A review of potentially low-cost sorbents for heavy metals. *Water Research, 33*(11), 2469-2479. doi: http://dx.doi.org/10.1016/S0043-1354(98)00475-8
- Barnhoorn, I. E. J., Bornman, M. S., Pieterse, G. M., & van Vuren, J. H. J. (2004). Histological evidence of intersex in feral sharptooth catfish (Clarias gariepinus) from an estrogen-polluted water source in Gauteng, South Africa. *Environmental Toxicology, 19*(6), 603-608. doi: 10.1002/tox.20068
- Beaulieu, Martin S. (2004). Manure Management in Canada. Retrieved from http://publications.gc.ca/Collection/Statcan/21-021-M/21-021-MIE2004001.pdf
- Beegle, Douglas. (1997). Estimating Manure Application Rates. Penn State Extention, The Pennsylvania State University, USA. Retrieved from http://pubs.cas.psu.edu/FreePubs/pdfs/uc151.pdf
- Brodowski, S., Rodionov, A., Haumaier, L., Glaser, B., & Amelung, W. (2005). Revised black carbon assessment using benzene polycarboxylic acids. *Organic Geochemistry, 36*(9), 1299-1310. doi: 10.1016/j.orggeochem.2005.03.011
- Carcaillet, C. (2001). Are Holocene wood-charcoal fragments stratified in alpine and subalpine soils? Evidence from the Alps based on AMS C-14 dates. Holocene, 11(2), 231-242. doi: 10.1191/095968301674071040
- Casey, Francis X. M., Larsen, Gerald L., Hakk, Heldur, & Šimůnek, Jiří. (2003). Fate and Transport of 17β-estradiol in Soil−Water Systems. *Environmental Science & Technology, 37*(11), 2400-2409. doi: 10.1021/es026153z

- Casey, Francis X. M., Simunek, Jirí, Lee, Jaehoon, Larsen, Gerald L., & Hakk, Heldur. (2005). Sorption, Mobility, and Transformation of Estrogenic Hormones in Natural Soil. *Journal of Environmental Quality, 34*(4), 1372-1379.
- Cheng, C. H., Lehmann, J., Thies, J. E., Burton, S. D., & Engelhard, M. H. (2006). Oxidation of black carbon by biotic and abiotic processes. *Organic Geochemistry, 37*(11), 1477-1488. doi: 10.1016/j.oeggeochem.2006.06.022
- Choi, Seul Min, Yoo, Sun Dong, & Lee, Byung Mu. (2004). Toxicological Characteristics of Endocrine-Disrupting Chemicals: Developmental Toxicity, Carcinogenicity, and Mutagenicity. *Journal of Toxicology and Environmental Health, Part B, 7*(1), 1-23. doi: 10.1080/10937400490253229
- Colucci, Michael S., Bork, Henry, & Topp, Edward. (2001). Persistence of Estrogenic Hormones in Agricultural Soils. *J. Environ. Qual., 30*(6), 2070-2076. doi: 10.2134/jeq2001.2070
- Das, B. S., Lee, L. S., Rao, P. S. C., & Hultgren, R. P. (2004). Sorption and Degradation of Steroid Hormones in Soils during Transport: Column Studies and Model Evaluation. *Environmental Science & Technology, 38*(5), 1460-1470. doi: 10.1021/es034898e
- ElSayed, Eman M., Prasher, Shiv O., & Patel, Ramanbhai M. (2013). Effect of nonionic surfactant Brij 35 on the fate and transport of oxytetracycline antibiotic in soil. *Journal of Environmental Management, 116*(0), 125-134. doi: http://dx.doi.org/10.1016/j.jenvman.2012.11.034
- Fan, Zhaosheng, Casey, Francis X. M., Hakk, Heldur, & Larsen, Gerald L. (2007). Persistence and fate of 17β-estradiol and testosterone in agricultural soils. *Chemosphere, 67*(5), 886-895. doi: http://dx.doi.org/10.1016/j.chemosphere.2006.11.040
- Gall, Heather E., Sassman, Stephen A., Lee, Linda S., & Jafvert, Chad T. (2011). Hormone Discharges from a Midwest Tile-Drained Agroecosystem Receiving Animal Wastes. *Environmental Science & Technology, 45*(20), 8755-8764. doi: 10.1021/es2011435
- Goeppert, Nadine, Dror, Ishai, & Berkowitz, Brian. (2014). Detection, fate and transport of estrogen family hormones in soil. *Chemosphere, 95*(0), 336-345. doi: http://dx.doi.org/10.1016/j.chemosphere.2013.09.039
- Grandjean, Philippe. (2001). GENERAL DISCUSSION: EFFECTS OF HORMONE DISRUPTERS IN MAN AND WILDLIFE. *APMIS, 109*(S103), S254-S260. doi: 10.1111/j.1600-0463.2001.tb05774.x
- Gulkowska, A., He, Y. H., So, M. K., Yeung, L. W. Y., Leung, H. W., Giesy, J. P., . . . Richardson, B. J. (2007). The occurrence of selected antibiotics in Hong Kong coastal waters. *Marine Pollution Bulletin, 54*(8), 1287-1293. doi: 10.1016/j.marpolbul.2007.04.008
- Hale, S. E., Hanley, K., Lehmann, J., Zimmerman, A. R., & Cornelissen, G. (2011). Effects of Chemical, Biological, and Physical Aging As Well As Soil Addition on the Sorption of Pyrene to Activated Carbon and Biochar. *Environmental Science & Technology, 45*(24), 10445-10453. doi: 10.1021/es202970x
- Hamer, U., & Marschner, B. (2002). Priming effects of sugars, amino acids, organic acids and catechol on the mineralization of lignin and peat. *Journal of Plant Nutrition and Soil Science-Zeitschrift Fur Pflanzenernahrung Und Bodenkunde, 165*(3), 261-268. doi: 10.1002/1522-2624(200206)165:3<261::aid-jpln261>3.0.co;2-i

- Hamer, U., Marschner, B., Brodowski, S., & Amelung, W. (2004). Interactive priming of black carbon and glucose mineralisation. *Organic Geochemistry, 35*(7), 823-830. doi: 10.1016/j.orggeochem.2004.03.003
- Hanselman, Travis A., Graetz, Donald A., & Wilkie, Ann C. (2003). Manure-Borne estrogens as Potential Environmental Contaminants: A Review. *Environmental Science & Technology, 37*(24), 5471-5478. doi: 10.1021/es034410+
- Herman, J. S., & Mills, A. L. (2003). Biological and hydrogeological interactions affect the persistence of 17β-estradiol in an agricultural watershed. *Geobiology, 1*(2), 141-151. doi: 10.1046/j.1472-4669.2003.00011.x
- Hockaday, W. C., Grannas, A. M., Kim, S., & Hatcher, P. G. (2007). The transformation and mobility of charcoal in a fire-impacted watershed. *Geochimica Et Cosmochimica Acta, 71*(14), 3432-3445. doi: 10.1016/j.gca.2007.02.023
- Hunt, J., DuPonte, M., Sato, D., & Kawabata, A. (2010). College of Tropical Agriculture and Human Resources, University of Hawai, Manoa. Retrieved from http://www.biocharmerchants.com/articles/University_of_Hawaii_article_1-3-11.pdf
- Jacobsen, Anne-Marie, Lorenzen, Angela, Chapman, Ralph, & Topp, Edward. (2005). Persistence of Testosterone and 17β-estradiol in Soils Receiving Swine Manure or Municipal Biosolids. *J. Environ. Qual., 34*(3), 861-871. doi: 10.2134/jeq2004.0331
- Jobling, S., Nolan, M., Tyler, C. R., Brighty, G., & Sumpter, J. P. (1998). Widespread sexual disruption in wild fish. *Environmental Science & Technology, 32*(17), 2498-2506. doi: 10.1021/es9710870
- Khanal, S. K. S. K. (2006). Fate, transport and biodegradation of natural Estrogens in the environment and engineered systems. *Environmental Science and Technology, 40*(21), 6537-6546.
- Kramer, R. W., Kujawinski, E. B., & Hatcher, P. G. (2004). Identification of black carbon derived structures in a volcanic ash soil humic acid by Fourier transform ion cyclotron resonance mass spectrometry. *Environmental Science & Technology, 38*(12), 3387-3395. doi: 10.1021/es030124m
- Kwon, S., & Pignatello, J. J. (2005). Effect of natural organic substances on the surface and adsorptive properties of environmental black carbon (char): Pseudo pore blockage by model lipid components and its implications for N-2-probed surface properties of natural sorbents. *Environmental Science & Technology, 39*(20), 7932-7939. doi: 10.1021/es050976h
- Lai, K. M., Johnson, K. L., Scrimshaw, M. D., & Lester, J. N. (2000). Binding of Waterborne Steroid Estrogens to Solid Phases in River and Estuarine Systems. *Environmental Science & Technology, 34*(18), 3890-3894. doi: 10.1021/es9912729
- Lee, Linda S., Strock, Troy J., Sarmah, Ajit K., & Rao, P. Suresh C. (2003). Sorption and Dissipation of Testosterone, Estrogens, and Their Primary Transformation Products in Soils and Sediment. *Environmental Science & Technology, 37*(18), 4098-4105. doi: 10.1021/es020998t
- Lehmann, J. (2007). Bio-energy in the black. *Frontiers in Ecology and the Environment, 5*(7), 381-387. doi: 10.1890/1540-9295(2007)5[381:bitb]2.0.co;2
- Lehmann, Johannes Joseph Stephen. (2009). *Biochar for environmental management : science and technology*. London; Sterling, VA: Earthscan.

- Leifeld, J., Fenner, S., & Muller, M. (2007). Mobility of black carbon in drained peatland soils. *Biogeosciences, 4*(3), 425-432.
- Liu, Zhaoyun, Demisie, Walelign, & Zhang, Mingkui. (2013). Simulated degradation of biochar and its potential environmental implications. *Environmental Pollution, 179*(0), 146-152. doi: http://dx.doi.org/10.1016/j.envpol.2013.04.030
- McClellan, A Tai, Deenik, Jonathan, Uehara, Goro, & Antal, Michael. (2007). Effects of flashed carbonized© macadamia nutshell charcoal on plant growth and soil chemical properties. *a a, 80*(100), 120.
- National Institute of Environmental Health Sciences. (2014). Endocrine Disrupters. Retrieved from http://www.niehs.nih.gov/health/topics/agents/endocrine/
- Nichols, D.J., Daniel, T. C., Moore, P. A., Edwards, D. R., & Pote, D. H. (1997). Runoff of Estrogen Hormone 17β-estradiol from Poultry Litter Applied to Pasture. *J. Environ. Qual., 26*(4), 1002-1006. doi: 10.2134/jeq1997.00472425002600040011x
- Panter, Grace H., Thompson, Roy S., & Sumpter, John P. (2000). Intermittent Exposure of Fish to estradiol. *Environmental Science & Technology, 34*(13), 2756-2760. doi: 10.1021/es991117u
- Peterson, E. W., Davis, R. K., & Orndorff, H. A. (2000). 17 β-estradiol as an Indicator of Animal Waste Contamination in Mantled Karst Aquifers. *J. Environ. Qual., 29*(3), 826-834. doi: 10.2134/jeq2000.00472425002900030019x
- Pietikainen, J., Kiikkila, O., & Fritze, H. (2000). Charcoal as a habitat for microbes and its effect on the microbial community of the underlying humus. *Oikos, 89*(2), 231-242. doi: 10.1034/j.1600-0706.2000.890203.x
- Qadeer, R., Hanif, J., Saleem, M., & Afzal, M. (1994). CHARACTERIZATION OF ACTIVATED-CHARCOAL. *Journal of the Chemical Society of Pakistan, 16*(4), 229-235.
- Rodionov, A., Amelung, W., Haumaier, L., Urusevskaja, I., & Zech, W. (2006). Black carbon in the Zonal steppe soils of Russia. *Journal of Plant Nutrition and Soil Science, 169*(3), 363-369. doi: 10.1002/jpln.200521813
- Sarmah, Ajit K., Northcott, Grant L., & Scherr, Frank F. (2008). Retention of estrogenic steroid hormones by selected New Zealand soils. *Environment International, 34*(6), 749-755. doi: http://dx.doi.org/10.1016/j.envint.2007.12.017
- Sarmah, Ajit K., Srinivasan, Prakash, Smernik, Ronald J., Manley-Harris, Merilyn, Antal, Michael Jerry, Jr., Downie, Adriana, & van Zwieten, Lukas. (2010). Retention capacity of biochar-amended New Zealand dairy farm soil for an estrogenic steroid hormone and its primary metabolite. *Australian Journal of Soil Research, 48*(6-7), 648-658. doi: 10.1071/sr10013
- Schmidt, M. W. I., & Noack, A. G. (2000). Black carbon in soils and sediments: Analysis, distribution, implications, and current challenges. *Global Biogeochemical Cycles, 14*(3), 777-793. doi: 10.1029/1999gb001208
- Shafizadeh, F. (1982). INTRODUCTION TO PYROLYSIS OF BIOMASS. *Journal of Analytical and Applied Pyrolysis, 3*(4), 283-305. doi: 10.1016/0165-2370(82)80017-x
- Snow, Daniel D., Cassada, David A., Papastavros, Efthimia, Bartelt, hunt, Shannon L., Li, Xu, . . . Sallach, J. Brett. (2013). Detection, Occurrence and Fate of Emerging

Contaminants in Agricultural Environments. *Water Environment Research, 85*(10), 869-888. doi: 10.2175/106143013X13698672321661

- Soto, Ana M., Sonnenschein, Carlos, Chung, Kerrie L., Fernandez, Mariana F., Olea, Nicolas, & Serrano, Fatima Olea. (1995). The E-SCREEN Assay as a Tool to Identify estrogens: An Update on Estrogenic Environmental Pollutants. *Environmental Health Perspectives, 103*, 113-122. doi: 10.2307/3432519
- Stafiej, Anna, Pyrzynska, Krystyna, & Regan, Fiona. (2007). Determination of anti-inflammatory drugs and Estrogens in water by HPLC with UV detection. *Journal of Separation Science, 30*(7), 985-991. doi: 10.1002/jssc.200600433
- Statistics Canada. (2006). A geographical profile of livestock manure production in Canada. Retrieved from http://www.statcan.gc.ca/pub/16-002-x/2008004/article/10751-eng.htm
- Stumpe, Britta, & Marschner, Bernd. (2010). Dissolved organic carbon from sewage sludge and manure can affect estrogen sorption and mineralization in soils. *Environmental Pollution, 158*(1), 148-154. doi: http://dx.doi.org/10.1016/j.envpol.2009.07.027
- Sumpter, J. P., & Jobling, S. (1993). Male sexual development in "a sea of oestrogen" (Vol. 342, pp. 124-125).
- Tryon, E. H. (1948). EFFECT OF CHARCOAL ON CERTAIN PHYSICAL, CHEMICAL, AND BIOLOGICAL PROPERTIES OF FOREST SOILS. *Ecological Monographs, 18*(1), 81-115. doi: 10.2307/1948629
- U.S. Geological Survey. (2014). Emerging Contaminants In the Environment. Retrieved from http://toxics.usgs.gov/regional/emc/
- Vos, J. G., Dybing, E., Greim, H. A., Ladefoged, O., Lambre, C., Tarazona, J. V., . . . Vethaak, A. D. (2000). Health effects of endocrine-disrupting chemicals on wildlife, with special reference to the European situation. *Critical Reviews in Toxicology, 30*(1), 71-133. doi: 10.1080/10408440091159176
- Warnock, D. D., Lehmann, J., Kuyper, T. W., & Rillig, M. C. (2007). Mycorrhizal responses to biochar in soil - concepts and mechanisms. *Plant and Soil, 300*(1-2), 9-20. doi: 10.1007/s11104-007-9391-5
- Wei, Liu, Shutao, Wang, Jin, Zhang, & Tong, Xu. (2014). Biochar influences the microbial community structure during tomato stalk composting with chicken manure. *Bioresource Technology, 154*(0), 148-154. doi: http://dx.doi.org/10.1016/j.biortech.2013.12.022
- Xuan, Richeng, Blassengale, Alma A., & Wang, Qiquan. (2008). Degradation of Estrogenic Hormones in a Silt Loam Soil. *Journal of Agricultural and Food Chemistry, 56*(19), 9152-9158. doi: 10.1021/jf8016942
- Ying, Guang-Guo, & Kookana, Rai S. (2005). Sorption and degradation of estrogen-like-endocrine disrupting chemicals in soil. *Environmental Toxicology and Chemistry, 24*(10), 2640-2645. doi: 10.1897/05-074R.1
- Ying, Guang-Guo, Kookana, Rai S., & Dillon, Peter. (2003). Sorption and degradation of selected five endocrine disrupting chemicals in aquifer material. *Water Research, 37*(15), 3785-3791. doi: http://dx.doi.org/10.1016/S0043-1354(03)00261-6

- Ying, Guang-Guo, Kookana, Rai S., & Ru, Ying-Jun. (2002). Occurrence and fate of hormone steroids in the environment. *Environment International, 28*(6), 545-551. doi: http://dx.doi.org/10.1016/S0160-4120(02)00075-2
- Yu, Chang-Ping, Deeb, Rula A., & Chu, Kung-Hui. (2013). Microbial degradation of steroidal estrogens. *Chemosphere, 91*(9), 1225-1235. doi: http://dx.doi.org/10.1016/j.chemosphere.2013.01.112
- Yu, Chang-Ping, Roh, Hyungkeun, & Chu, Kung-Hui. (2006). 17β-estradiol-Degrading Bacteria Isolated from Activated Sludge. *Environmental Science & Technology, 41*(2), 486-492. doi: 10.1021/es060923f
- Yu, Zhiqiang, & Huang, Weilin. (2005). Competitive Sorption between 17α-Ethinyl Estradiol and Naphthalene/Phenanthrene by Sediments. *Environmental Science & Technology, 39*(13), 4878-4885. doi: 10.1021/es048558k
- Yu, Zhiqiang, Xiao, Baohua, Huang, Weilin, & Peng, Ping'An. (2004). Sorption of steroid Estrogens to soils and sediments. *Environmental Toxicology and Chemistry, 23*(3), 531-539. doi: 10.1897/03-192
- Zackrisson, O., Nilsson, M. C., & Wardle, D. A. (1996). Key ecological function of charcoal from wildfire in the Boreal forest. *Oikos, 77*(1), 10-19. doi: 10.2307/3545580