Sorption of 17alpha-ethynylestradiol in different soil environments
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Sorption of 17α-Ethynylestradiol in Different Soil Environments

Abdellatif Al Shafie

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ABSTRACT

Sorption of 17α-Ethynylestradiol in Different Soil Environments

Abdellatif Al Shafie, Ph.D.
Concordia University, 2006

Endocrine Disrupting Compounds (EDCs) have the ability to negatively affect the endocrine system; subsequently, they became the focus of the current research and media awareness. Steroid estrogens (natural and synthetic) have been recognized as endocrine disrupting chemicals a number of studies have correlated these compounds to adverse effects in humans, wildlife and fish. Most of the research done thus far was focused on the fate, behavior and impact of these compounds within living organisms, or during their removal/treatment. However, knowledge about the behavior and partitioning of these compounds or their byproducts once they are released in the environment (particularly in soil media) is not yet very well established. The focus of this study was to identify and evaluate the extent of partitioning of 17α-ethynylestradiol (EE2), a synthetic estrogen steroidal hormone, to different soil constituents (clay minerals: illite, montmorillonite, iron oxide: goethite and soil organic humic acid). The study demonstrated the effect of variable experimental conditions, such as pH, oxygen availability and EE2 initial concentration on the degree of sorption of the steroidal estrogen to individual soil components. Moreover, the potential mobility of 17α-ethynylestradiol leaching from biosolids and natural fertilizers application was investigated.

Batch-equilibration methods were conducted at the nanogram level in an attempt to emulate current environmental concentrations. The outcome of this research showed that 17α-ethynylestradiol showed different affinities of binding to soil constituents. Partitioning onto clay minerals was greater than onto iron oxide or humic acid. Sorption of 17α-ethynylestradiol was generally rapid within 1 hour; however, uptake by clay minerals continued to occur over a period of 24 hours. Changes in soil particles' surface-charges as a result of changing pH values were demonstrated to have a great influence on the potential mobility of 17α-ethynylestradiol within soil media. Highest sorption values were achieved at the isoelectric points of individual soil constituent. Sorption was primarily governed by hydrophobic interactions with siloxane surfaces, coupled with the interaction of nonpolar 17α-ethynylestradiol with uncharged soil surface hydroxyl groups through hydrogen bonding. The results also implied that the affinity of the synthetic estrogen to partition to soil constituents within subsurface media is not expected to differ between aerated top soil regions and deep ground levels of air deficiency. Moreover, the introduction of leachate was found to reduce the affinity of 17α-ethynylestradiol to partition into illite, natural soil, and goethite; however, it increased the degree of sorption to montmorillonite. Finally, this study results suggest that montmorillonite possessed the greatest sorption capacities under a wide range of environmental conditions. Although adsorbing EE2 to clay minerals is expected to diminish the potential hazard of leaching out to groundwater, surface water bodies are still vulnerable to contamination through runoff.

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LIST OF SYMBOLS

Latin Symbols

a_i, b_i : Represent constants generated from experimental date.

C_e : Equilibrium concentration that is partitioned into the aqueous phase (µg/L).

C_i : Environnemental (initial) concentration (µg/L).

$C_{s,iep}$: Concentration (µg/kg) that is partitioned into the solid phase, at the isoelectric point.

C_s : Concentration that is partitioned into the solid phase (µg/kg).

K : Coefficient representing the change of sorption capacities as a result of wastewater application.

OM : Percentage of organic matter present in the applied liquid phase

pH : Solution pH at which sorption concentrations are to be estimated.

pH_{iep} : Solution pH designating the isoelectric point of the corresponding soil constituent.

SSA : Respective soil mineral specific surface area (m^2/g).

Greek Symbols

α	: Empirical parameter representing Freundlich sorption coefficient, μg/kg / $(\mu g/L)^n$, n being unitless.
β	: Empirical parameter representing Freundlich sorption constant, unitless.
γ	: Empirical coefficient that characterizes the curvature of a parabola.
δ	: Percentage of initial concentration that is partitioned into the solid phase.
λ_i	: Represents parameters to be estimated.

1.0 INTRODUCTION

Recently, the occurrence and fate of pharmaceutically active compounds in the aquatic environment has been recognized as one of the emerging issues in environmental chemistry and as a matter of public concern. Tons of pharmacologically active substances are used annually in human medicine for diagnosis, treatment and prevention of illness or to avoid unwanted pregnancy. Moreover, various prescription classes such as analgesics, antibiotics, anti-epileptics, anti-rheumatics, beta-blockers, chemotherapeutics, steroid hormones, and X-ray contrast media have been detected in the aquatic environment. In animal and fish farming, there is greater reliance on drugs (most administered as food additives) for preventing illness, or as growth promoters or parasiticides.

Many drugs are taken for long periods of time, even the major part of the user's life. Residues of pharmaceutically active compounds have been found as contaminants in sewage, surface, and ground and drinking water samples. These findings show that these compounds are not eliminated completely in the municipal sewage treatment works and are, thus, discharged as contaminants into the receiving water bodies. The modes of action of most pharmaceuticals in humans, animals and fish are often poorly understood; even more unknown is the possible effect on non-target receptor organisms (side effects). Moreover, the mixing of drugs might produce synergistic effects. However, the major concern associated with the presence of pharmaceutically active compounds in surface or ground water that serves as a source of drinking water is not necessarily acute effects on human health, but rather the manifestation of imperceptible effects on human or aquatic life that can accumulate over time to ultimately yield truly profound changes.

Medical drugs are developed with the intention of performing a biological activity; they are therefore important as environmental pollutants in terms of their effects and their fate. Among emerging contaminants, Endocrine Disrupting Compounds (EDCs) have become of particular concern they induce aquatic toxicity, dangerous for humans and wild animals; due to their biological effect, at very low concentrations. Therefore, there are current research needs to determine the occurrence, fate, bioavailability, and transport of these chemicals after their release into the environment.

Potential sources of EDCs found in the environment are numerous, primarily as a consequence of their secretion by wildlife and humans, in addition to the introduction of anthropogenic pharmaceuticals from the disposal of unused or expired drugs, and accidental spills during manufacturing or distribution (Figure 1.1). Due to the incomplete removal, the following sources of EDC are strongly suspected: a) treated water from wastewater treatment plants, b) biosolids generated at wastewater treatment plants, c) landfills, d) biosolids landfarming areas. Agricultural sites (exercise yards, manure landfarming areas) are also suspected to be an important source of contamination. All of them impose a potential hazard for groundwater, surrounding water bodies and by interning the food chain and eventually being consumed again by humans causing unintentional exposures.

Most of the research done thus far was focused on the fate, behavior and impact of these compounds within living organisms (surface water biota), or during their removal treatment (wastewater treatment plants). However, the major problem is that the behavior and distribution of these compounds or their byproducts once they are released in the environment is not yet very well understood. Therefore, understanding the partitioning of these hormones between liquid and solid phases is crucial for the prediction and assessment of their behavior and fates in environmental systems.

The aim of this research is to achieve a deeper insight in the partitioning of synthetic steroidal estrogenic compounds in the soil media.

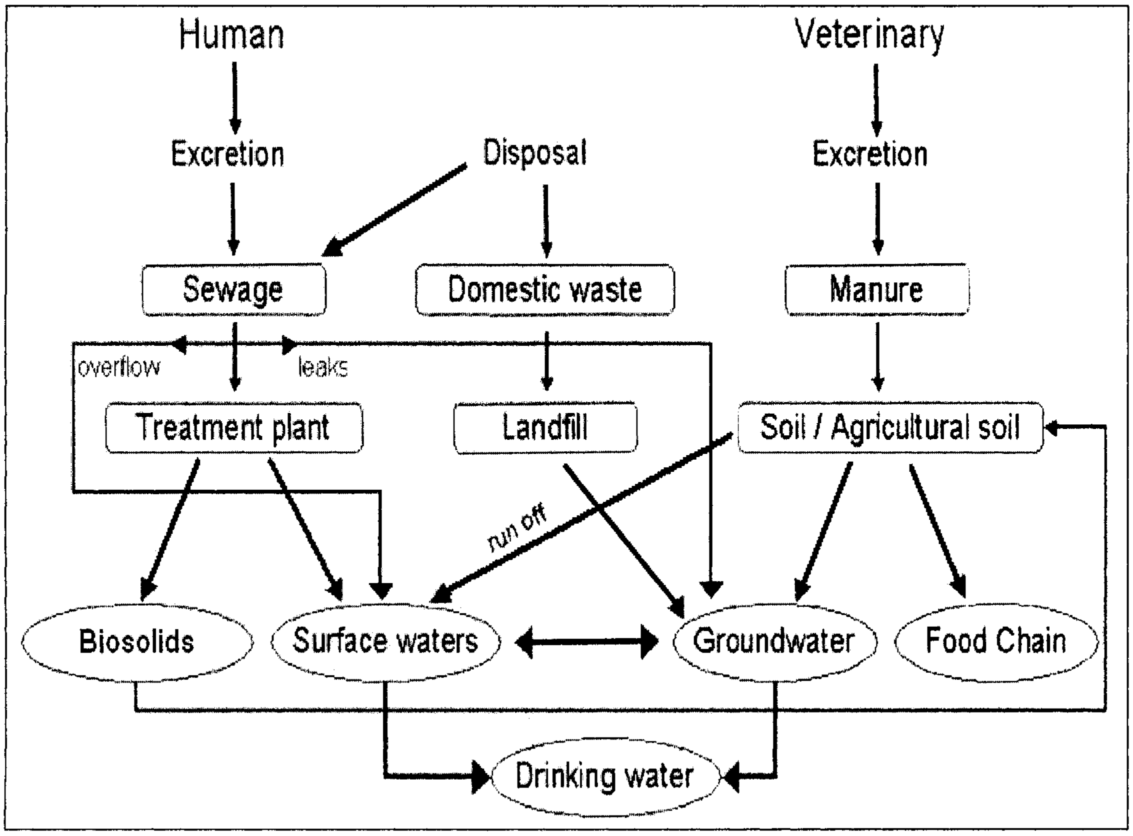


Figure 1. 1: Potential Sources and Pathways of EDC's in the Environment

2.0 LITERATURE REVIEW

2.1 Background

The presence of the endocrine disrupting compounds (EDCs) in our environment has become an emerging interest worldwide, because of their possible effects on ecosystems. These compounds may interfere with the reproduction of humans, livestock and wild-living animals. Adverse effects due to endocrine disruptors include decreased sperm count and development of hormonally sensitive carcinomas (female breast cancer, testicular and prostate cancers) in humans. In wildlife, endocrine disruptors induce reproductive abnormalities, feminization of fish and a decrease in the reproduction rate of birds (Belfroid et al., 1999; Ingrand et al., 2003; Ternes et al., 1999; Ying et al., 2004).

Wastewater treatment plants receive natural and synthetic EDCs from urban and industrial dischargers. Due to the incomplete removal of some of these compounds, wastewater and sewage treatment plant effluents are strongly suspected to be an important source of natural and synthetic estrogens contaminating the aquatic environment (Jobling et al., 1998; Alcock et al., 1999; Snyder et al., 1999; Baronti et al., 2000; Layton et al., 2000; Petrović et al., 2001; Snyder et al., 2004). Additionally, agriculture was also reported to potentially be a source of environmental EDCs (a threat to groundwater and runoff to surface water) through the use of certain pesticides, land application of sewage biosolids, or land application of animal manure containing elevated levels of hormones excreted by poultry or livestock (Arcand-Hoy et al., 1998; Layton et al, 2000; López de Alda and Barceló, 2000; Colucci et al., 2001; Colucci and Topp, 2001; Petrović et al., 2001; Ying et al., 2002; Tashiro et al., 2003).

Endocrine disrupting chemicals need to enter an organism before they can disrupt the organism's endocrine system. They exert their action by passing through the plasma membrane and binding to intracellular receptors (Chapman, 2003; Ying et al., 2002). Humans can be exposed to chemicals through a number of routes including the use of pharmaceuticals, drinking water and via food (Chapman, 2003; Ternes et al., 1999). They may also be exposed through breast milk, the air they breathe and the particles or vapors the air contains (Chapman, 2003).

A matter of some controversy relating to "endocrine disruptors" is the question of risks associated with exposure under low environmental levels (Chapman, 2003). There is evidence that low nanogram per liter concentration of some endocrine disruptors in water can adversely affect the reproduction biology of fish and other aquatic vertebrate species (Hanselman et al., 2003, Palace et al., 2002; Parrot et al., 2002).

2.2 Endocrine Disrupting Chemicals

Endocrine disrupting chemicals (EDCs) consist of naturally occurring and synthetic chemicals that may affect the balance of normal hormonal functions. In 1996, the European commission defined this class of compounds as an "exogenous substances that cause adverse health effects in an intact organism, or its progeny, consequent to changes in endocrine function" (Jeannot et al., 2002; López de Alda and Barceló, 2000; Petrović et al., 2001). Furthermore, the U.S. Environmental Protection Agency (U.S. EPA) has defined an environmental EDC as an "exogenous agent which interferes with the synthesis, secretion, transport, binding, action, or elimination of natural hormones in the body that are responsible for the maintenance of homeostasis, reproduction, development, and/or behavior'' (U.S. EPA 1997), as cited in Snyder et al. (2001, 2003).

The endocrine and reproductive effects of endocrine disrupting compounds (EDCs) are believed to be due to their ability to mimic the sex hormones estrogen and androgen (thereby producing similar responses to them), or antagonize effects of endogenous hormones by blocking their activities (i.e. be anti-estrogen or anti-androgen), or to interfere with the biosynthesis, transport or availability, or metabolism of endogenous hormones and hormone receptors. (Chapman, 2003; Colucci et al., 2001; Colucci and Topp, 2001; Holbrook et al., 2002; Petrović et al., 2001). There are numerous other definitions and opinions on what defines an EDC. However, it is generally accepted that the three major classes of endocrine endpoints are Estrogenic (compounds which mimic or block natural estrogen), Androgenic (compounds which mimic or block natural testosterone), and Thyroidal (compounds with direct or indirect impacts to the thyroid gland).

Among the EDCs there are five groups of compounds that are of priority within European Union and USA research activities: (1) Alkylphenol Polyethoxylates (APEO) and their associated metabolites (Holbrook et al., 2002; Ingrand et al., 2003; Kolpin et al., 2002;

Petrović et al., 2001; Routledge and Sumpter, 1996; Snyder et al., 1999; Ying et al., 2004), (2) Polychlorinated Compounds: dioxins, polychlorinated biphenyls (PCBs) and polycyclic aromatic hydrocarbons (PAHs), (Arcand-Hoy et al., 1998; Chapman, 2003; Colucci et al., 2001; Colucci and Topp, 2001; Ingrand et al., 2003; Petrović et al., 2001; Snyder et al., 1999; Ying et al., 2004), (3) Polybrominated Diphenyl Ethers, PBDEs, (Petrović et al., 2001), (4) Phthalates (Chapman, 2003; Petrović et al., 2001; Snyder et al., 1999; Tanaka et al., 2000; Ying et al., 2004), and (5) Steroid Sex Hormones (estrogens, androgens, progestogens), (Chapman, 2003; Holbrook et al., 2002; Ingrand et al., 2003; Kolpin et al., 2002; Petrović et al., 2001; Snyder et al., 1999; Ying et al., 2004). Of these groups, it appears that the Steroid Hormones represent the predominant form of estrogenic activity in wastewater effluents (Holbrook et al., 2002).

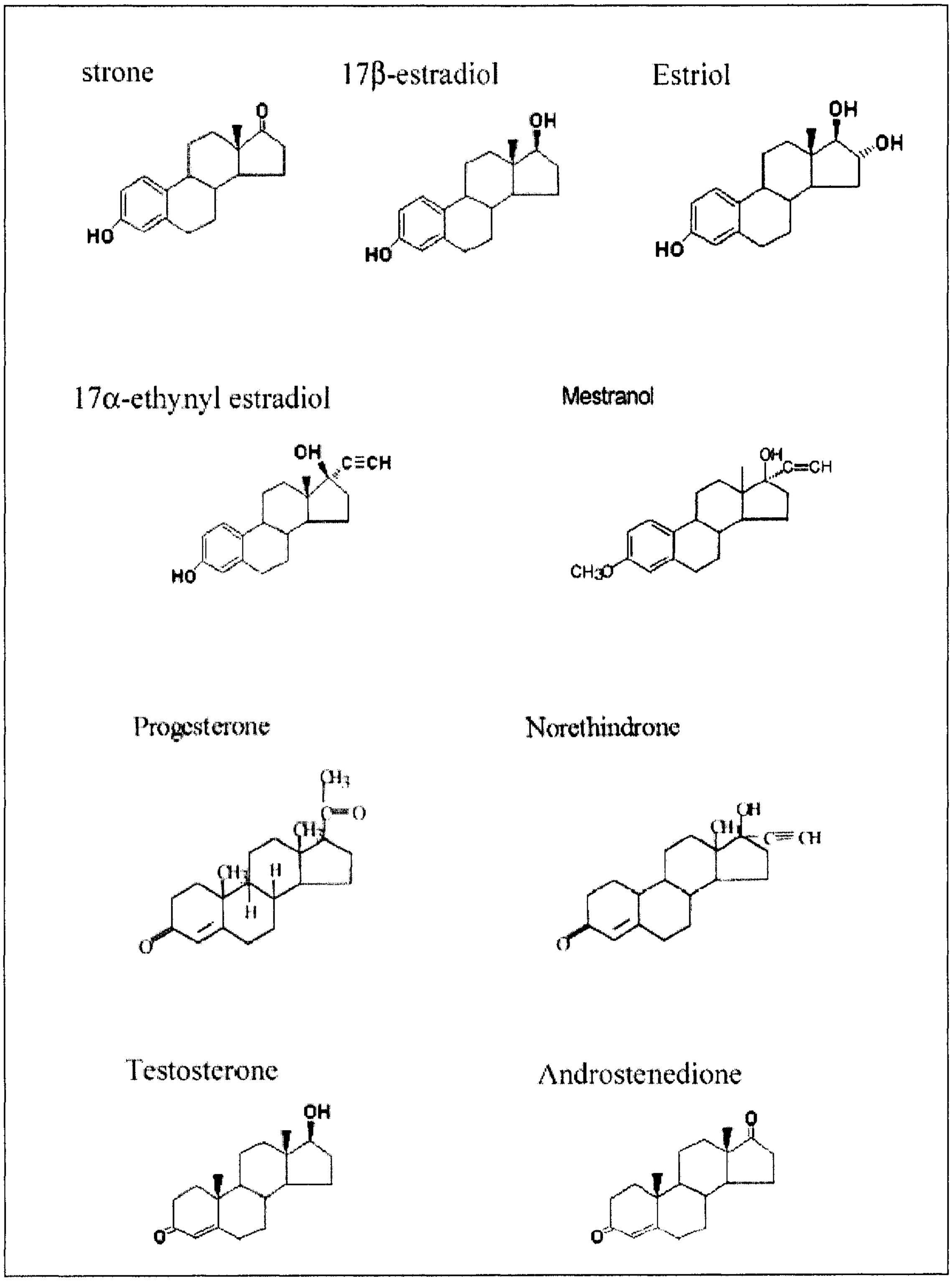
2.2.1 Steroid Hormones

This section concisely introduces steroid hormones and some of their characterizing features. It also gives a summary of some of their physicochemical properties.

2.2.1.1 Characterizing Features

Steroid hormones, Figure 2.1, are a group of biologically active compounds that are synthesized from cholesterol, a precursor of mammalian sexual steroids (Kolpin et al., 2002; Ying et al., 2002). Estrogens (estrone; E1, estradiol; E2, and estriol; E3) are predominantly female hormones, which are important for maintaining the health of the reproductive tissues, breasts, skin and brain (Mansell and Drewes, 2004; Ying et al., 2002). Androgens (testosterone and androsterone) are responsible for male reproductive development. They play an important role in tissue regeneration, especially the skin, bones and muscles (Mansell and Drewes, 2004; Ying et al., 2002). Progestogens (progesterone) can be thought of as a hormonal balancer, particularly of estrogens (López de Alda and Barceló, 2000; Ying et al., 2002). In addition, there are some synthetic steroids such as ethynylestradiol (EE2), norethindrone and mestranol (MeEE2) used as contraceptives (Colucci et al., 2001; Colucci and Topp, 2001).

Natural and synthetic hormones are used extensively for humans and animals. Estrogenic pharmaceutical products are the major ingredient in hormone replacement therapy treatments, and oral contraceptive formulations. Likewise, progestogens are used in the treatment of several other conditions, such as infertility. Moreover synthetic steroids are used worldwide as growth-promoting agents in veterinary medicine (Arcand-Hoy et al., 1998; Daeseleire et al., 1992; López de Alda and Barceló, 2000).



strone

17β-estradiol

Estriol

17α-ethynyl estradiol

Mestranol

Progesterone

Norethindrone

Testosterone

Androstenedione

Figure 2. 1: Molecular Structure of Steroid Hormones

(Arcand-Hoy et al., 1998; Lee et al., 2003; López de Alda et al., 2000&2002)

2.2.1.2 Physicochemical Properties

Steroid hormones are 18-carbon molecules with an aromatic A-ring as a distinctive part of their tetracyclic molecular framework. This phenolic A-ring is the structural component that characterizes the affinity of binding to the estrogen receptor (Arcand-Hoy et al., 1998; Hanselman et al., 2003). The key structural difference that distinguishes the steroid hormones from one another arises in the D-ring component, of their tetracyclic ring skeleton; owing to the type and stereo-chemical arrangement of the functional groups attached to Carbon-3, Carbon-16 and Carbon-17 positions (Figure 2.2). Estradiol can have either a hydroxyl group at Carbon-17 that points downward from the molecule (α-configuration) or a hydroxyl group that projects upward from the molecule (β-configuration). While estrone differs from estradiol by the carbonyl group at Carbon-17 rather than a hydroxyl group, estriol features hydroxyl groups at both Carbon-16 and Carbon-17 positions. Similarly, 17α-ethynylestradiol is structurally identical to E2, with the exception of an ethynyl substitution at Carbon-17. Moreover, conjugated steroids are analogous to their parent compounds, except for a sulfate and/or glucuronide substitution at Carbon-3 and/or Carbon-17 (Arcand-Hoy et al., 1998; Hanselman et al., 2003).

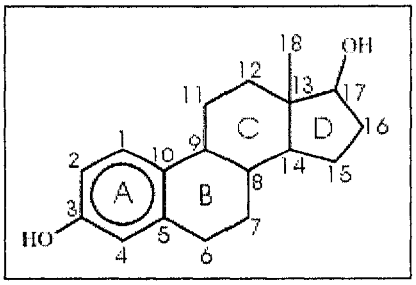


Figure 2. 2: Tetracyclic ring skeleton of steroid hormones (Arcand-Hoy et al., 1998; Hanselman et al., 2003)

Natural estrogens, namely estradiol, estrone and estriol, have water solubilities of approximately 13mg/L. Synthetic estrogenic steroids have much lower solubilities (e.g. 4.8mg/L for EE2). Typically, steroidal compounds are nonvolatile maintaining low vapor pressures ranging from (2.3E-10 to 6.7E-15) mmHg. In addition, they are generally considered weak acids with pK_a values ranging between 10.3 and 10.8. Steroids are relatively hydrophobic; log K_{ow} 2.8-4.7. Table 2.1 lists some of the physicochemical properties of these natural and synthetic steroids (Hanselman et al., 2003; Kuster et al., 2004; Lai et al., 2000; Lee et al., 2003; López de Alda et al., 2000&2002; Tabak et al., 1981; Ying et al., 2002). It can be seen that estrogens are hydrophobic organic compounds of low volatility. It is expected that their sorption onto soil or sediments will be a significant factor in reducing aqueous phase concentrations.

Table 2. 1: Physicochemical properties of steroid hormones

Compound	Formula	MW (g/mol)	WS (mg/L)	Log K_{ow}	MP (°C)	VP (mmHg)	pK_a
Estrone	$C_{18}H_{22}O_2$	270.36	13.0	3.43	259	2.3E-10	~10.5
Estradiol	$C_{18}H_{24}O_2$	272.37	13.0	3.94	171	2.3E-10	~10.6
Estriol	$C_{18}H_{24}O_3$	288.37	13.0	2.81	285	6.7E-15	~10.4
Ethynylestradiol	$C_{20}H_{24}O_2$	296.39	4.8	4.15	183	4.5E-11	~10.2
Mestranol	$C_{21}H_{26}O_2$	310.42	0.3	4.67	150	7.5E-10	~13.0
Progesterone	$C_{21}H_{30}O_2$	314.45	8.8	3.87	128	1.3E-06	na
Norethindrone	$C_{20}H_{26}O_2$	298.41	7.0	2.97	203	7.3E-09	na
Testosterone	$C_{19}H_{28}O_2$	288.40	18.3	3.22	155	na	na
Androsterone	$C_{20}H_{26}O_2$	286.40	39.0	na	173	na	na

* MW: molecular weight, WS: water solubility, MP: melting point, VP: vapor pressure, na: not available

(Hanselman et al., 2003; Kuster et al, 2004; Lai et al, 2000; Lee et al., 2003; López de Alda et al., 2000&2002; Tabak et al., 1981; Ying et al., 2003)

2.3 Fate and Behavior of Endocrine Disrupting Chemicals

In humans and animals, estrogens undergo various transformations mainly in the liver. They are frequently oxidized, hydroxylated, deoxylated and methylated prior to the final conjugation with glucuronic acid or sulphate. Therefore, estrogens are principally excreted as inactive polar conjugates (Belfroid et al., 1999; Ternes et al., 1999; Ying et al., 2002) and finally released into the environment. Although steroid conjugates do not possess a direct biological activity, they can act as precursor hormone reservoirs able to be reconverted to free steroids by bacteria in the environment (Baronti et al., 2000; Ternes et al., 1999a). Deconjugation was thought to happen in the human body because the gut contains high levels of the bacteria *Escherichia* coli, which are able to synthesize large amounts of the β-glucuronidase enzyme, which is capable of hydrolyzing acid glucuronides back to their primary compounds (Belfroid et al., 1999; Desbrow et al., 1998; Hanselman et al., 2003; Shi et al., 2004; Ternes et al., 1999b; Tyler and Routledge, 1998). As feces contain high levels of E. coli, it was suggested that deconjugation occurs preferentially in sewers, even before reaching the sewage treatment works (Alcock et al., 1999; Baronti et al., 2000; Panter et al., 1999; Ternes et al., 1999b; Ying et al., 2002; Zuehlke et al., 2004). Moreover, it is been reported that deconjugation continued to occur during waste water treatment processes (Alcock et al., 1999; Desbrow et al., 1998; Larsson et al., 1999; López de Alda and Barceló, 2000; Ternes et al., 1999b; Ying et al., 2002; Zuehlke et al., 2004), and in the environment (Desbrow et al., 1998; López de Alda and Barceló, 2000; Routledge et al., 1998; Zuehlke et al., 2004). Once in the environment, EDCs may undergo a series of processes, such as dilution, photolysis, biodegradation, and sorption to solids and bed sediments, which can contribute to their elimination and decrease of their aqueous concentrations in environmental water bodies.

2.3.1 Dissipation of Endocrine Disrupting Chemicals

Synthetic hormones are generally more stable in the environment than are natural hormones and have greater potency (Esperanza et al., 2004; Colucci and Topp, 2001; Snyder et al., 1999; Snyder et al., 2004; Ternes et al., 1999a; Tabak et al., 1981). In the Brazilian sewage treatment plants (STPs), the observed removal rates ranged from 64% to 78% for EE2 and from 92% to 99.9% for E2, whereas a German sewage treatment work (STW) failed to remove EE2, and removed E2 at a very low rate, only 64% (Ternes et l., 1999a). As recently reviewed, the removal of endocrine-disrupting chemicals in activated sludge treatment works suggested that the activated sludge treatment efficiently removed E2 ~ 87%, and EE2 ~ 85% (Baronti et al., 2000; Johnson and Sumpter, 2001). Furthermore, an input/output balance of estrogenic active compounds in a major municipal sewage plant in Germany showed that the load of estrogenic activity of the wastewater was reduced by about 90% in the sewage plant (Körner et al., 2000). Its worth mentioning that in the Japanese STPs, the removal rates were reported to be more than 99% in autumn and from 7% to >99% in summer. The reason behind this large difference in removal rates it still unclear (Nasu et al., 2001). Colucci et al. (2001) studied the persistence and pathways of dissipation of 17ß-estradiol in soil in laboratory microcosm incubations. 17β-Estradiol was oxidized to estrone in both autoclaved and nonsterile loam, silt loam, and sandy loam soils, suggesting an abiological transformation (Colucci et al., 2001). They have also reported that the mineralization (cleavage of the phenolic ring) of estradiol in the soils tested was relatively slow compared with the rates of dissipation. Similarly, Colucci and Topp (2001) demonstrated the persistence of 17α-ethynylestradiol in agricultural soil in a laboratory setting. The hormone was rapidly dissipated in loam, sandy loam, and silt loam soils under a range of moisture and temperature conditions. However, the stability of 17α-ethynylestradiol in sterile soil, decreased removal in the absence of oxygen, and the response of dissipation kinetics to variation in temperature and moisture suggested that the removal was microbially mediated (Colucci and Topp, 2001).

"Removal during sewage treatment" is used as a collective term to describe the disappearance of chemicals due to processes such as biodegradation and adsorption on sludge and solids. Accordingly, it was not determined whether the estrogenic activity contained in the influent wastewater was either biodegraded during the wastewater or biosolids treatment processes or was unavailable to the extraction/detection procedure (Holbrook et al., 2002).

2.3.2 Biodegradation of Endocrine Disrupting Chemicals

Due to the endocrine-disrupting potency of natural and synthetic estrogens, there has been an increasing interest in biodegradation of these estrogens by using microorganisms. Activated sewage sludge contains microbial species capable of degrading both natural and synthetic steroid compounds. The biodegradation and transformation of unconjugated estrogens has been studied in soil, water, and manure for several years. Rapid biodegradation of E2 has been reported in aerobic experiments with activated sludge and biosolids (Layton et al. 2000; Lee and Liu, 2002; Onda et al., 2003; Ternes et al. 1999a). The batch experiments showed that while in contact with activated sludge the natural estrogen 17β-estradiol was oxidized to estrone, which was further eliminated in approximate linear time dependence. It was further demonstrated that E2 was largely degraded biologically in the denitrifying (Andersen et al., 2003) and aerated nitrifying tanks of the activated sludge system (Andersen et al., 2003; Shi et al., 2004; Vader et al., 2000). This natural estrogen was also found to be readily biodegradable in river water and sediments (Jürgens et al., 2002; Williams et al., 1999), in seawater and marine sediment, and groundwater and aquifer sediment (Ying and Kookana, 2003; Ying et al., 2003) under aerobic conditions.

However, EE2 was found to be more persistent in the environment (Jürgens et al., 2002; Layton et al., 2000; Ternes et al., 1999b). While, Ternes et al. (1999) observed no significant reduction of synthetic EE2 concentrations in aerobic batch experiments; Layton et al. (2000) reported that the mineralization of EE2 was 25–75-fold less than that of the natural estrogen E2. The solubility of EE2 in pure water and sewage-treatment water was reported to be 3-fold less soluble than natural steroidal estrogens. This fact is believed to contribute to the increased resistance of EE2 to biodegradation as compared with natural steroidal estrogens (Desbrow et al., 1998). Slow degradation in marine sediment was observed for EE2 under aerobic conditions and for E2 under anoxic conditions (Ying and Kookana, 2003; Ying et al., 2003). EE2 was slowly degraded in the aerated nitrifying tank (Andersen et al., 2003; Shi et al., 2004) whereas, denitrifying conditions failed to degrade it (Andersen et al., 2003). Most recently, Ying et al. (2004) investigated the role of the treated sewage effluent on degradation of E2 and EE2 in aquifer sediments. E2 was rapidly biodegraded, under aerobic conditions, with or without the supplement of effluent. EE2, on the other hand, was found to be persistent under both aerobic and anaerobic conditions, as well as in the presence of effluent. The addition of effluent in the incubation media slightly enhanced the relative rate of E2 degradation only under anaerobic conditions.

In summary, the removal of EDCs during the activated sludge processes is variable and may be related to temperature (Ternes et al., 1999b), sludge age (Vader et al., 2000), hydraulic retention time (Johnson and Sumpter, 2001), and/or sewage composition (Layton et al., 2000). Although a complete understanding of EDC behavior during biological wastewater treatment is presently lacking, adsorption to organic matter may facilitate their removal from biological wastewater treatment systems or during their transport (Huang and Sedlak, 2001). In addition, ozonation, UV-radiation, membrane filtration, advanced oxidation process, and granulated activated carbon adsorption are potential treatments that might improve the effectiveness of estrogen removal in a STP (Chapman, 2003; De Rudder et al., 2004; Onda et al., 2002). However, implementation of these techniques would increase the cost of wastewater treatment. Alternatively, understanding the fate of estrogens within the environment might yield removal methods based on better management or minor modifications of current practices.

2.3.3 Adsorption of Endocrine Disrupting Chemicals

Understanding the partitioning of natural and synthetic steroidal estrogens between liquid and solid phases is critical for the prediction of their fates in environmental systems. Since estrogens are hydrophobic organic compounds of low volatility, it is likely that sorption will be a significant factor in reducing aqueous phase concentrations. The fate of estrogens once bound to solids is an important consideration in terms of transportation, degradation, and potential exposure to organisms (Díaz-Cruz et al., 2003; Hanselman et al., 2003; Holbrook et al., 2002; Huang and Sedlak, 2001; Lai et al., 2000; Layton et al, 2000; Mansell and Drewes, 2004; Petrović et al., 2001; Ying et al., 2003).

The distribution and partitioning of estrogenic steroids in the environment is determined by their physicochemical properties and site-specific environmental conditions. Moreover, sorption depends on the structure and position of functional groups of the sorbate, sorbent binding site availability and competition for binding sites, the chemical characteristics of the sorbate, mineralogical composition, mineral particle size and surface area, organic matter content, cation exchange capacity, microbial activity of the sorbent, salinity of the aqueous phase, infiltration velocity, particle transport, residence time, and ground-water hydraulics (Casey et al., 2003; John et al., 2000; Johnson et al., 1998; Lai et al., 2000; Verstraeten et al., 2003). For example, estrogens in saline waters, e.g. the estuarine or marine environment, were shown to exhibit greater removal from the aqueous phase than in freshwaters. It is likely that increased removal of estrogens is due to aggregation and flocculation in the higher ionic strength medium, resulting in high rates of sedimentation (Johnson et al., 1998; Lai et al., 2000).

A number of different mechanisms, which are suspected to be responsible for estrogens sorption, include sorption to organic matter, surface adsorption to mineral constituents, ion exchange, complex formation with metal ions such as Ca^{2+}, Mg^{2+}, Fe^{3+} or Al^{3+}, and H-bonding. However, sorption to organic matter has been frequently reported being the most important, indicating hydrophobic partitioning as the dominant sorption mechanism (Johnson et al., 1998; Lai et al., 2000; Lee et al., 2003; Shore et al., 1993). The octanol-water (K_{ow}) and organic carbon (K_{oc}) partition coefficients are frequently used indicators of the tendency of compounds to partition to organic matter. The greater these coefficient values are for a given compound, the greater its tendency to partition to organic matter, e.g. organic carbon rich matrices (Díaz-Cruz et al., 2003; Holbrook et al., 2002; Lai et al., 2000; López de Alda et al., 2002; Ternes, 2001).

Lai et al. (2000) investigated the influence of various environmental conditions on sorption of estradiol, estrone, estriol, ethynylestradiol, and mestranol onto sediments from the United Kingdom. One hour shaking was enough to achieve maximum sorption values. Elevated estrogen concentrations resulted in a decrease of removal levels; however, increased sediment loads induced greater removal. They correlated estrogen sorption with total organic carbon content, consistent with hydrophobic sorption mechanism; nevertheless, they also demonstrated that iron oxide (in the absence of organic carbon) was able to attain appreciable sorption capacities. Additionally, Lai et al. (2000) suggested that estrogenic compounds competed amongst each other for binding site, a phenomenon that might be applicable for a wide range of hydrophobic complexes. Casey et al. (2003) studied the fate and transport of the natural hormone, E2, in soil water systems. Their batch equilibrium experiments indicated high sorption affinity with correlations to mineral particle size and organic matter content. The sorption affinity appeared to be associated with the surface area and/or cation exchange capacity of soil.

Moreover, sorption and dissipation of steroid hormones was examined in soils and fresh sediments (Lee et al., 2003). Sorption equilibrium was attained within a few hours. Sorption isotherms and their computed parameters suggested hydrophobic partitioning as the dominant sorption mechanism. Lee et al. (2003) concluded that major fraction of the steroid hormone will be associated with solid phases, and hence, limiting the threat of ground water contamination; as leaching from soils is speculated to be insignificant. On the other hand, surface waters remain vulnerable through the inputs of runoff of soil and land applied biosolids. Sorption of selected endocrine disrupting chemicals was also studied under estuarine conditions (Bowman et al., 2002). Experimental work signified colloidal particles as stronger sorbents than sediment particles. They also demonstrated the association between sorption capacities and particle sizes, suggesting direct correlation with particulate organic matter and specific surface areas.

Sorption of natural and synthetic estrogen hormones to individual soil constituents was recently simulated (Shareef et al., 2006; Van Emmerik et al., 2003). Both studies agreed that clay minerals had greater sorption capacities than iron oxides. It was suggested that sorption to montmorillonite involved intercalation of estrogens into the interlayer spaces of the clay mineral. This assumption was further promoted by the results of desorption experiments, where little or no estrogen was desorbed from montmorillonite in comparison to other clay minerals or iron oxides. Conversely, the effect of pH on sorption has not been fully established. Van Emmerik et al. (2003) observed little pH dependence on sorption except for the case of iron oxide, while Shareef et al. (2006) showed that uptake of EDCs was little influenced by pH conditions with the exception of montmorillonite. As such, variable mechanisms have been suggested for sorption of estrogens to individual soil components.

Although the K_{oc} values, as well as K_{ow} values of these estrogenic steroids, suggest their hydrophobic nature and high binding with sediment/soil particles, these compounds have been widely reported not only in surface water but also in ground water. Furthermore, in almost all laboratory-based experiments, sorption was evaluated without additions of biosolids. The information thus gained does not allow assessment of the effects of the chemical, physical, and microbiological changes that can occur in a soil following biosolids application. Clearly, there is a need to better understand estrogenic steroids behavior in different environmental media.

2.4 Soil Media

A soil environment consists of two major elements: a porous medium through which solutions and suspensions flow and a solid medium characterized as a potential filter material that filters out numerous wastes from the proceeding water body. Consequently, it is essential for contaminant management to investigate the contaminants interaction with soil. This section provides a quick overview of soil characteristics that have potential control over these interactions.

2.4.1 Soil Composition and Properties

Soils normally contain soil solids, fluids and gaseous phases (Table 2.2). Soil solids are mainly composed of organic and inorganic components. The inorganic component mostly constitutes of alumino-silicates, i.e. oxides of aluminum and silicon, which can range from highly crystalline to amorphous substances. The fractions considered here include the clay minerals and oxides and hydrous oxides. On the other hand, soil organics originate from vegetation and animal sources, and range from relatively unaltered plant tissues to highly humified material (Olphen, 1977; Yong et al., 1992; Yong, 2001).

2.4.1.1 Clay Minerals

Primary minerals are the resulting unaltered form of the parent rock due to physical and/or chemical weathering processes. Secondary minerals represent the altered products

Table 2. 2: Soil Media Composition

Soil Media	**Gaseous Phase**	Air and gas
	Fluid Phase	Water with dissolved solutes
	Solid Fractions	Soil Organics • Humic substances (humic acid) • Soil polysaccharides
		Inorganic Crystalline • Oxides and hydrous oxides of iron, aluminum, and silicon (goethite) • Primary and secondary minerals (kaolinite, illite, montmorillonite) • Carbonates, sulphates, phosphates, sulphides
		Inorganic Non-crystalline • Hydrous oxides of iron, aluminum, and silicon • allophane

* After Yong, 2001

of the physical, chemical and/or biological processes. These secondary minerals possess large specific surface areas, due to their very small particle size, along with the significant electric charges on their surfaces; features that make them important in the development of contaminants interaction and accumulation mechanisms in soil. The main building blocks for all secondary minerals consist of two basic crystal structures, the silica tetrahedron and alumina octahedron. These crystals are the backbone of the tetrahedral and octahedral sheets (Figure 2.3). The bonding between these two sheets, by sharing a layer of oxygen atoms between them, form what is known as the elemental unit layer. Clay minerals are derived by differing the stacking of these unit layers. A wide variety of secondary minerals are found in the soil, some of which are introduced in this section.

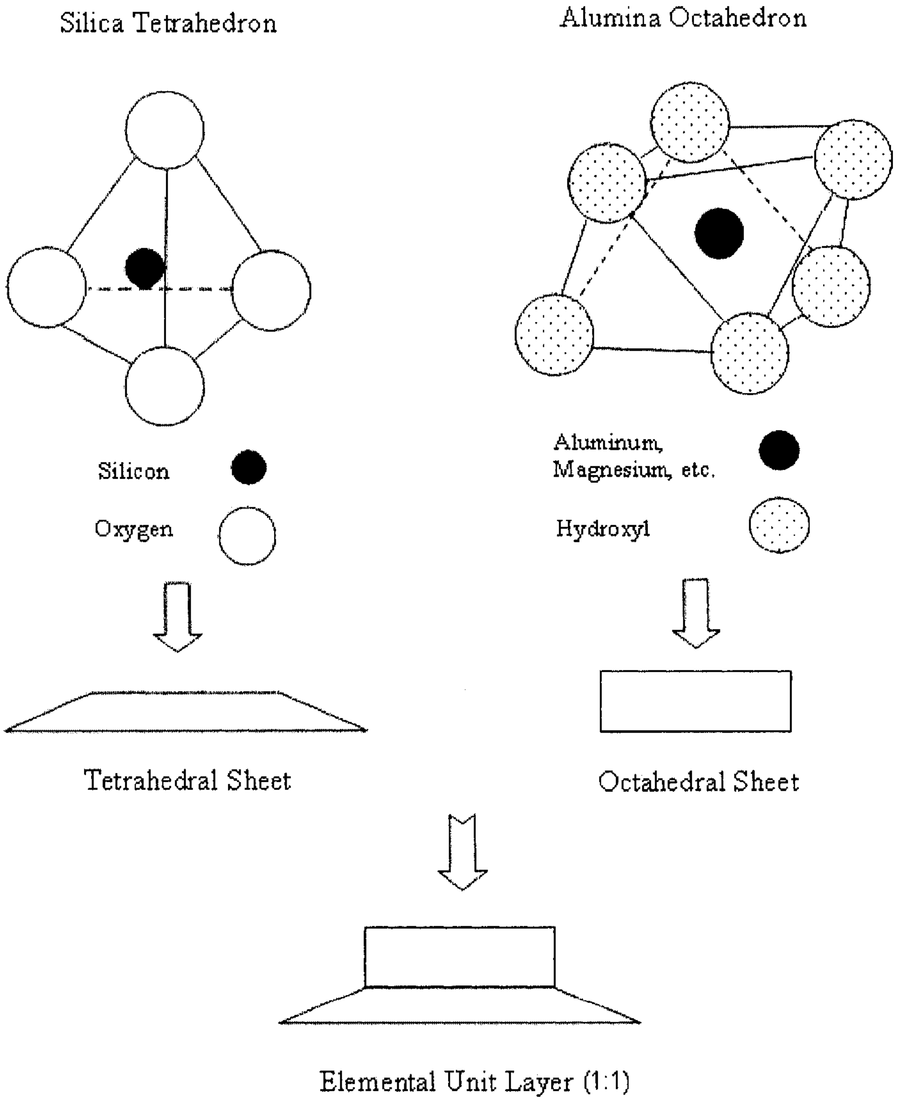


Figure 2. 3: Basic Building Blocks for Clay Minerals (Adapted from Mitchell, 1993; Yong et al., 1992)

Kaolinite

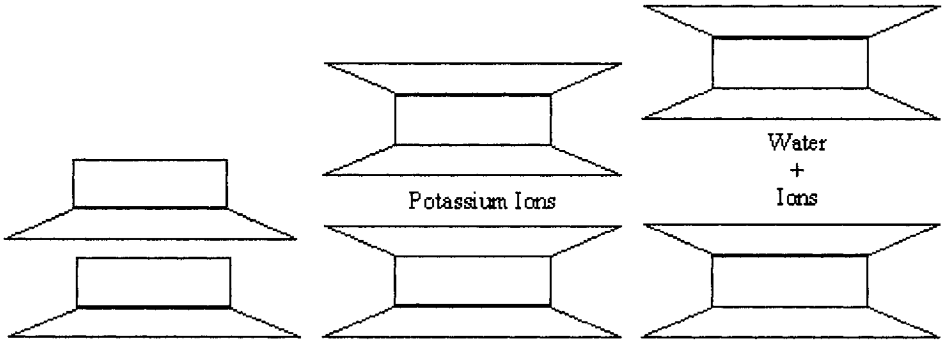
This mineral is abundant in soils that have undergone considerable weathering in warm, moist climates. The structure of kaolinite is formed by staking a single sheet of alumina octahedral on top of the silica tetrahedral sheet to give a (1:1) unit layer (Figure 2.4). The structural formula of kaolinite is $Si_4Al_4O_{10}(OH)_8$. Repeating layers are held together by both van der Waals forces and hydrogen bonding between hydroxyls from the alumina sheet on one layer, and oxygen from the silica sheet on the opposite layer. The interlayer bonds are strong enough to prevent interlayer swelling. Since kaolinite is non-expanding clay, its interaction with environmental contaminant is only expected to be through its external surfaces. The specific surface area of kaolinite is between 10 - 20 m^2/g, and its cation exchange capacity (CEC) is about 5 to 15 cmol/kg. There are limited isomorphous substitutions in the clay lattice; thus, the exchange capacity is attributed primarily to the edges of the particles, which feature pH dependent charge properties (Mitchell, 1993; Olphen, 1977; Yong et al., 1992; Yong, 2001).

Illite

Illite occurs principally in sedimentary rocks in temperate and in arid regions. The chemical structure of illite is notably different from kaolinite in that the building layers consist of an alumina sheet packed in between two silica sheets. These stacked (2:1) unit layers are held together bonding through potassium ions (Figure 2.4), which are just the right size to fit into the hexagonal holes of the silica sheet. The interlayer bonding is sufficiently strong to restrict the access of water and polar liquids into the interlayer regions. The structural formula of illite is $K_2(Si_6Al_2)Al_4O_{20}(OH)_4$. Isomorphous substitutions in the clay lattice (aluminum for silicon in the silica sheet) and the broken bonds at the edges account for the net negative electric charge on the clay mineral. Generally, illites have properties intermediate between kaolinite with low activity and montmorillonite with high activity. The cation exchange capacity for illite is in the range of 15 - 40 cmol/kg and the specific surface area for the mineral is about 70 - 100 m^2/g (Mitchell, 1993; Olphen, 1977; Yong et al., 1992; Yong, 2001).

Montmorillonite

Montmorillonite occurs in the environment as a weathering product from silica poor rocks. The chemical structure of montmorillonite is similar to that of illite consisting of two silica sheets sandwiching an alumina sheet. The primary difference, however, is that there are no potassium ions to hold the building layers together (Figure 2.4). Accordingly, the resulting structure is only weakly bonded by van der Waals forces, and water molecules are easily penetrated into the interlayers causing the structure to expand (Figure 2.5). This process is normally referred to as the swelling of montmorillonite. Another difference is isomorphous substitutions, which mainly occur in the alumina sheet where aluminum is being substituted with magnesium, iron, zinc, nickel, lithium, or other cations. Additionally, up to 15 percent of silicon in the tetrahedral sheet could be replaced with aluminum. The charge deficiencies arising from such substitutions are balanced by exchangable cations occurring between the unit layers and on the surfaces of the particles. The type and extent of these substitutions govern the strength of the bond that holds the layers and hence determines the degree of swelling. The general formula of the half-cell unit of montmorillonite is $(Na,Ca)_{0.33}(Al,Mg)_2Si_4O_{10}(OH)_2.n(\text{interlayer})H_2O$. Montmorillonite has cation exchange capacity (CEC) of 80-150 cmol/kg and surface area that ranges from 600-800 m^2/g, of which, as much as 80% is attributed to internal surfaces (Mitchell, 1993; Olphen, 1977; Yong et al., 1992; Yong, 2001). Due to its properties, montmorillonite is expected to play a major role in the transport and fate of environmental pollutants within subsurface.



	Kaolinite	Illite	Montmorillonite
CEC (cmol$_c$/kg)	5 - 15	15 - 40	80 - 150
External surface area (m^2/g)	10 - 20	70 - 100	70 - 120
Internal surface area (m^2/g)	-	-	550 - 650
Interlayer spacing (nm)	0.7	1.0	1.0 - 2.0
Swelling	Low	Low to none	High
Bonding	Hydrogen	Potassium ions	Van der Waal's bonds

Figure 2. 4: Secondary Clay Minerals

(After Brandy and Weil, 1996; Mitchell, 1993)

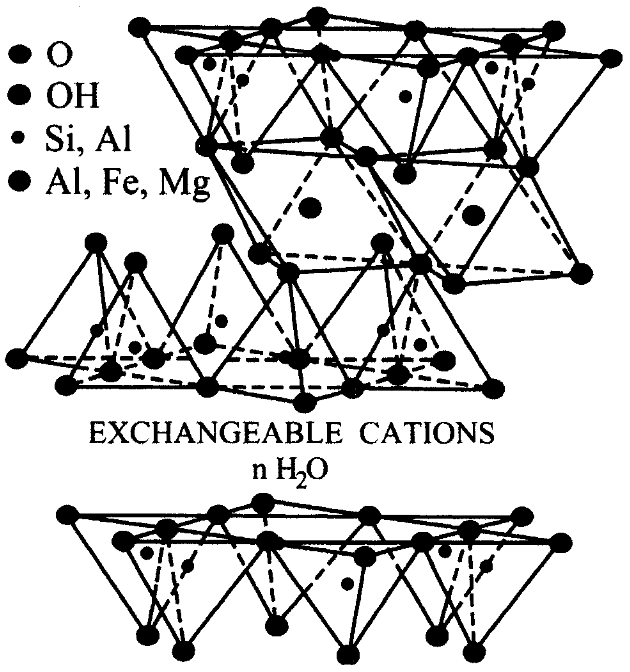


Figure 2. 5: Molecular Structure of Montmorillonite

2.4.1.2 Oxides and Hydrous Oxides

This group of minerals features oxides, hydroxides, and hydrous oxides of metals such as iron, aluminum, manganese, and silicon. This class of soil constituents comprises common crystalline minerals such as gibbsite (γ-$Al(OH)_3$), hematite (α-Fe_2O_3), and goethite (α-FeOOH). Figure 2.6 shows the molecular structure of goethite. They are normally found in regions of greatly weathered tropical soils. These minerals typically possess large surface areas, and they differ from layer silicates in the absence of isomorphous substitutions. Hydrous metal oxides are primarily amphoteric; characterized by the broken bonds, which in the presence of water, are satisfied with protons or hydroxyl ions of dissociated water molecules. Thus, they can carry positive or negative surface charge depending on the chemical properties of the soil solution. Consequently, these minerals might embrace a significant influence of the chemistry of a soil solution and might exert a pronounced impact on the fate and behavior of substances in the soil environment (Yong et al., 1992; Yong, 2001).

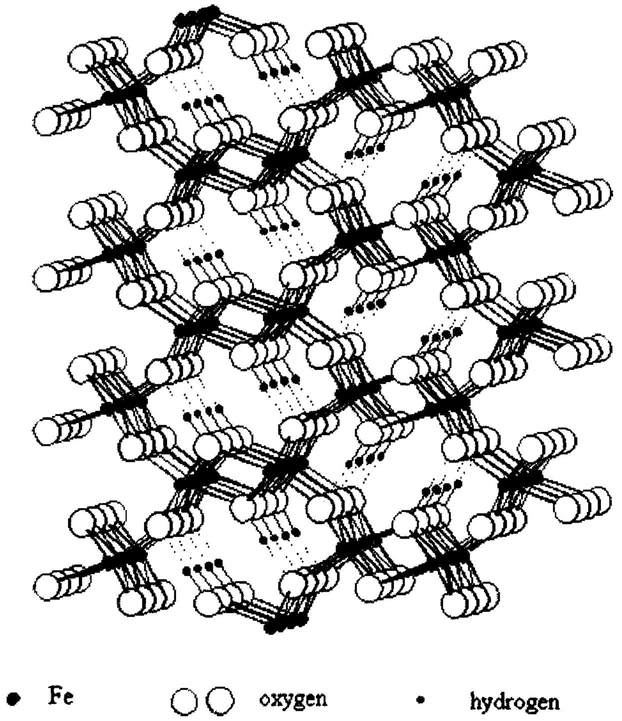


Figure 2. 6: Molecular Structure of Goethite

2.4.1.3 Soil Organics

Soil media comprises a wide range of organic material. These compounds mainly consist of carbon and hydrogen. The type of skeletal carbon bonds attributes to different chemical structures of these compounds. They could be formulated in alycyclic (saturated) rings, aromatic (non-saturated) rings, or as chains. The major characteristic of this group of material arises from their possession of functional groups. Typical functional groups include carboxyl (COOH), phenolic (ring-OH), alcoholic (OH), carbonyl (C=O), and amines (NH_x). The functionality and extent of activity of these functional groups provide the basis for the interaction with different soil constituents as well as with contaminants. These function groups are further characterized by their ability to produce positive and negative charges on the organic surface and as a function of ambient pH. Since most of the functional groups of humic substances dissociate between pH 5 and 7, the molecular structures are expected to exhibit a net negative charge in soils. The most common soil organics are humic substances (Figure 2.7) including humic acids ($C_{187}H_{186}O_{89}N_9S$), fulvic acids ($C_{135}H_{182}O_{95}N_5S_2$), and humins. The distinction between these components could be achieved following their solubility in strong acid and base solutions. For instance, introducing humic substances into aqueous sodium hydroxide solution will separate the insoluble humin fraction. Subsequently, acidifying the aqueous solution down to pH 1 with concentrated hydrochloric acid will precipitate the humic acid fraction out, leaving the soluble organic fulvic acid remaining in solution. Humic substances are materials that have a large molecular weight, significant surface charge, and high specific surface area; characteristics which make them a very vital soil constituent for the adsorption and retention of potentially toxic metals and organic pollutants (Sposito, 1989; Yong et al., 1992; Yong, 2001).

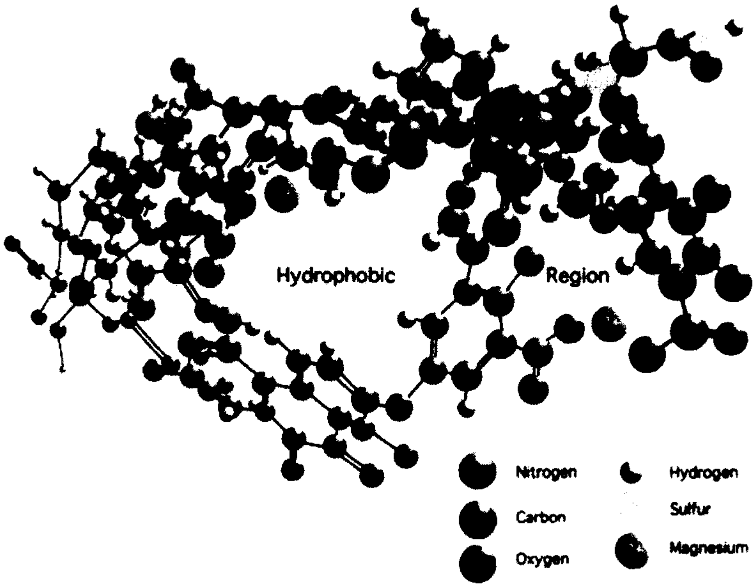


Figure 2. 7: Molecular Structure of Humic Material

2.4.2 Soil Surface Electric Charge

Most clay particles possess a negative electric charge on their surfaces. This electric charge originates from the combination of two distinct sources; fixed charges and variable charges. Fixed charges refer to charges arising from isomorphous substitutions; where one ion substitutes for the other in the clay lattice during the crystallization of the mineral. The difference in the balance between the positive valances of the substituting and substituted ions controls the resulting negative charge on the clay lattice. The main substitutions found are aluminum for silicon in the silica sheet, and ions such as magnesium, iron or lithium substituting for aluminum in the alumina sheet. This fixed charge is considered to account for most of the negative charge in minerals, except for kaolinite, where isomorphous substitutions within the crystal lattice are relatively small, limiting the magnitude of its effective surface charge (Olphen, 1977; Yong et al., 1992;

Yong, 2001). Variable charges, on the other hand, are the major contributor of surface electric charges of a variety of soil constituents including oxide and hydrous oxide minerals, a large number of non-crystalline inorganics and soil organics. These variable charges are developed mainly due to imperfections and distortions at the surface, especially the broken bonds at the edges. Soil constituents possessing variable charge surfaces are highly dependent on the ambient pH. Positive and negative charges could be developed on the surface according to the concentration of hydrogen ions of the aqueous environment (Olphen, 1977; Yong et al., 1992; Yong, 2001). As a result, the sum of all positive and negative charge contributions will regulate the resulting net electric charge on the particle surface. Consequently, while aqueous pH has a significant role in defining the resulting sign of the surface electric charge, clay particles could be left with net positive surface charge at one point and with net negative charge at another point. The switchover between these two states occurs at a point where all positive and negative charges balance each other, and the surface charge at this point is independent of the solution electrolyte concentration. The point of zero charge, at which the transition between positive and negative surface charges occur is usually referred to as the isoelectric point. The identification of this point for different soil constituents is of great importance in predicting their interaction with ions of water and other polar liquids, metals, and other inorganic and organic toxins (Mcbride, 1994; Olphen, 1977; Yong et al., 1992).

2.4.3 Soil-Contaminant Interaction

Contaminants behavior within subsurface soil media is greatly influenced by the type chemistry of the soil constituents, the contaminants and the pH of the system. The nature and activity of the functional groups, their configuration, and their physical and chemical properties are of great importance in investigating the interactions between organic chemicals and soil colloids.

2.4.3.1 Sorption

Sorption is the term used to describe the surface process through which solutes (ions, molecules and compounds) are accumulated at the interface between liquid and solid phases. In this process, dissolved solutes from leachates engage through physical and chemical interactions and become bonded to the surface of the various soil constituents. Physical sorption refers to the interaction between contaminants and soil surfaces due to electrostatic attraction as a result of the charge deficiencies of the soil constituents. Chemical adsorption or chemisorption, on the other hand, refers to high affinity, specific adsorption owing to chemical bonding forces. However, it is not always easy to distinguish between these interaction-mechanisms while studying contaminant sorption within soil media. Different mechanisms are responsible for the resulting interaction between contaminants and soil surfaces, some of which include: ionic forces between polar molecules and charged soil constituents, van der Waals forces which are the most common bonding mechanism for non-polar molecules, hydrogen bonding that may dominate the bonding type between soil organic matter and soil particles, and strong chemical bonding resulting from the valence forces between ions that penetrate the coordination shell of the structural atoms and structural cations through oxygen and hydroxide groups. The extent of adsorption is influenced by the availability of adsorption sites, which is highly dependent on the surface area or porosity of the adsorbent. Since a single site cannot accommodate two or more different adsorbates, the adsorption process is essentially competitive. Moreover, different adsorption sites possess different affinity and adsorption energies due to the fact that the surfaces of most soils are heterogeneous. This heterogeneity is principally responsible of the nonlinearity of adsorption isotherms (Chiou, 2002; Yong et al., 1992; Yong, 2001). Huang (1996) showed that a microscopic surface roughness could affect the sorption of a small N_2.molecules. "Sorption on the interlayer surface of bentonite exhibits diffusion controlled behavior".

2.4.3.2 Sorption Isotherms

Adsorption is usually described by constructing its respective sorption isotherm. Sorption isotherms are normally expressed graphically by drawing the concentration of the contaminant adsorbed to the soil phase against the equilibrium concentration of the contaminant in the aqueous phase of the soil solution. Different models are available in the literature to define sorption isotherms (Chiou, 2002; Essington, 2004; Sposito, 1989; Yong et al., 1992; Yong, 2001). The Langmuir-type isotherm, Equation (2.1), assumes adsorption to reach a limit (sorption maxima) based on the hypotheses that the surface of the adsorbent is uniform; implying that all adsorption sites have equal energies, and adsorption only occurs in a monolayer, i.e. only the free surface of the adsorbent:

$$C_s = \frac{abC_e}{1 + bC_e} \tag{2.1}$$

where C_s is the concentration of adsorbed contaminants, C_e is the equilibrium concentration of contaminants in the aqueous phase, and a, b are rate coefficients determined empirically from experimental data. Based on the characteristics of Langmuir model, a is defined as the adsorption maxima having the same units as C_s. Moreover, the adsorption constant b is an indicator of the intensity of the adsorption isotherm. The product of the adsorption maxima times the adsorption constant is a measure of the slope of the adsorption isotherm as the equilibrium concentration, C_e, approaches zero.

Unlike the Langmuir model, the Freundlich isotherm takes into account the heterogeneity of the available sites and their relative adsorption energies. Furthermore, this model does not assume limited sorption, i.e. reaching adsorption maxima. The relationship describing Freundlich-type isotherm is given in Equation (2.2):

$$C_s = K_f * C_e^n \tag{2.2}$$

where C_s and C_e are again the concentration of adsorbed contaminants and the equilibrium concentration of contaminants in the aqueous phase, respectively. The model parameter K_f is the Freundlich sorption coefficient, while n is the Freundlich sorption constant. Both Freundlich isotherm parameters are determined empirically and are functions of soil material. The parameter n is indicative of the response of the adsorption capacity of the soil to increased loadings of chemical pollutants. For example, when n is less than 1, it indicates that increasing pollutant loadings will successively decrease the adsorbed amount of the pollutant on the solid phase. In other words, this is suggestive of the assumption that available mechanisms for sorption are being exhausted. However, in the case that n equals 1, linearity is obtained, and the Freundlich isotherm is reduced to the linear isotherm, Equation (2.3):

$$C_s = k_d * C_e \qquad (2.3)$$

where k_d is defined as the equilibrium distribution coefficient. In this special case, one concludes that the sorbed fraction of the chemical pollutant is a constant proportion of the available pollutant.

3.0 OBJECTIVES

The aim of this research is to achieve a deeper insight in the partitioning of synthetic steroidal estrogenic compounds (17α-ethynylestradiol) in the soil media. In an attempt to achieve this objective, the associated objectives are formulated as follows:

(1) Evaluation of the correlation between representative soil particles surface and the maximum achievable partitioning of the synthetic estrogen.

(2) Assessment of the influence of soil particles' surface-charge changes on the equilibrium partitioning of the synthetic estrogen in the subsurface.

(3) Assessment of the synthetic estrogen equilibrium partitioning in conditions simulating aerated topsoil and deep ground level with oxygen deficiency.

(4) Determination of the potential mobility of the synthetic estrogen leaching from biosolids or manure applications.

(5) Development of an analytical procedure that attains maximum extraction and detection efficiencies of the low concentration synthetic estrogenic compound.

(6) Development of an empirical model that predicts the synthetic estrogen partitioning in soil media under variable environmental conditions.

4.0 EXPERIMENTAL WORK

This section introduces the experimental approach, chemicals and materials used in this research, and the methodology employed to achieve the research objectives.

4.1 Experimental Approach

The focus of this research was to gain a greater insight into the distribution and behavior of synthetic steroidal estrogens within subsurface soil media. Understanding the partitioning of these synthetic hormones between liquid and solid phases is crucial for the prediction and assessment of their behavior and fates in environmental systems. For this matter, the synthetic hormone 17α-ethynylestradiol (EE2) was elected as a representative steroidal estrogenic compound. As discussed in Chapter 2, 17α-ethynylestradiol is a widely used pharmaceutical that constitutes the major ingredient of hormone replacement therapy and oral contraceptive formulations. This synthetic estrogen was found to be more potent than natural estrogens (Mansell, and Drewes, 2004; Snyder et al., 2001; Thorpe et al., 2003) and it was also found to be highly persistent in the environment (Jürgens et al., 2002; Layton et al., 2000; Ternes et al., 1999b). EE2 has been detected in the nanogram per liter range in discharged domestic effluents, rivers, drinking, surface and ground waters (Baronti et al., 2000; Belfroid et al., 1999; Desbrow et al., 1998; Huang and Sedlak, 2001; Laganá et al., 2004; Routledge et al., 1998; Snyder et al., 1999; Ternes et el., 1999; Williams et al., 2003; Zuehlke et al., 2004). At these low concentrations, it was evident that EE2 may exert adverse effects, especially on animals and wildlife (Majewski et al., 2002; Palace et al., 2002; Parrott and Wood, 2002; Thorpe et al., 2003). As a result of its substantial potency and estrogenic activity, 17α-ethynylestradiol (EE2) has been chosen as the probe compound in this study.

EE2 is a hydrophobic compound possessing low aqueous solubility and high K_{ow}. Based on its physicochemical properties (Table 2.1) the synthetic steroid hormone is hypothesized to be principally associated with the solid phase. To study the behavior of EE2 in subsurface soil it was essential to investigate its interaction with individual soil constituents. As it was defined in Chapter 2, soil components that might have a great role in controlling the extent of interaction with EE2 include: soil clay minerals, oxides and hydrous oxides, and soil organics. The most common representative members of each of these soil components were selected to evaluate the correlation between soil particles surface area and the maximum achievable rates of the synthetic estrogen partitioning. Kaolinite was selected to represent the group of clay minerals that have 1:1 (one silica layer to one alumina layer) structure. Illite, having 2:1 mineral structure, was used as a representative of the family of mica-like clay minerals. Furthermore, montmorillonite which belongs to the smectite-vermiculite group also has 2:1 mineral structure. This clay mineral was chosen to represent the group of hydrous aluminum silicates, which possess the characteristics of swelling clays. Goethite was selected as the principal constituent of the hydrous-oxide mineral fraction of soils. Humic substances were considered to represent soil organic material, for this purpose, humic acid was chosen as one of the target sorbents in this research. Additionally, interaction of EE2 with a sample of natural soil was also investigated.

Different mechanisms could be responsible for sorption of the synthetic estrogen to soil minerals and soil organic matter. Non-polar 17α-ethynylestradiol is expected to primarily interact via hydrophobic attraction, hydrogen bonding, or by ion exchange. Most soil surfaces possess electrical charges, part of which is directly influenced by the chemistry (especially pH) of the pore fluid. At low pH, there is an excess of hydrogen ions in pore fluid, which are sorbed on soil surfaces yielding a net positive charge. Conversely, at alkaline range, the rising concentration of hydroxyl in pore fluid will drive soil particles to release their protons increasing the negative charge on these surfaces. As such, EE2 interaction with these surfaces is expected to be affected by changes in the resulting charges, as a function of aqueous pH. The affinity of EE2 partitioning and hence its mobility might be significantly controlled by such changes.

Moreover, environmental conditions are also likely to change with depth of the subsurface soil. Topsoil is typically aerated and oxygen is abundant in this region. However, with depth oxygen molecules are depleted and deep ground levels are characterized by their oxygen deficiency status. The availability of oxygen molecules within soil media might have an influence on the mobility of EE2 in subsurface soils.

One of the major introduction sources of EE2 into the environment is by leaching from biosolids, sludge, and recycled water used in agriculture. The hydrophobic estrogen is expected to readily interact with dissolved organics integrated within biosolids, which facilitate its partitioning into aqueous phase and significantly increase its solubility. Thus, the application of such material with the chemistry of their complex matrix is anticipated to greatly influence the affinity and mobility of EE2 in the subsurface. Accordingly, sorption experiments simulating characteristic environmental conditions in isolated systems were designed to demonstrate their impact on the distribution and behavior of the synthetic estrogen. Two case studies were investigated, in which the effect of the application of wastewater biosolids on the sorption of EE2 on different soil components and natural soil samples was considered. The studied samples represented thickened activated sludge, generated during the metabolism of microorganisms in secondary treatment process.

In summary, it is hypothesized that the non-polar, hydrophobic, synthetic steroidal estrogenic hormone (EE2) will be primarily associated with the solid phase once it is released into the environment. The extent of its interaction with different soil constituents is anticipated to vary as these constituents have different structures, functional groups, and surface properties. Moreover, the degree of interaction of this compound with individual soil constituents was speculated to be influenced by the electrical charges on their surfaces. Most of these charges are pH dependent. Clayey soils are typically negatively charged at high pH. This negative charge is reduced as pH values are decreased till there is no more charge on the surface (point of zero charge) at the pH of the isoelectric point. If pH further decreased below that point, the sign of the surface charge would give positively charged soil particles. Consequently, it is hypothesized that the extent of EE2 adsorption, and hence, it mobility in the subsurface to be directly affected by changes in environment pH.

In an attempt to evaluate these aspects and potential response to changes in environmental conditions, the experimental approach was designed to consist of two phases. The first phase, investigating partitioning phenomena of EE2 to soil components, consisted of five stages. In these stages, the mass balance was evaluated based on measuring EE2 equilibrium concentration in the aqueous phase assuming the remaining EE2 concentration to be associated with the solid phase. The second phase, investigating changes taking place on soil components and within EE2 in various environmental conditions, consisted of two stages.

Figure 4.1 shows a schematic approach of the five-stage plan of experiments conducted in Phase 1. The first stage of the study investigated sorption kinetics and equilibration times required for the partitioning of EE2 into individual soil components. The second stage illustrated the effect of hormone low concentrations on the extent of partitioning, and sorption isotherms were modeled accordingly. Stage 3 studied the effect of pH on the behavior of partitioning of EE2 into individual soil components. Anaerobic conditions prevailed in the set of experiments of stage 4. Finally, stage 5 demonstrated two case studies, which examined the potential effect of the application of leachate from biosolids on the partitioning of EE2 in soil, in addition to the effect of the presence of the organic materials integrated within these matrices, under variable environmental conditions.

The first stage of Phase 2 assessed potential changes taking place within clay and oxides samples when EE2 is sorbed under variable pH. The second stage of investigation permitted to assess the range of changes within non-adsorbed EE2 under variable pH.

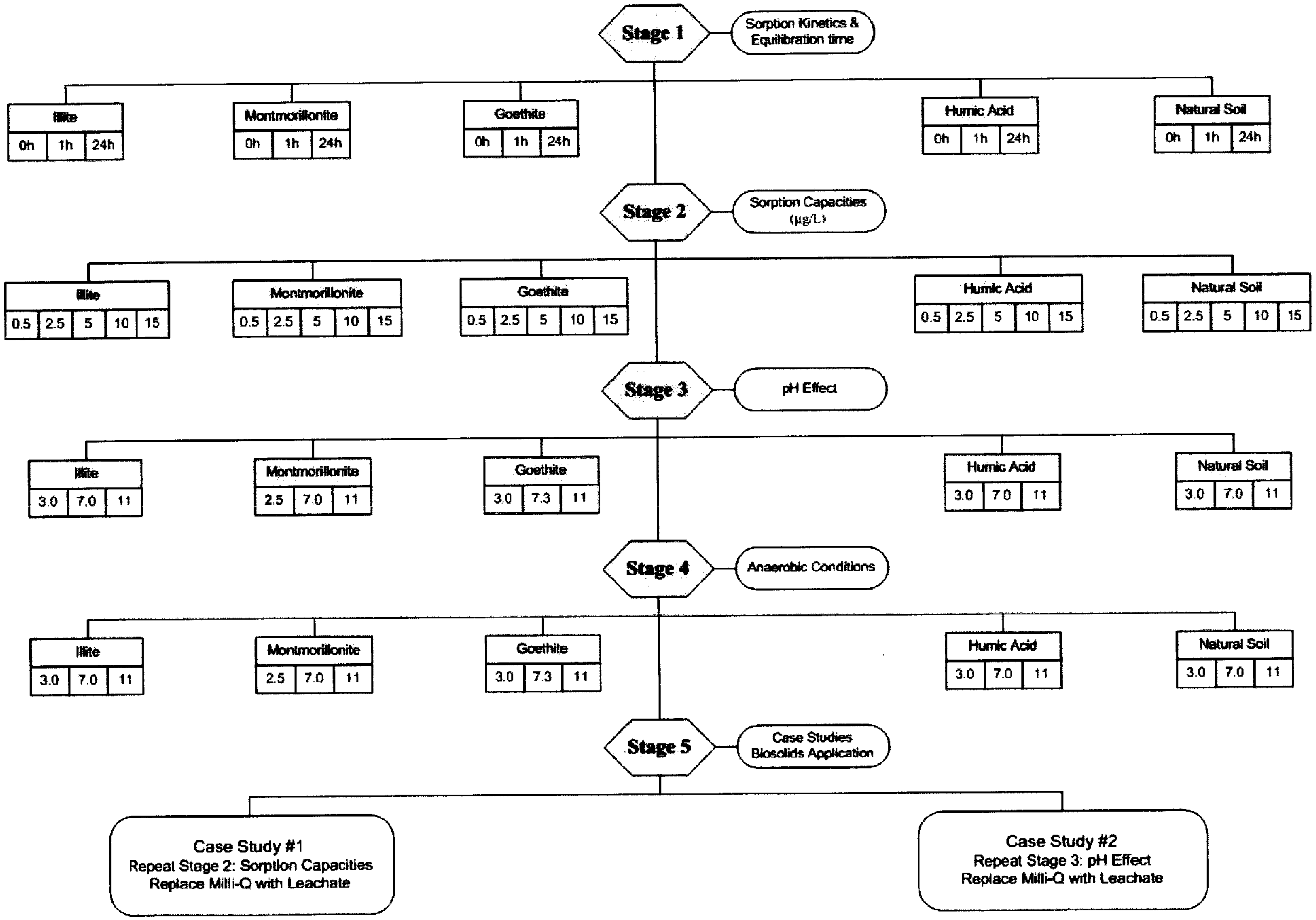


Figure 4. 1: Schematic Approach of Experimental Work

4.2 Chemicals and Materials

17α-Ethynylestradiol ≥98% purity was obtained from Sigma-Aldrich, Oakville, ON. Internal standard (D4), 17a-Ethynylestradiol-2,4,16,16-d4, was purchased from CDN Isotopes, Point-Claire, QC. HPLC-grade water, methanol, and acetonitrile (Fisher Scientific, Ottawa, ON), and dichloromethane (Sigma-Aldrich, Oakville, ON) were all analytical reagents of greater that 99% purity. Stock solutions of both EE2 and the internal standard were prepared in methanol, and were kept under refrigerated conditions (-16 °C) when not in use. Dilutions and added volumes were carefully selected to mimic environmental concentrations while ensuring a range of aqueous concentrations above the detection limit, and minimizing the volume fraction of methanol in experiments to avoid its interference with partitioning processes or during chromatographic analysis. Most of the target sorbents were obtained from Sigma-Aldrich, Oakville, ON. Some of the properties of these sorbents are listed in Table 4.1 and Table 4.2. Finally, the sludge material was collected from the Ottawa wastewater treatment plant, Ottawa, ON. Some selected characterizing parameters of this sludge are listed in Table 4.3.

Table 4. 1: Selected Properties of Soil Minerals

Sorbent	Surface Area [a,b] m^2/g	Swelling Index [c]	Isoelectric Point	CEC [a,b] cmol/kg
Kaolinite	10 - 20	0.08	4.3[d] , 4.7[e]	5 - 15
Illite	70 - 100	0.4	7.5[e]	15 - 40
Montmorillonite	600 - 800	0.9	2.5[d]	80 - 150
Goethite	50	-	7.3[d] , 9.0[e]	up to 100

[a]Brandy and Weil, 1996; [b]Mitchell, 1993; [c]Elektorowicz and Ju, 1997; [d]Appelo and Postma, 1993; [e]Essington, 2004

Table 4. 2: Characteristics of Natural Soil

Soil Mineralogy	Metal Content	Other Parameters
• Quartz 4.6% • Feldspar 21% • Dolomite 3.2% • Amphibole 6.0% • Illite 49.8% • Chlorite 14.1% • Kaolinite 1.3%	• Fe: 273 mg/kg • K: 176 mg/kg • Ca: 250 mg/kg • Ni: 31 mg/kg • Pb: 9 mg/kg	• Carbonate Content 4.5% • Organic Matter 1.3% • CEC 21meq/100g • Specific Gravity 2.75 • Sulfate 0.6ppm as SO_4^{-2} • pH 7.6

Hakimipour, 2001

Table 4. 3: Characterizing Parameters of Sludge

pH	**~ 6.61**
COD	**1938.8 (mg/L)**
Sulfate	**93736 (mg/L)**
Chloride	**229.87 (mg/L)**
Total Solids	**6.37 (%)**
Fixed Solids	**29.79 (%)**
Moisture Content	**93.63 (%)**
Volatile Solids/ Organic Matter Content	**70.21 (%)**

Huang (2006)

4.3 Analytical Techniques

Given the low concentrations (sub-μg/L to low-μg/L) to be detected, the analytical step had to be as sensitive and as selective as possible. According to some studies, this is a critical step in terms of loss of target analytes. Several recent studies have focused on identifying an analytical procedure for the analysis and detection of steroid hormones in various environmental ecosystems (Baronti et al, 2000; Cathum and Sabik, 2001; Ferguson et al., 2001; Ingrand et al., 2003; Kuch and Ballschmiter, 2001; López de Alda and Barceló, 2000; López de Alda and Barceló, 2001; Petrović et al., 2001; Snyder et al., 2003; Ternes et al., 2002; Ying et al., 2002; Zuehlke et al., 2004). However, a unique methodology has not yet been defined for such practices. It was amongst the objectives of this research to develop an analytical procedure that attains maximum extraction and detection efficiency of the synthetic estrogenic compound, 17α-ethynylestradiol, from aqueous media.

Extraction and Analysis

Unless otherwise indicated, all experimental sample tubes were centrifuged at 2700 rpm for 30 minutes, and the supernatant (30 mL) was collected and extracted by solvent extraction. Precisely before commencing the extraction step, the internal standard, D4, was added to collected supernatants, with concentrations comparable to those of EE2 that are expected to prevail in the aqueous phase. All aqueous phases were liquid-liquid extracted, three times, with 3 mL dichloromethane (DCM), by shaking vigorously for 30 seconds at each extraction step. The aliquots of the organic layer were collected in glass vessels and taken to dryness using vacuum rotary dryer. After dryness, glass vessels were carefully washed, three times, with trace volumes (100 μL) of DCM, and the collected aliquots were transferred to HPLC macro vials. These vials were then dried under a stream of nitrogen gas, and the residues were finally resuspended in 50 μl solvent mixture of 40% acetonitrile, and taken for analysis.

All samples were analyzed with high performance liquid chromatography (HLPC) system (Hewlett Packard, Series 1100), which was equipped with a reversed-phase column (Luna C8 (2), 3 micron, 50 x 1 mm) from Phenomenex, Torrance, CA. The mobile phase was Milli-Q water and acetonitrile, 2 mM NH_4OAc (ammonium acetate) pH 7.4 (adjusted with NH_4OH) at volume ratio of (60:40), delivered at a constant flow rate of 50 μL/min. The injection volume of standards and samples was 50 μL and the total run time for an HPLC analysis was 15 minutes. The wash between injected samples was done periodically as follows: 15 min with 98% acetonitrile, 2 mM NH_4OAc, and then 15 min with 40% acetonitrile, 2 mM NH_4OAc.

The synthetic estrogen, 17α-ethynylestradiol, was detected using a Micromass-Quattro II mass spectrometer (MS), with a Z-Spray electrospray source. The two ions at m/z 295.2 and 299.2 in negative ionization mode were monitored. In general, optimum signal was obtained with the ESI capillary voltage set to 3.00 kV, the cone set to 30 V, and the source temperature set to 120 °C. The drying gas (nitrogen) flow rate was 300 mL/min at 200 °C.

EE2 was quantified in sample extracts relative to its respective deuterium-labeled surrogate analogue, D4. This stable-isotope dilution quantitation method served as an automatic correction for losses of the hormone during extraction or sample preparation, as well as for variations in instrument response from injection to injection. A five-point quantitative calibration curve was constructed for the hormone, EE2 and the, D4-labeled standard across an array of concentrations, in 40% acetonitrile/water, and was found to be linear ($R^2 > 0.99$) for the range of concentrations applied in this research. Generally, all samples and measurements were done in triplicates or duplicates as stated in the following sections. Statistical analyses were performed for these measurements to find the respective means and standard deviations. Experimental errors were calculated and displayed on all graphical representations illustrated in this study.

4.4 Methodology and Experimental Protocol

This section provides a detailed description of the steps taken for sample preparation and experimental procedure performed at every stage of the study.

4.4.1 Phase 1

4.4.1.1 Stage 1: Sorption Kinetics and Equilibration Time

Objectives

The expected results from this proposed set of experiments include the assessment of the time required for the equilibration of each studied system, which would ascertain the time element required to design the remaining batch-experiments throughout the rest of this research. Additionally, kinetics of sorption of EE2 to various soil samples were demonstrated, which are likely to provide a better understanding of rates and extent of mechanisms responsible for sorption of the estrogenic hormone to individual soil constituents. Moreover, the results of this set of experiments could also assist the evaluation of the correlation between particles surface and the maximum achievable rates of partitioning of EE2 into solid phases.

Procedure

A batch-equilibration method was used to measure the sorption of 17α- ethynylestradiol by various soil constituents from aqueous solution. Samples consisting of clay particles (illite, montmorillonite), oxides (goethite), humic acid, and natural soil were weighed (3.5 g) individually in a preweighed glass centrifuge tubes (50 mL) equipped with Teflon-Silicon discs and screw-caps. Milli-Q water was added to each tube in a volume of 35 mL. Finally, all sample tubes were spiked with the hormone solution to give a final concentration in each tube of 0.5 μg/L (equivalent to 5 ng/g of dry soil). Soil mass (g) to solution volume (mL) ratio was fixed at (1:10) throughout the course of this research. Tubes were capped and mixed on a mechanical end-over-end shaker (10 rpm) at controlled temperature of 30 °C. Kinetic experiments were conducted by measuring EE2 concentration periodically for 24 hours starting immediately after the addition of the hormone. At each sampling time, a sample tube, for each soil constituent, was sacrificed and taken for analysis. Sample tubes were centrifuged and the supernatant was collected for extraction and analysis. Samples were sacrificed for analysis at times 0 h, 1 h, and 24 h. All samples and measurements were done in triplicates. Experiments included analysis of EE2 in the solution without the addition of soil samples. These samples served as controls to check against the compound loss by volatility or sorption to laboratory equipment.

4.4.1.2 Stage 2: Assessment of Sorption Capacities

Objectives

This group of experiments was designed to assess soil constituents' sorption capacities and the response of EE2 partitioning to changes in the initial hormone concentration, while all other parameters (pH, temperature, time, etc.) remain constant. The results of the experiments are expected to give an indication for the acceptable range of concentrations to be applicable for the remaining experimental stages. Solid EE2 concentrations were plotted against aqueous EE2 concentration, and the data were fitted with different sorption isotherms to find the model that best represents the obtained results.

Procedure

A series of batch-equilibrium tests were performed, in which samples consisting of clay particles (illite, montmorillonite), oxides (goethite), humic acid, and natural soil were weighed (3.5 g) individually in glass tubes (50 mL), and an aqueous solution was added to each tube in a volume of 35 mL. The pHs of the suspensions were measured before commencing the test to indicate the solution chemistry of individual suspensions. These pH values were 8.5, 7.5, 8.0, 5.7, and 8.2 for illite, montmorillonite, goethite, humic acid, and natural soil, respectively. The hormone solution, with a proper dilution, was added to give the desired final concentration in each tube. Five concentrations were used in the sorption tests: 0.5, 2.5, 5, 10, and 15 μg/L (equivalent to: 5, 25, 50, 100, and 150 ng/g of dry soil). This challenging low range of concentrations was designated for this study to virtually simulate environmental levels of EE2. The lowest concentration was carefully conceived to be as low as possible while insuring that the aqueous concentration to be measured at the end of the partitioning test will not jeopardize the detection limit of the analytical technique (section 4.3). Test tubes were then capped and mixed on an end-over-end shaker at a preset temperature of 30 °C, for one hour, at which time, enough sorption (> 90%) has taken place, as was confirmed in Stage 1 of this research. At the end of the experiment, sample tubes were centrifuged, and the supernatant was collected, extracted and analyzed for EE2 concentration as described in section 4.3. All experiments were run in triplicates.

4.4.1.3 Stage 3: pH Effect

Objectives

The results of this stage of the study are expected to evaluate the influence of changes in soil particles' surface charges (as a result of changes in soil-suspension pH over the range between acidic and alkaline conditions) on the partitioning of the synthetic estrogen in the subsurface. Furthermore, the results of these experiments are expected to improve the understanding of rates and extent of mechanisms responsible for sorption of the estrogenic hormone to individual soil constituents.

Procedure

A series of batch-equilibration experiments were performed, in a similar manner to Stage 1 defined in section 4.4.1.1, with a number of modifications, to assess the effect of variation of suspension pH on the partitioning of EE2 between aqueous and solid phases. Samples consisting of clay particles (illite, montmorillonite), oxides (goethite), humic acid, and natural soil were weighed (3.5 g) individually in glass tubes (50 mL), and Milli-Q water was added to each tube in a volume of 35 mL. The pH of the suspension was adjusted to the required value by the addition of acetic acid (AcOH) or sodium hydroxide (NaOH), and the suspension was pre-equilibrated for 1 h. After equilibration, the hormone solution, with a proper dilution, was added to give a final concentration in each tube of 10 μg/L (equivalent to 100 ng/g of dry soil). Tubes were capped and mixed on an end-over-end shaker at controlled temperature, 30°C, for one hour. At the end of the experiment, sample tubes were centrifuged, and the supernatant was collected, extracted and analyzed for EE2 concentration as described in section 4.3. All experiments were run in duplicates. Additionally, control samples were analyzed under the same conditions of each investigated soil sample, with the exception that no soil mass was added to the controls.

For each soil sample, sorption of EE2 was studied under three suspension pH values, carefully chosen to reflect the effect of variation of aqueous chemistry and soil-surface charges on the rate and extent of sorption of the organic estrogen, EE2, to various soil constituents. For each soil constituent, three pH values were chosen to represent; neutral conditions (~7), acidic range (~3), and basic range (~11), respectively. The selection of the studied pH values for individual soil components was made with respect to the pH at their isoelectric points (Table 4.1), whenever possible.

4.4.1.4 Stage 4: Effect of Anaerobic Conditions

Objectives

The results of these experiments would verify whether or not the presence or absence of oxygen has an affect on the rates or mechanisms of sorption of EE2 on solid matrices, or whether any abiotic transformation of EE2 is expected to take place under these conditions. Accordingly, the potential influence of the availability or lack of oxygen on the mobility of EE2 in the subsurface matrix could be evaluated.

Procedure

This array of experiments suggested the execution of the samples illustrated in Stage 3, under anaerobic conditions. All samples were prepared under an atmosphere of CO_2-free nitrogen. Prior to spiking the samples with the hormone solution, all test tubes were purged with a stream of nitrogen gas for 30 min, to ensure the total evacuation of O_2 and its replacement with N_2. At the end of the 30 min, and while being under the nitrogen gas, hormone solutions were added to test tubes, with proper dilutions, to achieve the intended initial concentrations. Tubes were then tightly capped and shaken for the same time period as described in the Stage 3. After which, samples were centrifuged, and the supernatants were extracted and analyzed as described in section 4.3.

4.4.1.5 Stage 5: Case Studies: Effect of Biosolids Application

Objectives

This stage of the research sought to reproduce environmental conditions, in which the fate of EE2 is controlled by its distribution amongst the aqueous phase, the soil, and the applied biosolids. Consequently, these results would enhance the ability to assess the hazard of contaminating water bodies following common agricultural practices.

Procedure

The partitioning of EE2 between aqueous and solid phases was investigated in the presence of biosolids. Sludge was collected from the Wastewater Treatment Plant in Ottawa, ON, after secondary treatment and thickening stages. In lab, samples were centrifuged at 30,000g for 60 min, and the separated supernatant (leachate) was stored at 4 °C. Before commencing the tests, the slurry of sludge was extracted and analyzed to check the possibility of being already contaminated with 17α-ethynylestradiol. Two case studies were demonstrated.

4.4.1.5.1 Case 1

In the first case study, batch-equilibrium experiments were performed, as discussed in Stage 2, with illite, montmorillonite, goethite, and natural soil (representing the solid phase); however, the centrifuged leachate represented the aqueous phase. Here again, samples were prepared in the same manner as described previously (section 4.4.1.2), maintaining a soil mass (g) to solution volume (mL) ratio at (1:10). Investigated concentrations included: 2.5, 5, 10, and 15 μg/L (equivalent to: 25, 50, 100, and 150 ng/g of dry soil). Test samples were equilibrated for the required period, then, they were centrifuged and analyzed for EE2 concentrations in the extracted aqueous phase. Due to the complexity of the investigated matrix, a filtration step was required prior to any HPLC injection. After extracting the aqueous phase with DCM, the collected organic aliquots were evaporated and redissolved in the HPLC elution solvent, as discussed in section 4.3. However, an extra step was added, in which the resuspended material was filtered using 0.2 μm PTFE filters of 4.0 mm diameter. The filtered aliquots were then injected and analyzed for EE2 concentrations as described in section 4.3. It was assumed that the presence of the biosolids within the studied samples might significantly affect the partitioning pattern and sorption rate, as these substances posses elevated amounts of organic matter, microorganisms and a complex matrix of other materials. All experiments were run in duplicates.

4.4.1.5.2 Case 2

The second case study involved studying the partitioning of EE2 between solid phases and the centrifuged leachate, under variable environmental conditions, characterized by different pH conditions. Following the same approach explained in Stage 3, the last set of experiments was designed accordingly. Batch-equilibration tests were executed, with illite, montmorillonite, goethite, and natural soil representing the solid phase, and the centrifuged leachate to represent the aqueous phase. Soil samples were weighed (3.5 g) individually in preweighed centrifuge glass tubes (50 mL), and leachate was added to each tube in a volume of 35 mL. The pH of the suspension was adjusted to the required value by the addition of acetic acid (AcOH) or sodium hydroxide (NaOH), and the suspension was pre-equilibrated for 1 h. After equilibration, the hormone solution, with a proper dilution, was added to give a final concentration in each tube of 10 μg/L (equivalent to 100 ng/g of dry soil). Tubes were capped and mixed on an end-over-end shaker at controlled temperature, 30°C, for one hour. At the end of the experiment, sample tubes were centrifuged, and the supernatant was collected, extracted, filtered and analyzed for EE2 concentration as described in section 4.3.

4.4.2 Phase 2

4.4.2.1 Stage 1: FTIR Analysis

Objectives

The results of this stage of the study are expected to allow for the evaluation of changes taking place on soil components under various environmental conditions. Potential changes of soil components surfaces in response to changes in aqueous pH or as a result of interacting with EE2 were demonstrated. This evaluation would, in turn, provide a better understanding of the mechanisms responsible for sorption of the estrogenic hormone to individual soil constituents.

Procedure

Fourier Transform Infrared Spectroscopy (FTIR) was used to evaluate potential changes that might take place on soil components under various environmental conditions. Samples consisting of clay minerals (illite, montmorillonite) and oxides (goethite) were employed in a series of batch-equilibration experiments in a similar manner to Stage 3 of Phase 1, as described in section 4.4.1.3. These batch experiments were performed once with the addition of EE2 and another time without the introduction of EE2. At the end of the batch tests, solutions were centrifuged and the remaining solid phases were collected for further analysis. The collected soil samples were air dried in a fume hood for 24 – 48 hours. The dried samples were then manually ground into homogenous powders, which were taken for FTIR analysis. All analyses were performed using Nicolet Nexus 470 FTIR spectrometer equipped with smart miracle AMTIR crystal. The analyses were done for the range of wavelengths between 4000 cm^{-1} and 400 cm^{-1}.

4.4.2.2 Stage 2: UV/Vis Analyses

Objectives

This stage of the study was designed to investigate changes taking place within EE2 in various environmental conditions. The results of this stage are expected to illustrate the behavior of dissolved EE2 under different aqueous chemistry (i.e. pH). These results are, therefore, anticipated to reflect upon EE2 behavior in the aqueous phase of the batch equilibration experiments suggested in Phase 1 of this research (section 4.4.1).

Procedure

UV/VIS analysis was intended to assess the behavior of EE2 in the supernatant of batch adsorption experiments (Phase 1); however, due to the extremely low concentration of EE2, it was difficult to perform the UV/VIS analysis on these samples. Alternatively, elevated concentrations of dissolved EE2 were particularly prepared for this stage. Solutions of EE2 were prepared in distilled water to give a final concentration of 4 ppm. The pH of these solutions were adjusted to the required values (3, 7, and 11) by the addition of acetic acid (AcOH) or sodium hydroxide (NaOH) and taken for analysis.

5.0 RESULTS

This section describes the general trend of the partitioning process of 17α-ethynylestradiol (EE2), between liquid and solid phases. Overviews of each batch test, summarizing initial experimental condition, test settings, and observations are also given. A tabulated representation of the results could be found in Appendix A.

5.1 Results from Phase 1

5.1.1 Stage 1: Sorption Kinetics and Equilibration Time

Batch equilibration method was used to study sorption kinetics and equilibration time required for the partitioning of EE2 into individual soil components. The results of this stage showed that partitioning onto clay minerals was greater than onto the iron oxide and humic acid (Figure 5.1).

Figure 5.1 also shows that while sorption to goethite and humic acid reached equilibrium within the first hour of the experiment, sorption to clay minerals continued to increase slowly over the remaining 24 hours. However, in all cases more than 90% of the total sorption was achieved after one hour of shaking, which made it reasonable to adjust the length of the batch test of the following stages to one hour.

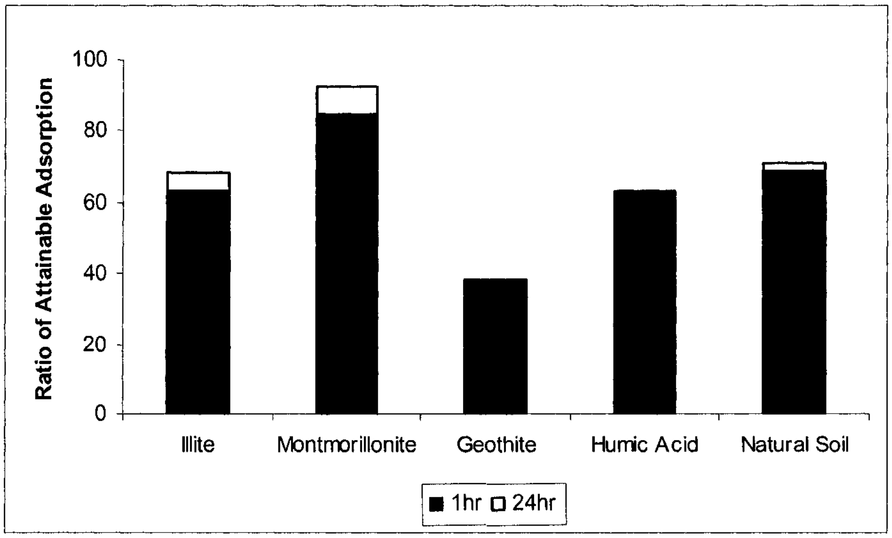


Figure 5. 1: Equilibration Time of EE2 Adsorption to Soil Constituents

Due to unavoidable analytical complexity, outputs of the HPLC-MS assay for kaolinite soil samples were inadequate for comprehensive analysis. The obtained chromatography was obscured by an unknown peak (probably organic impurities) that overlapped with the peak of interest, which made it difficult to extract quantitative results of tests performed with kaolinite throughout the intended research described in Chapter 4.

5.1.2 Stage 2: Assessment of Sorption Capacities

This group of experiments consisted of a series of batch-equilibrium tests performed to assess the response of EE2 partitioning to changes in the initial hormone concentration (0.5, 2.5, 5, 10, and 15 μg/L), while all other parameters (time of 1 hour, temperature of 30 °C, etc.) remain constant. The pHs of the suspensions were unchanged. Measured pH values were 8.5, 7.5, 8.0, 5.7, and 8.15 for illite, montmorillonite, goethite, humic acid, and natural soil, respectively. Figure 5.2 illustrates that montmorillonite exhibited highest sorption capacity, followed by natural soil, then closely by illite and humic acid (80%, 70%, 60%, and 60%, respectively). Goethite showed the lowest sorption capacity of 40%. The effect of increasing initial EE2 solution concentration on the extent of the uptake of EE2 by individual soil constituents is shown in Figure 5.3.

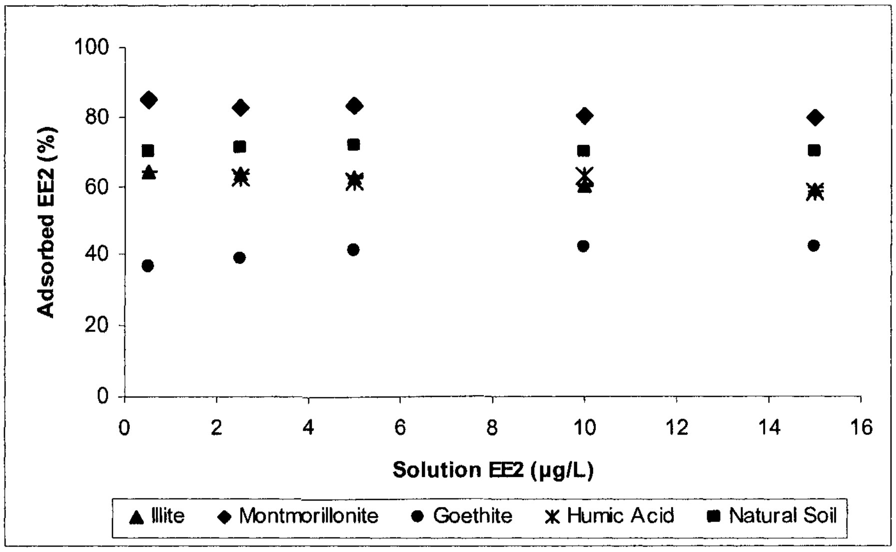


Figure 5. 2: Soil-Constituent Sorption Capacities

Sorption isotherms were constructed for each of these soils and it was generally found that diagrams of sorbed concentrations versus aqueous concentrations were best fitted on a log-log scale with $R^2 > 0.99$. Accordingly, the Freundlich model, Equation (2.2), was found to best represent the obtained results, and was followed to construct sorption isotherms of individual soil constituents.

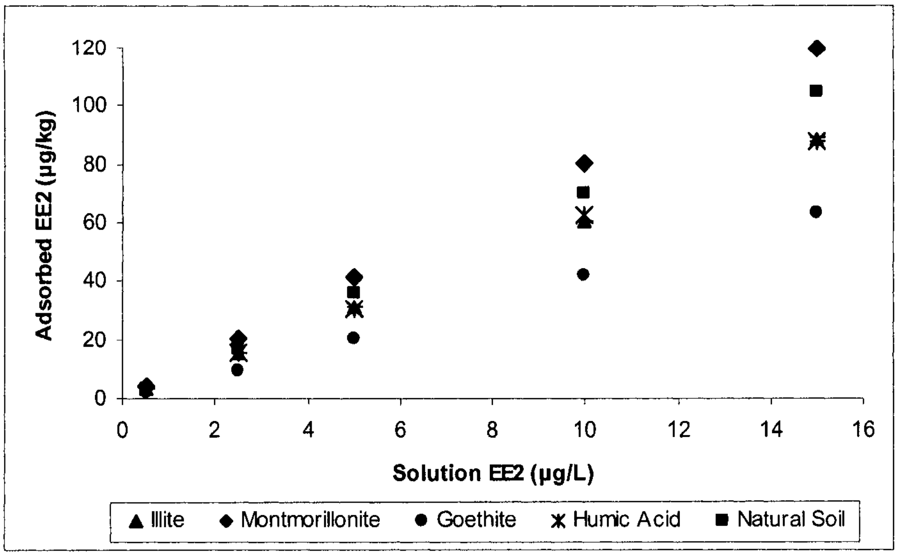


Figure 5. 3: Concentration effect on EE2 Adsorption to Solid Phase

Table 5.1 summarizes the parameters of sorption isotherm as calculated based on Freundlich model, Equation (5.1):

$$C_s = K_f * C_e^n \qquad (5.1)$$

Where,

C_s : Concentration of EE2 adsorbed by the solid phase, µg/kg.

C_e : Equilibrium solution concentration, µg/L.

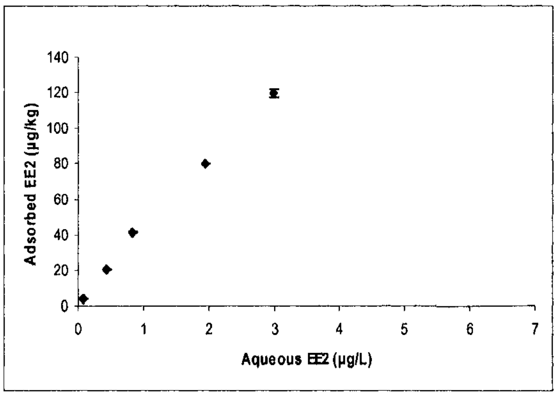
K_f : Freundlich sorption coefficient, µg/kg / (µg/L)^n.

n : Freundlich sorption constant, unitless.

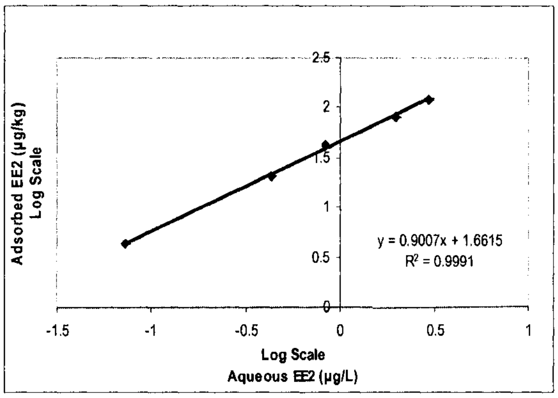
Table 5. 1: Parameters of Freundlich Isotherm

Soil Material	K_f	n
Montmorillonite	45.87	0.90
Natural Soil	24.33	0.99
Humic Acid	17.10	0.93
Illite	16.80	0.94
Goethite	6.32	1.08

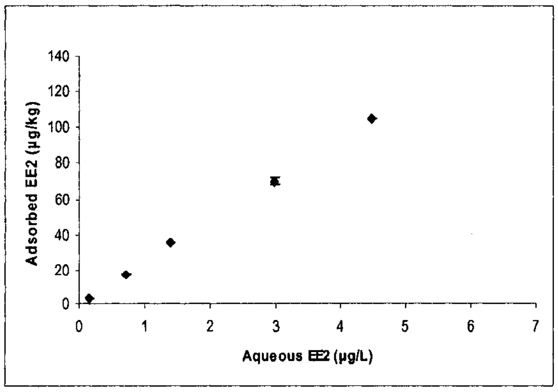
Figure 5.4 demonstrates multiple-concentration sorption isotherms for EE2 along with the Freundlich model for montmorillonite, natural soil, humic acid, illite, and goethite.



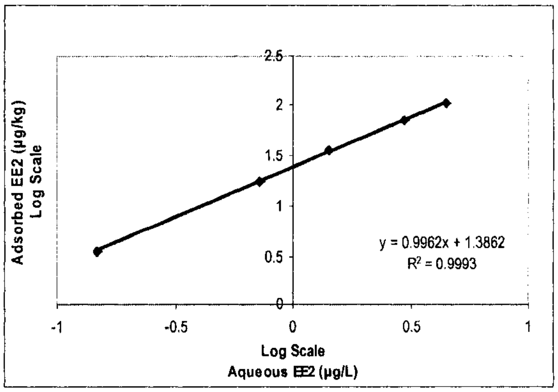
a-1) Sorption isotherm of EE2 to montmorillonite



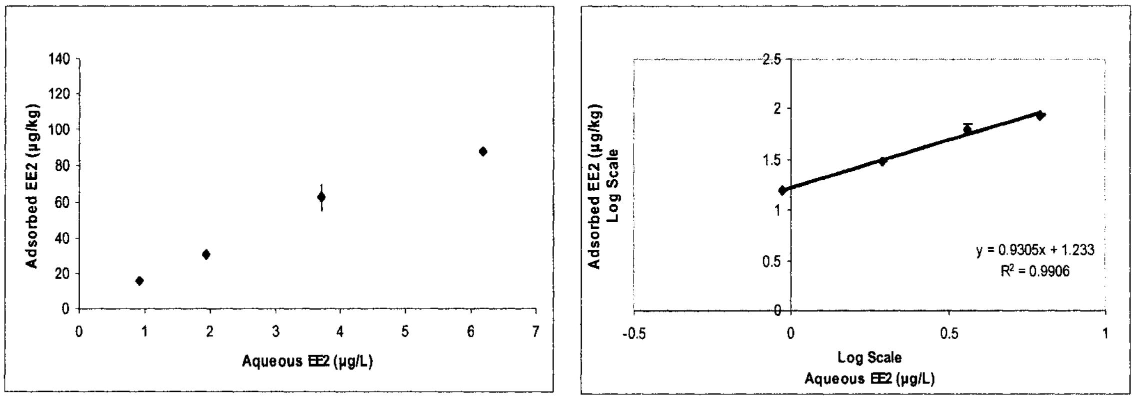
a-2) Freundlich isotherm of EE2 sorption to montmorillonite



b-1) Sorption isotherm of EE2 to natural soil

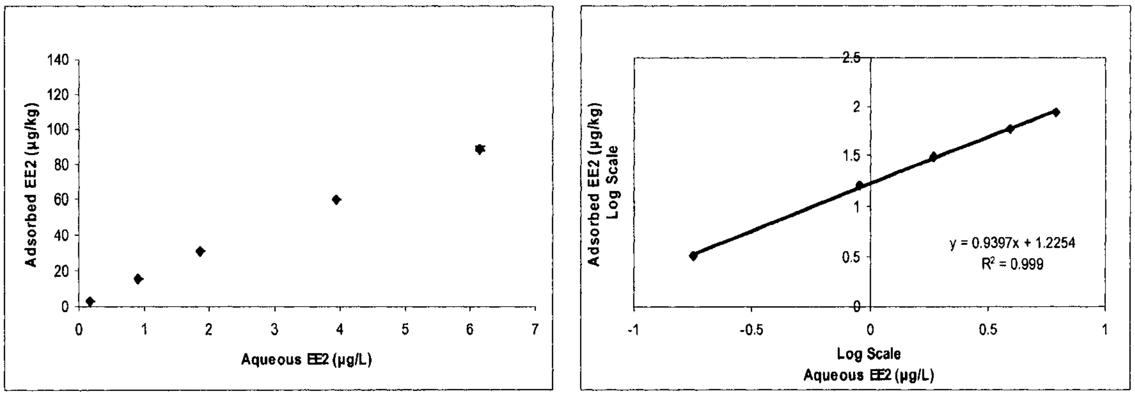


b-2) Freundlich isotherm of EE2 sorption to natural soil



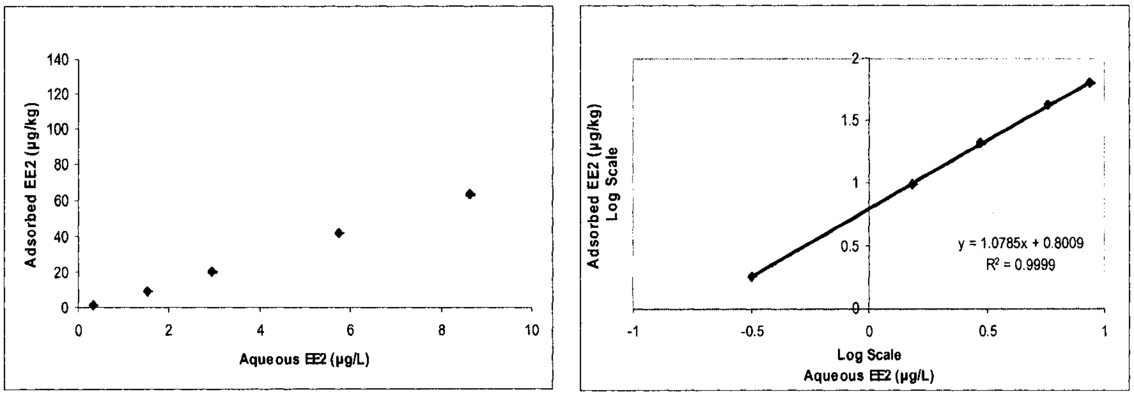
c-1) Sorption isotherm of EE2 to humic acid

c-2) Freundlich isotherm of EE2 sorption to humic acid



d-1) Sorption isotherm of EE2 to illite

d-2) Freundlich isotherm of EE2 sorption to illite



e-1) Sorption isotherm of EE2 to goethite

e-2) Freundlich isotherm of EE2 sorption to goethite

Figure 5. 4: Multiple Sorption Isotherms for Soil Constituents motmorillonite (a), natural soil (b), humic acid (c), illite (d), goethite (e)

5.1.3 Stage 3: pH Effect

The effect of pH on the behavior of the partitioning of EE2 onto individual soil components was investigated. Acidic, neutral, and alkaline solution environments were acclimated, and the partitioning of EE2 to clay minerals, iron oxide, humic acid and the natural soil was tested in a batch equilibration experiment. Results showed that while illite (Figure 5.5) displayed the highest sorption capacity (ca. 63%) around neutral pH (pH ~ 7.0), and decreased sorption as pH diverged from the neutral condition in either direction (i.e. pH ~ 4 or pH ~ 11), the clay mineral, montmorillonite, exhibited maximum sorption in the acid range (ca. 99%), and decreased steadily as solution pH departed away towards more basic state (Figure 5.6).

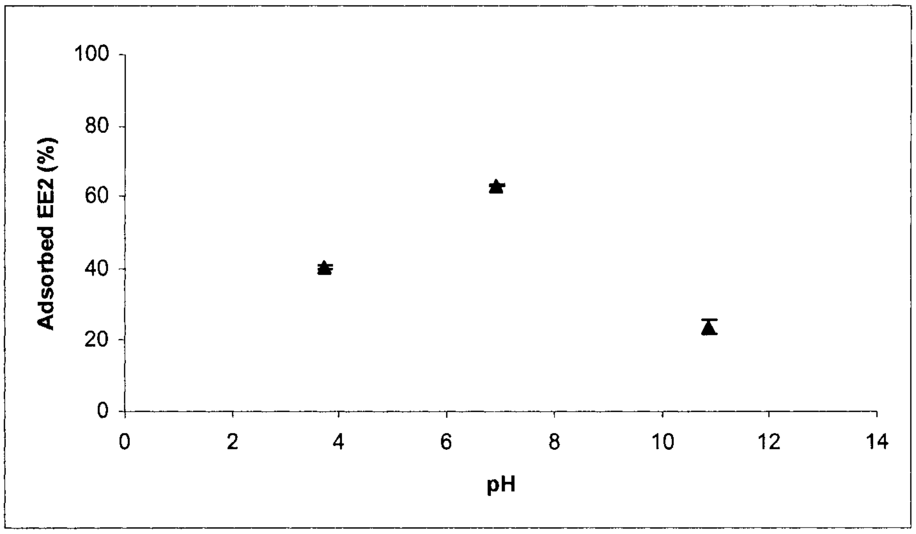


Figure 5. 5: pH effect on EE2 Adsorption to Illite

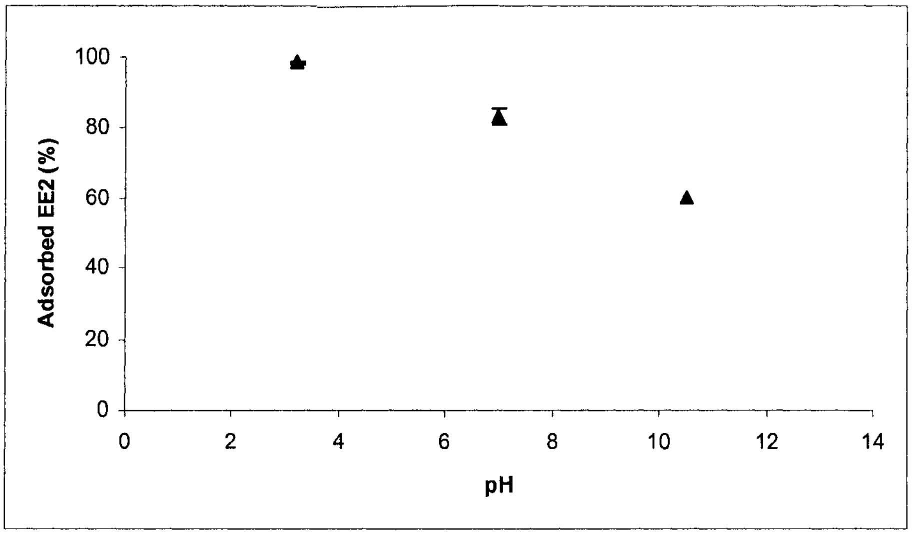


Figure 5. 6: pH effect on EE2 Adsorption to Montmorillonite

It can be seen from Figure 5.7 that the uptake of EE2 onto goethite was the greatest around neutral solution pH (ca. 52%), but declined as solution pH was changed either above or below pH 7.0.

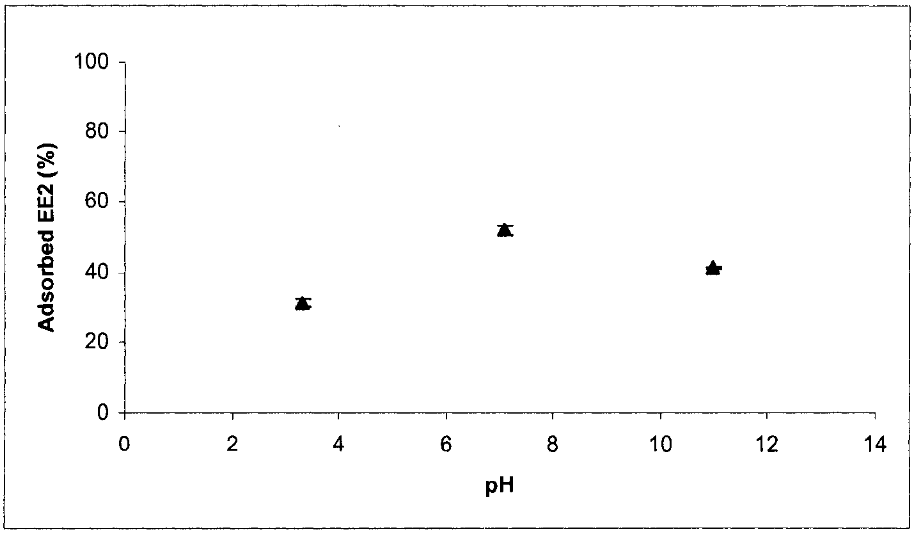


Figure 5. 7: pH effect on EE2 Adsorption to Goethite

Comparably, EE2 adsorption to natural soil samples peaked at mid-range pH (ca. 68%) with significant reduction at high pH (pH ~ 11), as shown in Figure 5.8. The natural soil sample has 50% illite by composition, which might explain the similarity between the behaviors of the two sample tests.

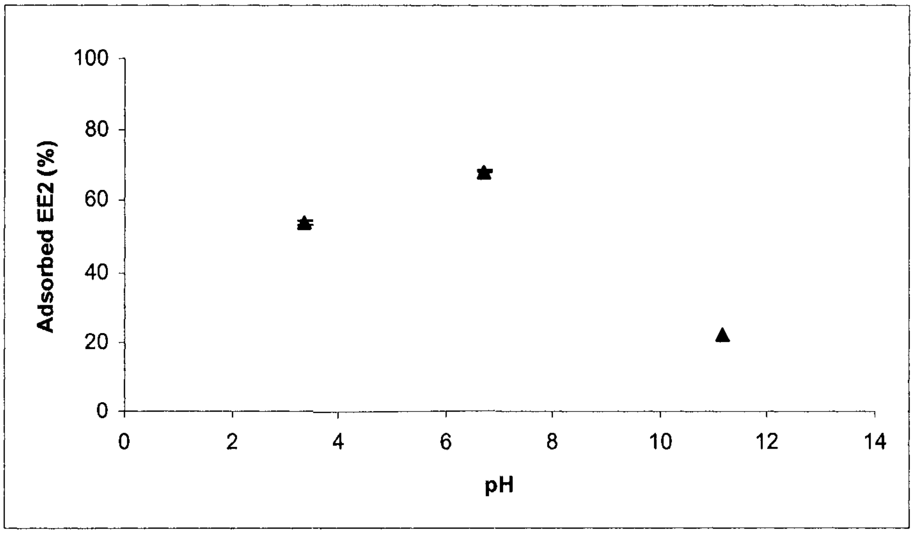


Figure 5. 8: pH effect on EE2 Adsorption to Natural Soil

Uptake by humic acid was particularly distinct from other results. There was slight change in adsorption capacities between basic and neutral solution pH. Nevertheless, a large increase (ca. 99% adsorption) was observed as solution pH was dropped below 7.0 to represent substantial acid-state environment (Figure 5.9).

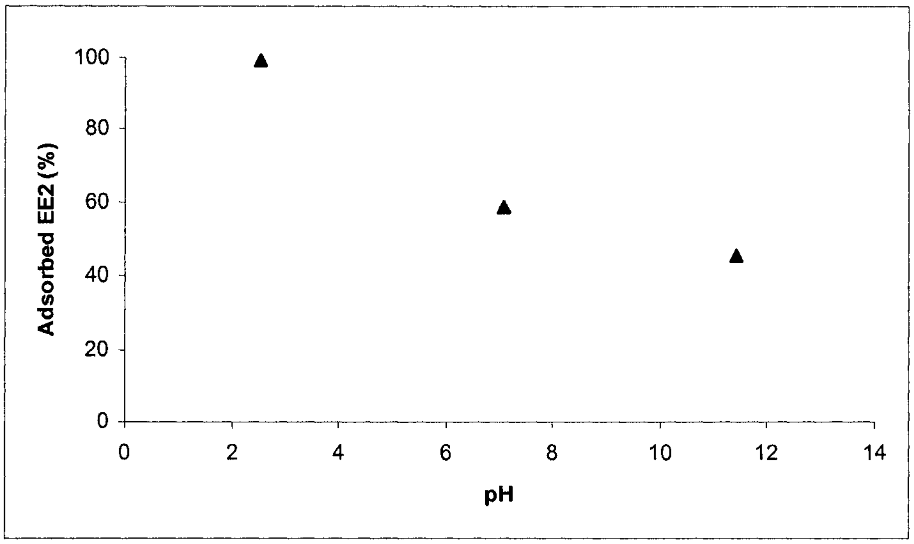


Figure 5. 9: pH effect on EE2 Adsorption to Humic Acid

5.1.4 Stage 4: Effect of Anaerobic Conditions

The series of experiments of the previous stage was re-conducted after a principal modification on the experimental protocol, which insured maintaining anaerobic conditions throughout samples preparation and testing. The prevailed anaerobic environment was investigated to assess the potential effect of the presence versus the absence of oxygen on the uptake of EE2. The outcome of tests done under anaerobic conditions did not differ much from what was previously observed with the presence of oxygen. This trend was witnessed for illite, montmorillonite, humic acid and natural soil, as can be seen in Figure 5.10, Figure 5.11, Figure 5.12, and Figure 5.13, respectively. The observed little variations might reflect the different rughness of porous and non-porous media that can better accommodate N_2 than EE2. In other words, the reason could be the competition with N_2 for available adsorption places.

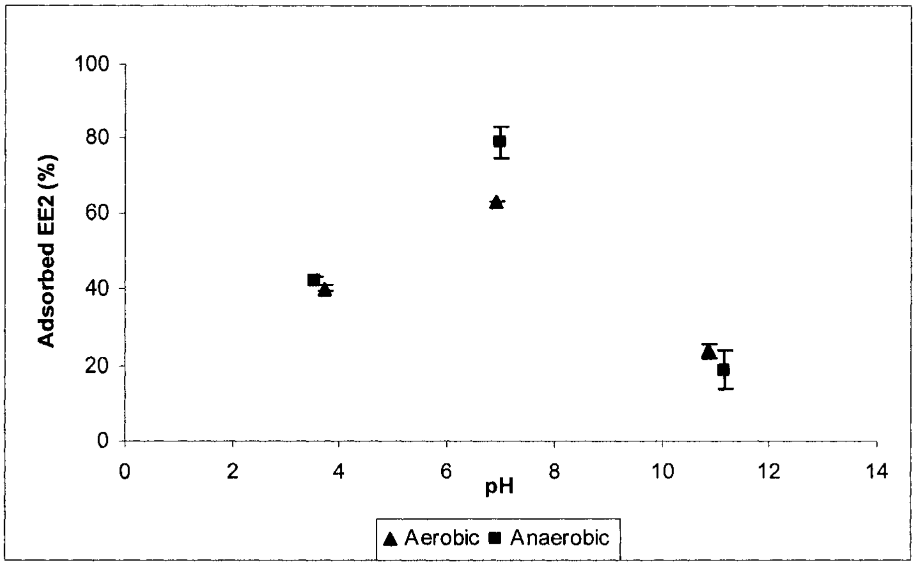


Figure 5. 10: pH effect on EE2 Adsorption to Illite, Aerobic vs. Anaerobic

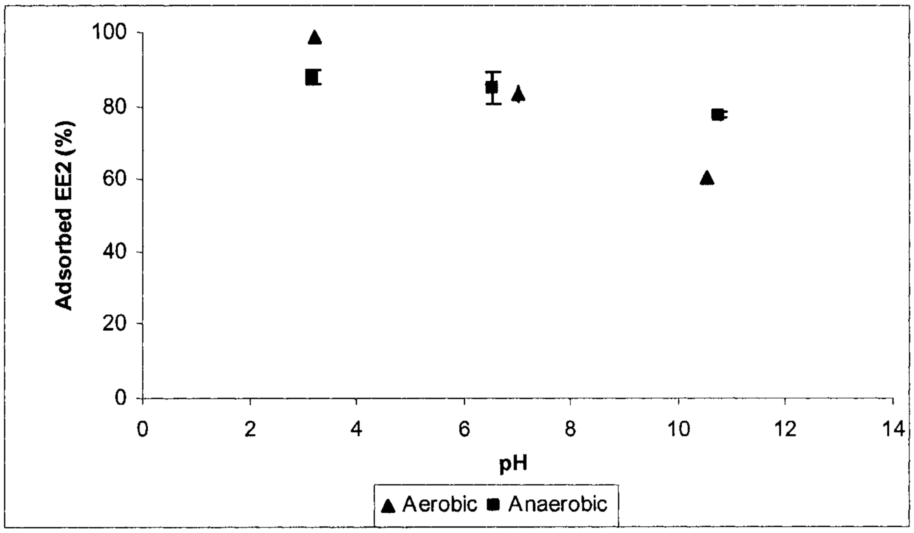


Figure 5. 11: pH effect on EE2 Adsorption to Montmorillonite, Aerobic vs. Anaerobic

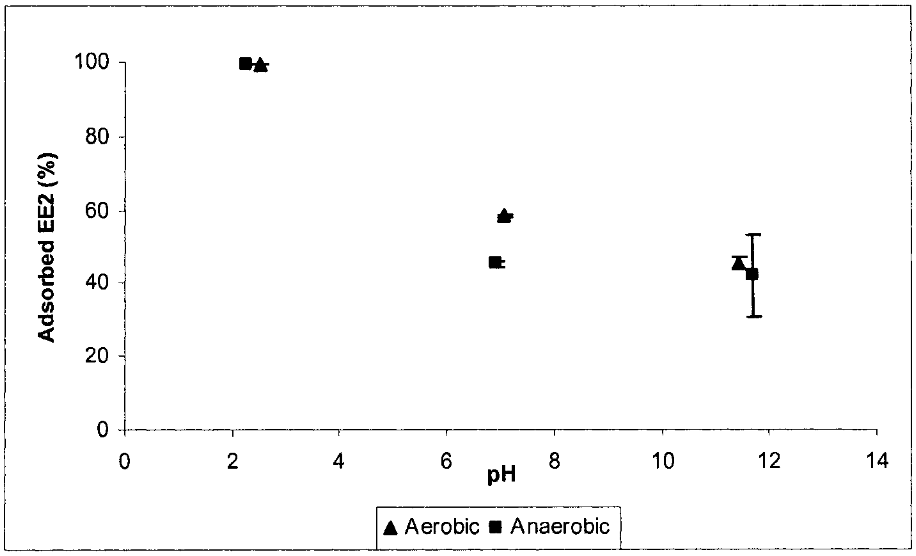


Figure 5. 12: pH effect on EE2 Adsorption to Humic Acid, Aerobic vs. Anaerobic

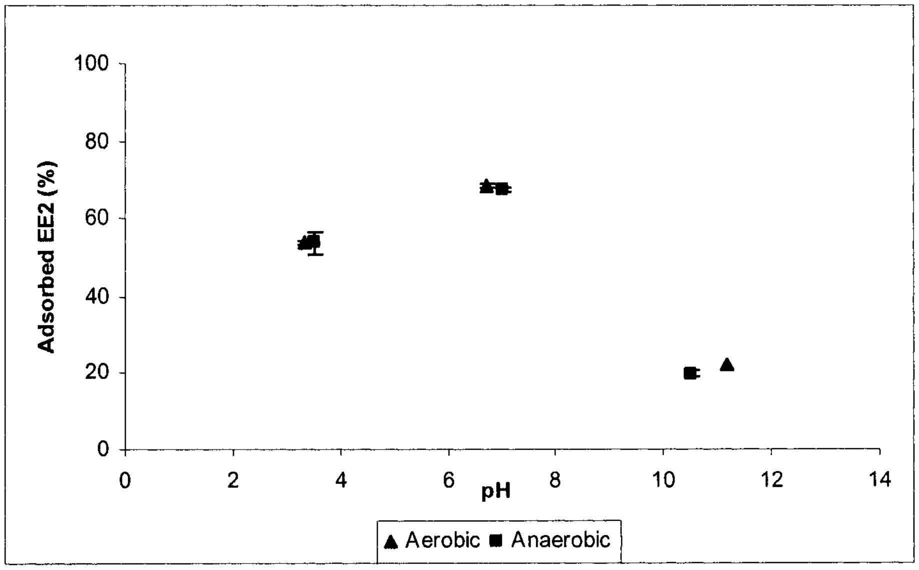


Figure 5. 13: pH effect on EE2 Adsorption to Natural Soil, Aerobic vs. Anaerobic

Conversely, goethite demonstrated a considerably distinctive behavior as shown in Figure 5.14. A drop in adsorption capacity was observed under anaerobic and basic environment, and huge increase was noticed when tests were performed under anaerobic and acidic conditions, in relative to values previously achieved in the presence of oxygen.

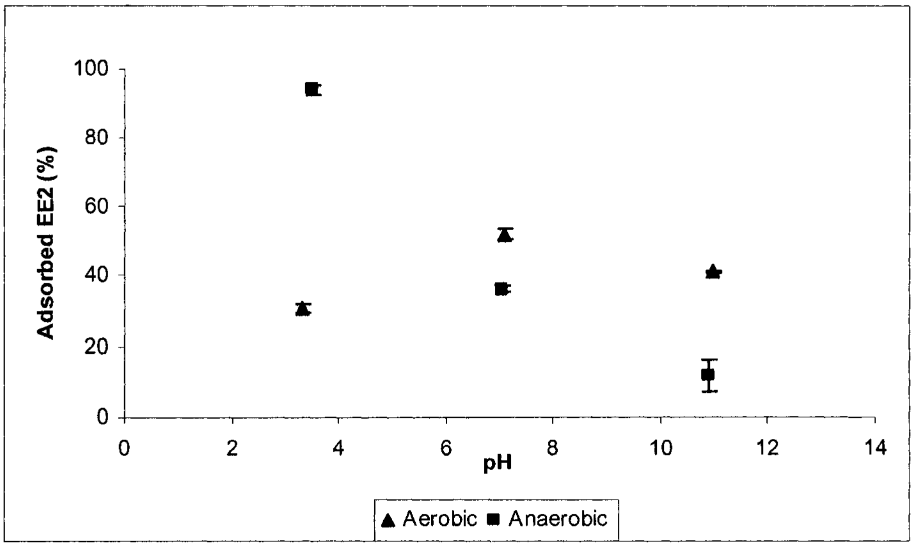


Figure 5. 14: pH effect on EE2 Adsorption to Goethite, Aerobic vs. Anaerobic

5.1.5 Stage 5: Case Studies: Effect of Biosolids Application

In this stage, two case studies were investigated to assess the effect of biosolids application on the partitioning behavior of the synthetic estrogen, EE2, between liquid and solid phases. The first case study focused on studying the response of the portioning process to changes in initial hormone concentrations. The second case study investigated the effect of changing environmental conditions, i.e. pH, on EE2 partitioning. The results of these two case studies are presented in the following sections.

5.1.5.1 Case Study 1: Effect of Concentration

In comparison with results previously found in Stage 2, where Milli-Q water was the representing liquid phase, it could be noticed that sorbed EE2 values were slightly lower when batch tests were done with leachate from biosolids. This slight decrease was in the range of 5% for illite (Figure 5.15) to 10% for natural soil (Figure 5.16) tests. However, a more pronounced decline was observed for goethite, where up to 30% less EE2 sorbed to the iron oxide (Figure 5.17).

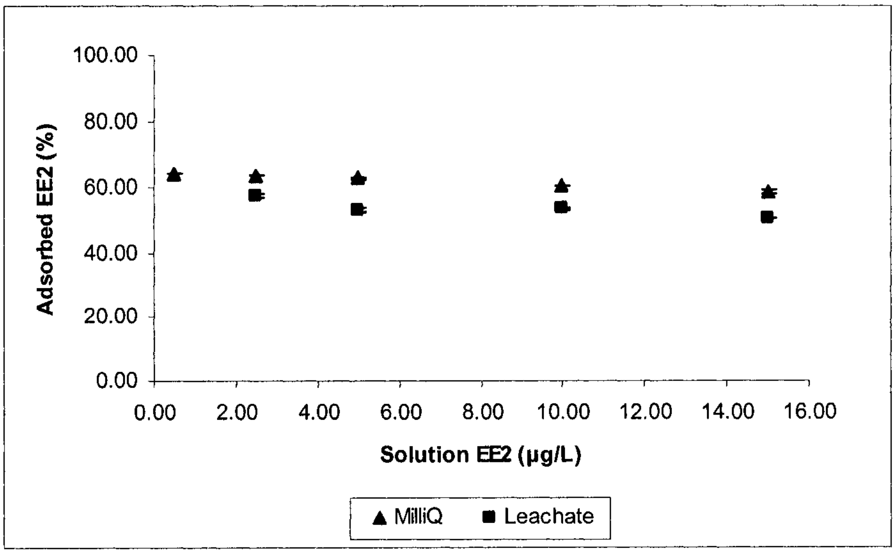


Figure 5. 15: Concentration effect on EE2 Adsorption to Illite, Milli-Q vs. Leachate from Biosolids

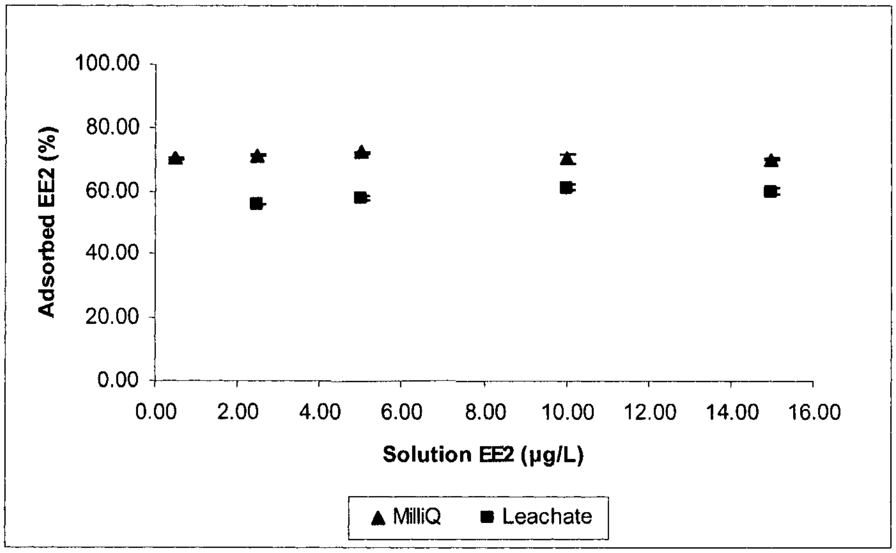


Figure 5. 16: Concentration effect on EE2 Adsorption to Natural Soil, Milli-Q vs. Leachate from Biosolids

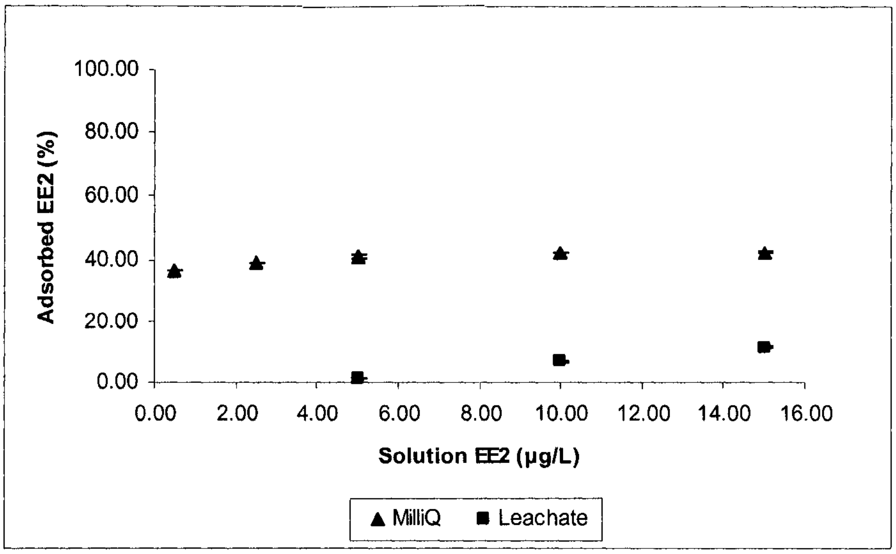


Figure 5. 17: Concentration effect on EE2 Adsorption to Goethite, Milli-Q vs. Leachate from Biosolids

EE2 partitioning to montmorillonite, on the other hand, was greater in the presence of biosolids. The results show that almost 98% of the hormone was associated with the solid phase across the range of studied concentrations (Figure 5.18).

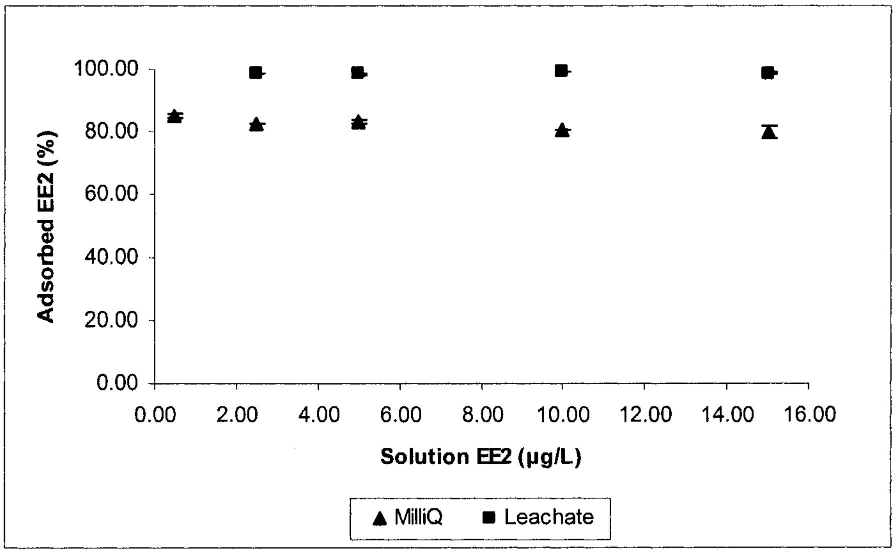


Figure 5. 18: Concentration effect on EE2 Adsorption to Montmorillonite, Milli-Q vs. Leachate from Biosolids

5.1.5.2 Case Study 2: Effect of pH

The earlier observations (Section 5.1.5.1) were further confirmed by the results of the second case study, which in turn assessed the portioning of EE2 between liquid and solids phases under variable pH environments. Here again, illite (Figure 5.19) and natural soil (Figure 5.20) tests demonstrated reduced sorption values with leachate from biosolids in comparison to same tests performed with Milli-Q water. Maximal sorption values with leachate were ca. 50% for both illite and natural soil.

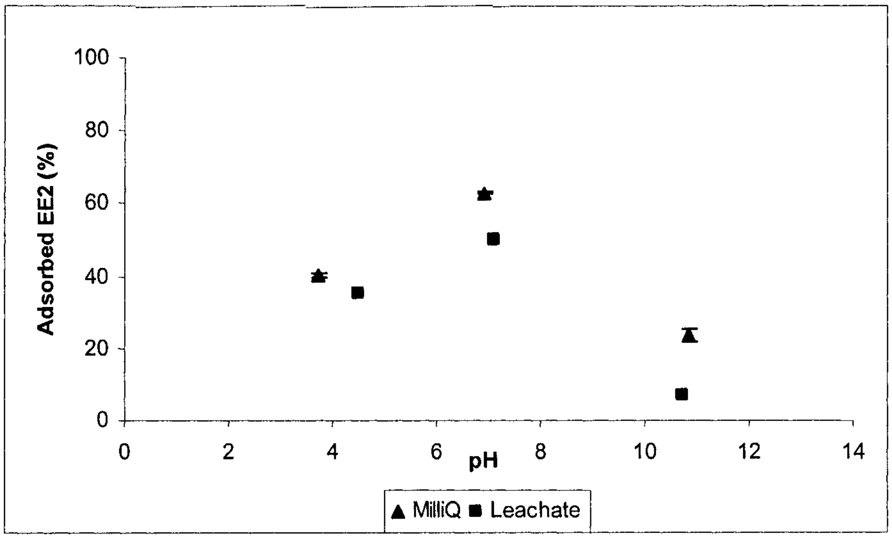


Figure 5. 19: pH effect on EE2 Adsorption to Illite, Milli-Q vs. Leachate from Biosolids

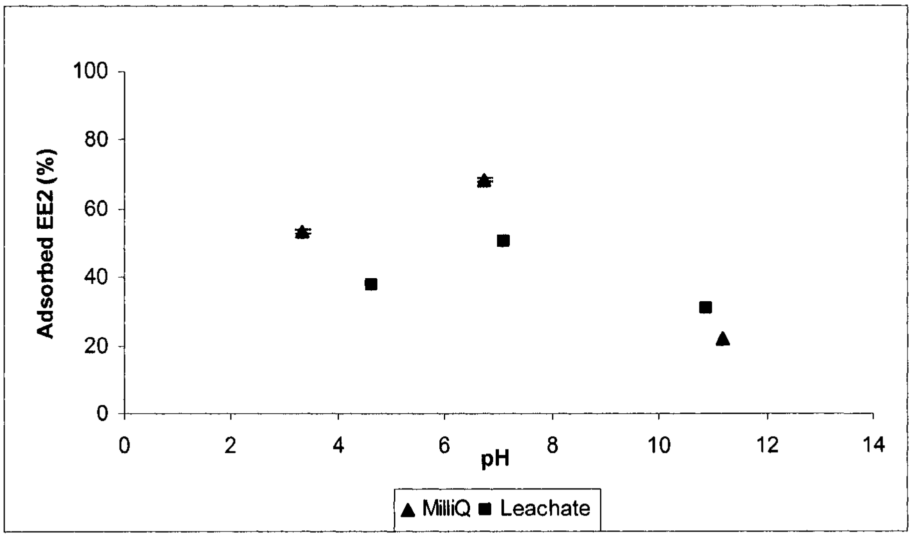


Figure 5. 20: pH effect on EE2 Adsorption to Natural Soil, Milli-Q vs. Leachate from Biosolids

Goethite also exhibited similar partitioning behavior, with respect to changes in the solution pH, to those found earlier in Stage 3 (Figure 5.21). Highest sorption rates were achieved at neutral pH (ca. 21%) with decreasing sorption capacities as either acidic or basic states become more evident; however, current sorption values were significantly less in the presence of leachate from biosolids.

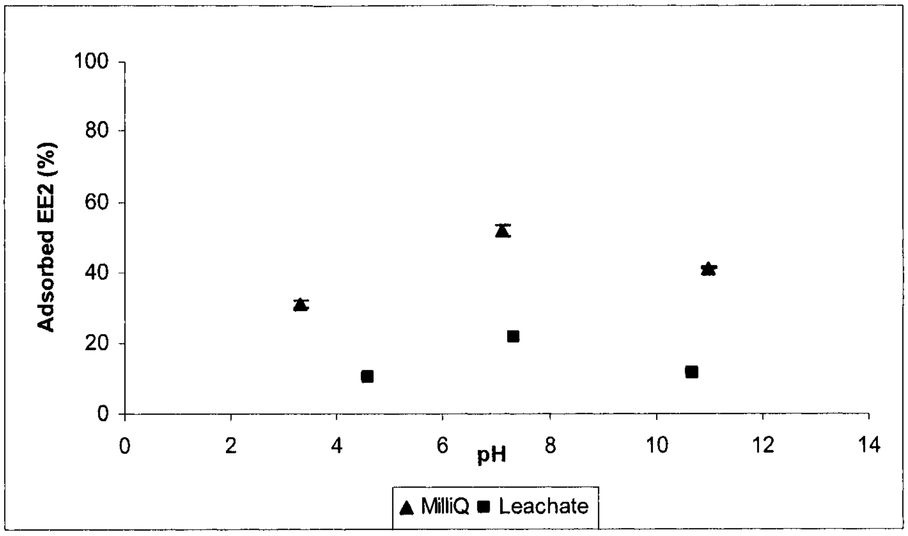


Figure 5. 21: pH effect on EE2 Adsorption to Goethite, Milli-Q vs. Leachate from Biosolids

Analogous to the trend seen in the results of the first case study for EE2 partitioning behavior onto montmorillonite in the presence of leachate from biosolids, the results of this current case study also shows that while influenced by the changes of solution pH, EE2 partitioning onto clay mineral, montmorillonite, was simultaneously affected by the presence of leachate from biosolids, in the sense that, overall sorption values where relatively higher, and nearly all EE2 concentrations (ca. 100%) were associated with the solid phase, as shown in Figure 5.22.

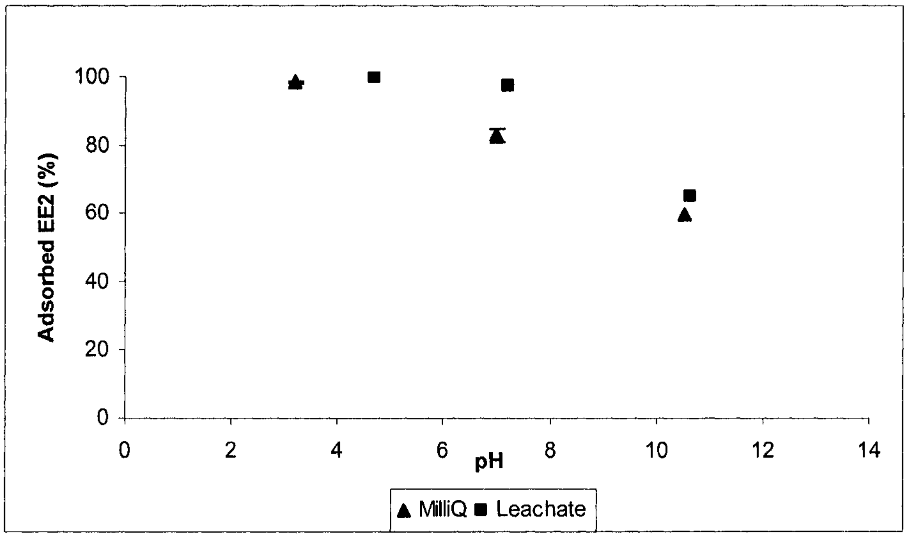


Figure 5. 22: pH effect on EE2 Adsorption to Montmorillonite, Milli-Q vs. Leachate from Biosolids

5.2 Results from Phase 2

5.2.1 Stage 1: FTIR measurements of mineral surface changing

Fourier Transform Infrared Spectroscopy (FTIR) analysis was used to investigate potential changes to mineral surfaces due to changes in pH or as a result of the anticipated interaction of the steroid estrogen (EE2) with these surfaces. Figure 5.23 shows the FTIR spectra of the clay mineral illite tested under different pHs (3, 7, 8.5, and 11). This spectrum displays the absorption of various infrared light wavelengths by the specific molecular components and structure. For example, the absorption band at ~ 1000

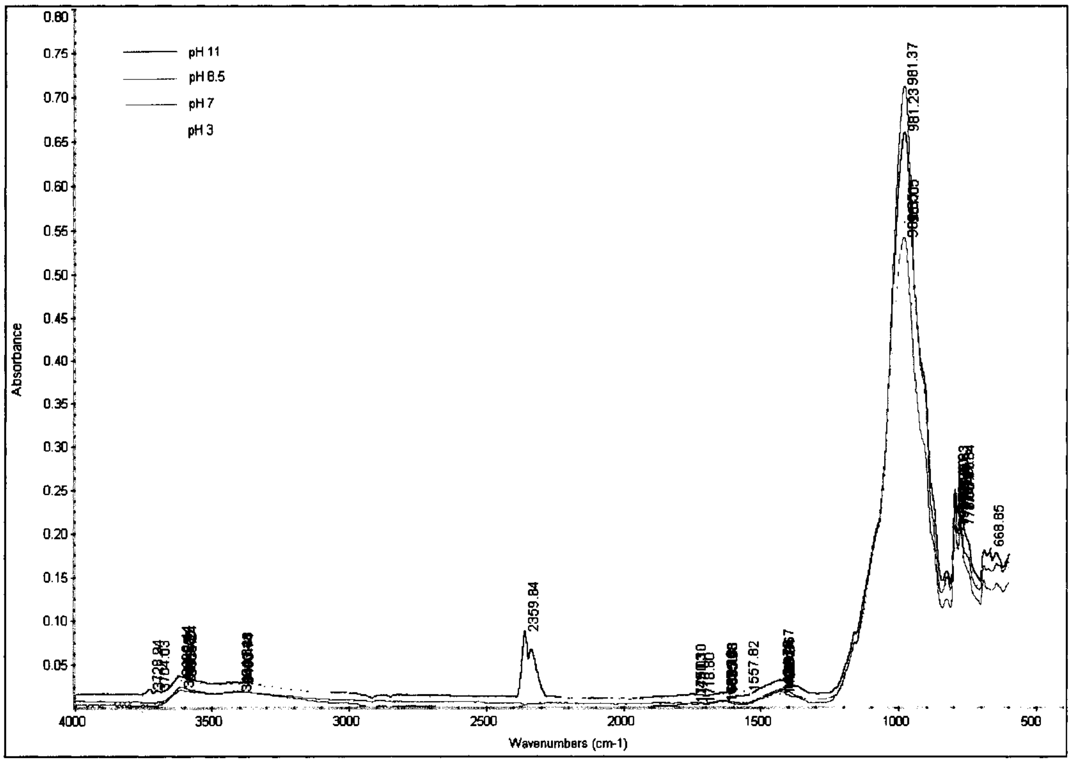


Figure 5. 23: FTIR Spectrum of Illite under Variable pH (full range)

cm^{-1} wavenumber is associated with (–Si–O–Si–) bonds of the clay mineral. Furthermore, absorption bands in the range of 3800 – 3000 cm^{-1} wavenumbers are representative of the OH groups integrated within the mineral structure and the bulk water interrelated within the mineral sheets. Figure 5.24 shows a larger image of these latter absorption bands (related to OH groups) to investigate their absorption intensities, which might vary representing potential changes occurring in the mineral surface as a result of altering pH values. The absorption band shown at wavenumber ~ 3600 cm^{-1} is responsible for stretching vibrations of OH groups integrated within the mineral structure, i.e. it represents the vibration frequencies of dimers in the dioctahedral environment. However, the bands shown at wavenumber 3400 cm^{-1} are associated with the bulk water incorporated within the mineral sheets.

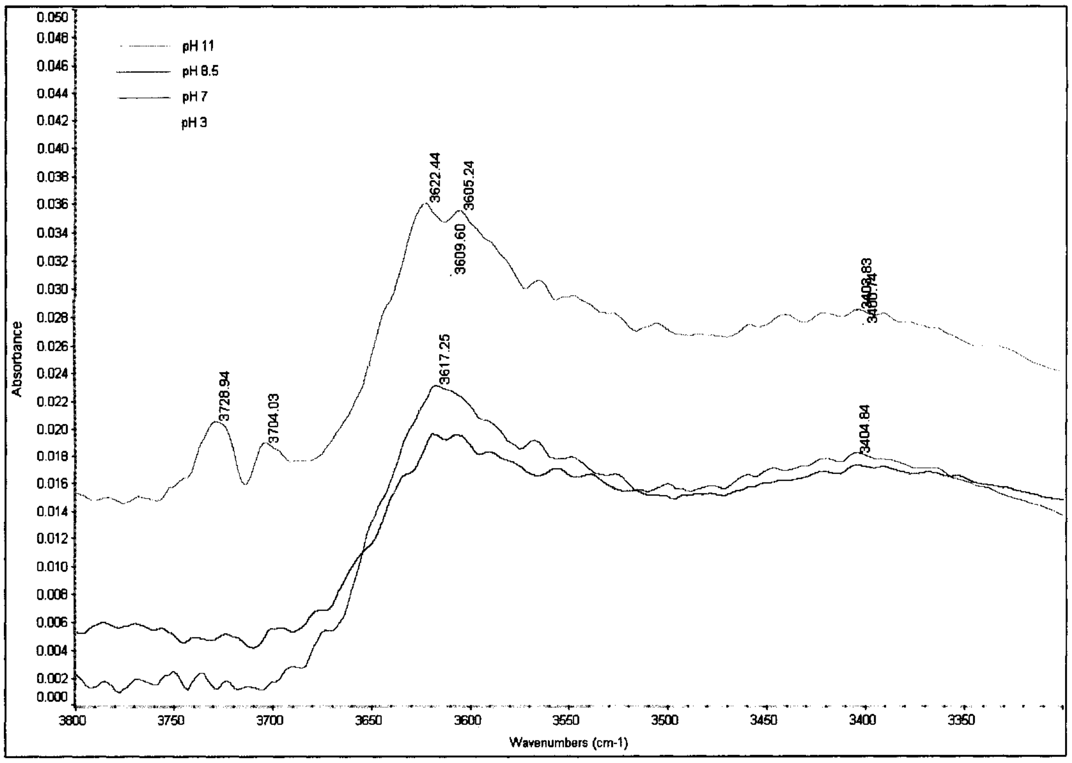


Figure 5. 24: FTIR Spectrum of Illite under Variable pH (wavenumbers 3800 – 3000 cm^{-1})

Figure 5.24 demonstrates that most of the detected OH groups were abundant within the mineral structure (dimers). It also shows that altering pH values had a pronounced effect on changes in the mineral arrangement as the ratio between OH of dimers (3600 cm^{-1}) and bulk OH groups (3400 cm^{-1}) varied in response to changes in pH values (Table 5.2).

Table 5. 2: FTIR Results for Illite

pH	**3**	**7**	**11**
Band Height (3600 cm^{-1})	0.0131	0.0156	0.0156
Band Height (3400 cm^{-1})	0.0031	0.0035	0.0030
Ratio (3600 / 3400)	4.226	4.571	5.20

The FTIR spectrum of the clay mineral montmorillonite (Figure 5.25) shows that montmorillonite has a molecular structure similar to that of illite (Figure 2.23). The –Si–O–Si– bonds are identified by the absorption band at wavenumber ~ 1000 cm^{-1}, and the range of wavenumbers (3800-3000 cm^{-1}) refer to the vibration frequencies of dimers and bulk OH groups of the montmorillonite structure. However, the major difference between the molecular structures of montmorillonite in comparison to that of illite is the ratio between OH groups of dimers and the bulk water OH's as represented by their absorption intensities (wavenumbers ~ 3600 and 3400 cm^{-1}, respectively). Figure 5.26 demonstrates that the calculated ratios (Table 5.3) were found lower in the case of montmorillonite relative to the ratios associated with illite. This difference coincides with the fact the montmorillonites are typical swelling clay minerals that provide a greater access for bulk

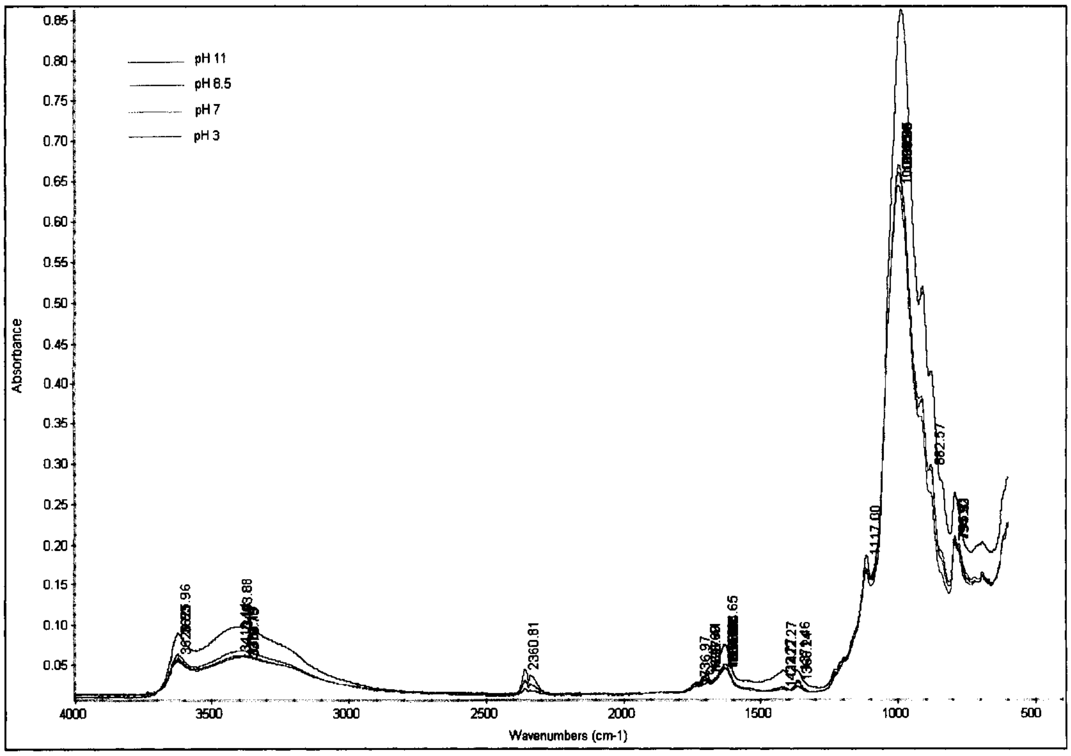


Figure 5. 25: FTIR Spectrum of Montmorillonite under Variable pH (full range)

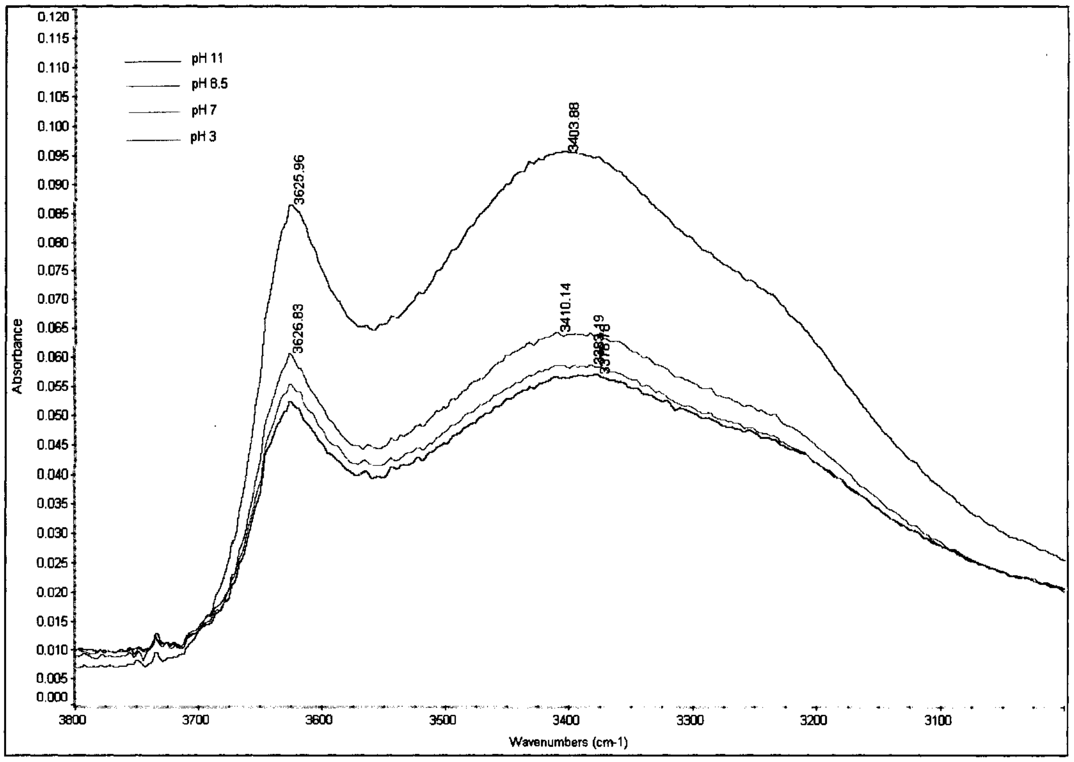


Figure 5. 26: FTIR Spectrum of Montmorillonite under Variable pH (wavenumber 3800 – 3000 cm^{-1})

water to penetrate within their mineral sheets. Here again, the calculated ratios show that the molecular structure of montmorillonite was influenced in response to changes in pH values, as was also the case with illite.

Table 5. 3: FTIR Results for Montmorillonite

pH	**3**	**7**	**11**
Band Height (3600 cm^{-1})	0.023	0.024	0.041
Band Height (3400 cm^{-1})	0.010	0.011	0.021
Ratio (3600 / 3400)	2.300	2.182	1.950

Figure 5.27 demonstrates the FTIR spectra of iron oxide (goethite) prepared under different pH values (3, 7, 9, and 11). The spectrum is quite different than montmorillonite or illite, as the structure of this mineral does not consist of the former siloxane surfaces.

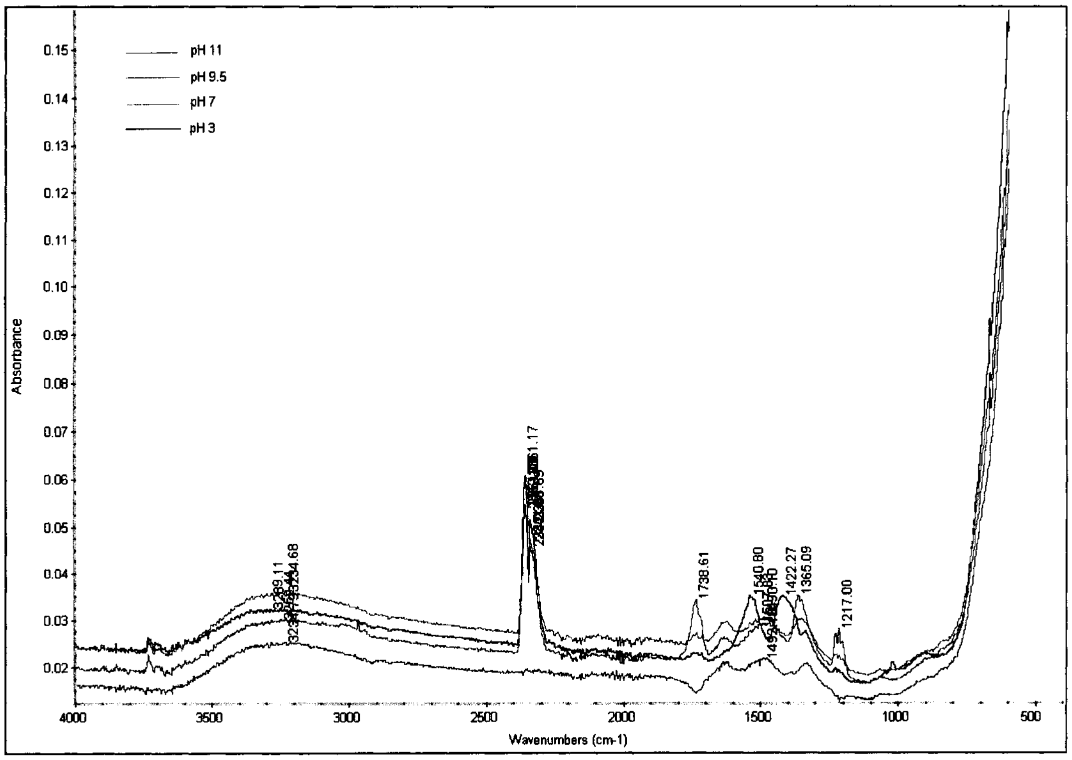


Figure 5. 27: FTIR Spectrum of Goethite under Variable pH (full range)

Goethite was found to be the mineral most influenced by changes in pH values. It can be seen that the intensity, location and size of the vibration absorption bands of OH groups are significantly different than what was observed with clay minerals (montmorillonite and illite). The change in goethite structure can be further illustrated through Figure 5.28. The introduction of sodium hydroxide (NaOH) and acetic acid (AcOH) to change pH values is more evidently expressed in the region 1800 – 1000 cm^{-1}. For example, in the spectra with pH value ~ 3, new bands at 1532 and 1420 cm^{-1}were observed. These bands could be assigned to acetate of iron (Fe), i.e. symmetrical and asymmetrical vibrations of C(O)O of this salt.

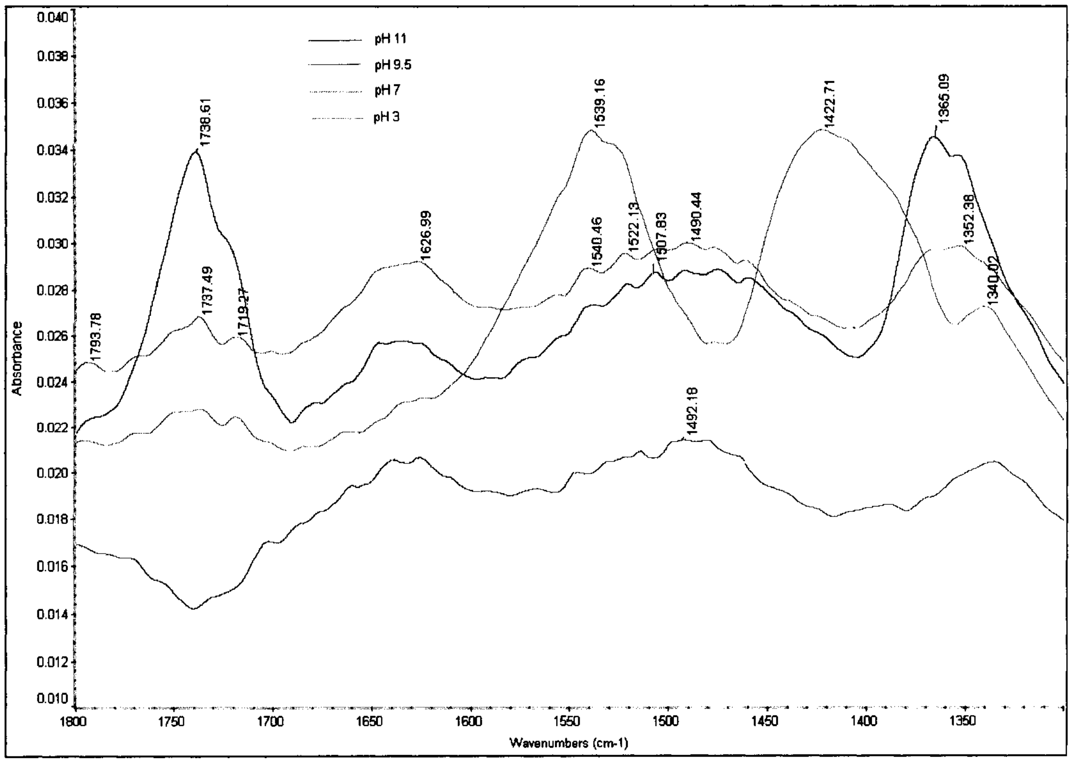


Figure 5. 28: FTIR Spectrum of Goethite under Variable pH (wavenumbers 1800 – 1300 cm^{-1})

To investigate the potential changes in the surfaces of minerals as a result of adsorbing the steroid estrogen (EE2), it was essential to evaluate the molecular structure of EE2 using FTIR analysis (Figure 5.29). As shown in the spectrum, absorption bands in the range of 4000 – 1500 cm^{-1} wavenumbers are typically due to functional groups (e.g. -OH, CH_3). The region between 1500 - 400 cm^{-1} wavenumbers is referred to as the fingerprint region. The spectrum shows that EE2 molecules are held together by very strong hydrogen bonding as can be seen by the high intensities representing the OH groups of these molecules.

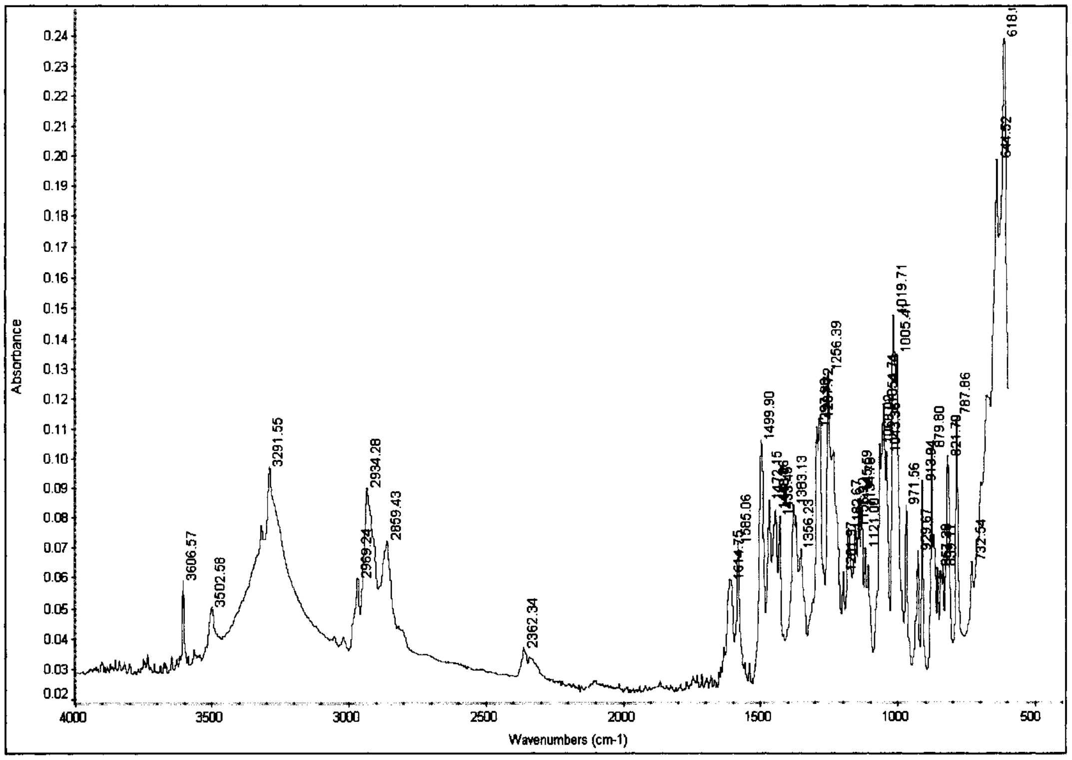


Figure 5. 29: FTIR Spectrum of 17α-Ethynylestradiol

Figure 5.30 displays the spectrum of illite samples collected after batch adsorption test with EE2, as described in section 4.4.2.1. As shown in the spectrum, there is no direct detection of EE2 within the resulting frequencies, and this is due to the very small concentrations (μg/L) of EE2 used in these batch tests. However, the effect of introducing EE2 through batch experiments and its subsequent uptake by the clay mineral illite has indirectly influenced the resulting spectra; results are better shown in Figure 5.31.

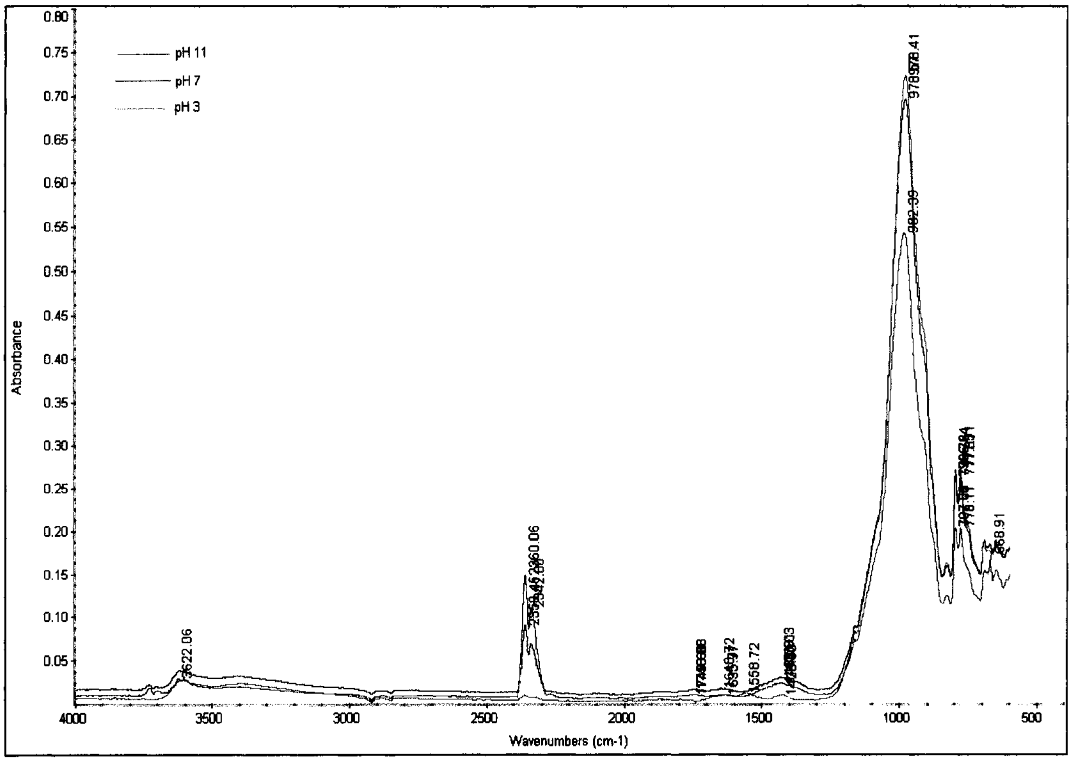


Figure 5. 30: Spectrum of Illite Spiked with EE2 (full range)

The calculated ratios between bands at 3600 cm^{-1} to bands at 3400 cm^{-1} (Table 5.4) were found to be somewhat different from those found earlier for the uncontaminated illite samples (Table 5.2). These differences are indicative of the indirect influence of interactions between EE2 and the hydroxyl groups of illite. Additionally, there are new vibration bands detected at wavenumbers 3700 – 3750 cm^{-1}. These new bands reflect the incorporation of a single molecule (represented by its OH group) into the structure of illite. This single molecule could be in fact the replacement of potassium ions holding illite layers together by EE2 molecules during the uptake process.

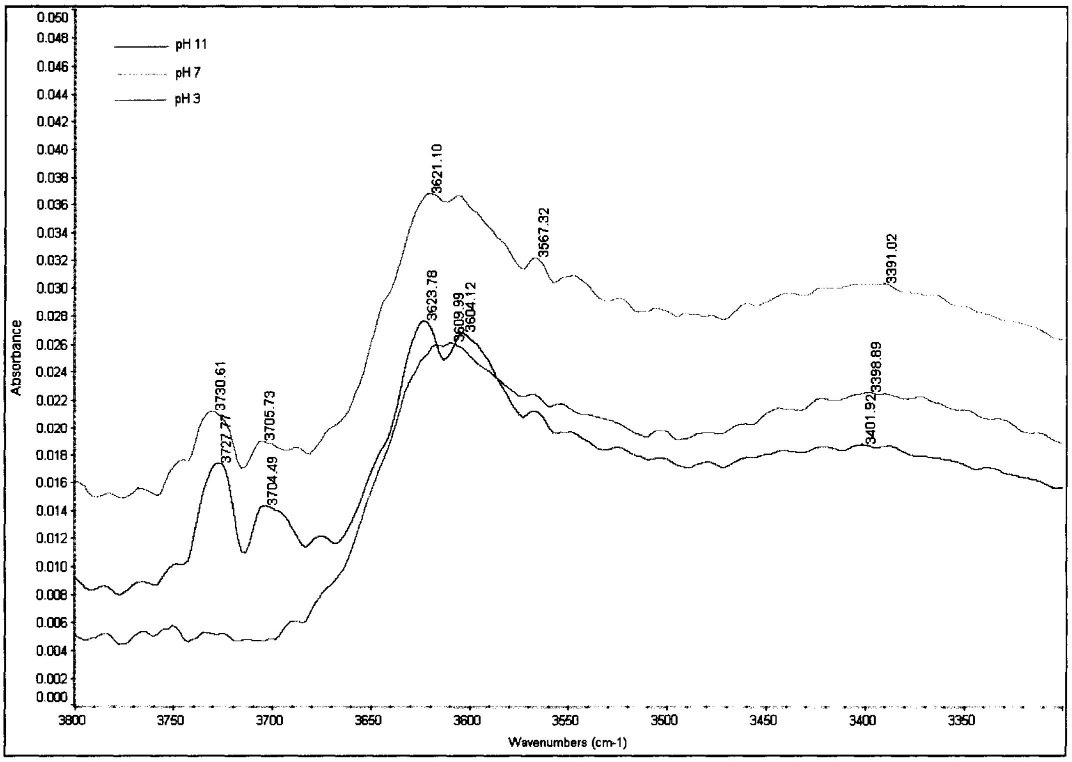


Figure 5. 31: Spectrum of Illite Spiked with EE2 (wavenumbers 3800 – 3000 cm^{-1})

Table 5. 4: FTIR Results for Illite Spiked with EE2

pH	**3**	**7**	**11**
Band Height (3600 cm^{-1})	0.0148	0.0156	0.0144
Band Height (3400 cm^{-1})	0.0035	0.0031	0.0024
Ratio (3600 / 3400)	4.229	5.032	6.000

Figure 5.32 shows the FTIR spectra of montmorillonite samples previously exposed to trace concentrations of EE2 in batch equilibrium experiment, section 4.4.2.1. Here again, the presence of EE2 was not directly detected in the spectra because of it is particularly

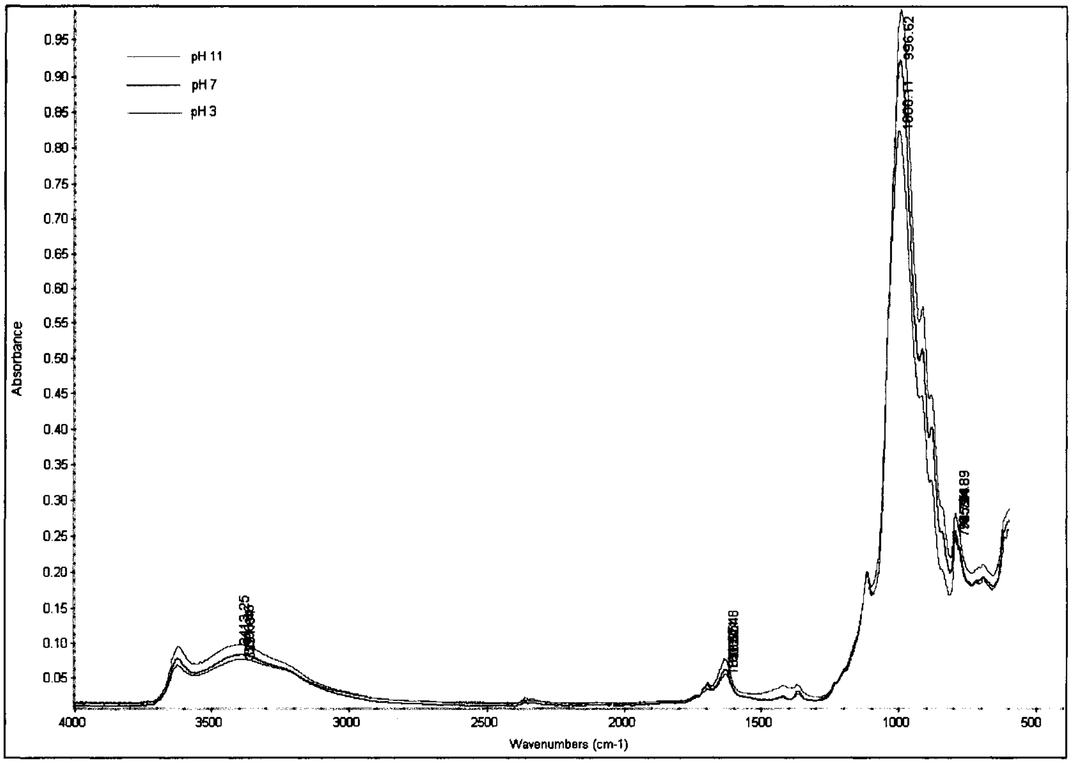


Figure 5. 32: FTIR Spectrum of Montmorillonite Spiked with EE2 (full range)

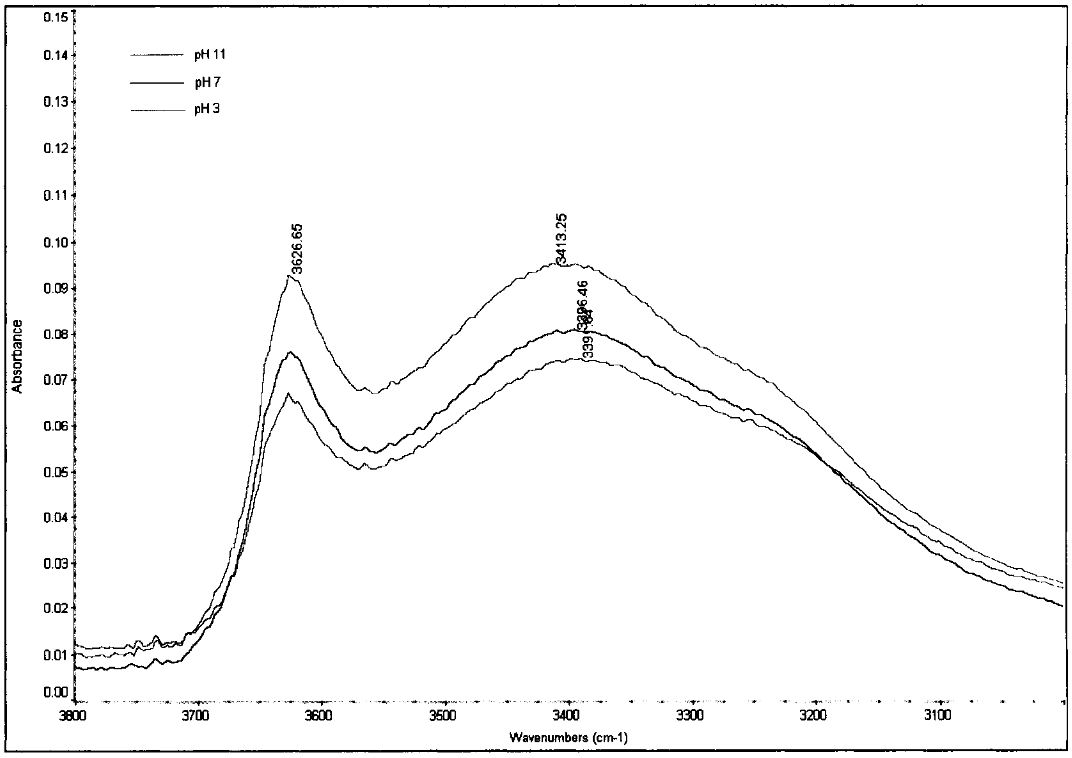


Figure 5. 33: FTIR Spectrum of Montmorillonite Spiked with EE2 (wavenumbers 3800 – 3000 cm^{-1})

low concentration at microgram per liter levels. However, its indirect influence was shown in Figure 5.33, and it is more evident in the new calculated ratios (Table 5.5).

Table 5. 5: FTIR Results for Montmorillonite Spiked with EE2

pH	**3**	**7**	**11**
Band Height (3600 cm^{-1})	0.029	0.037	0.044
Band Height (3400 cm^{-1})	0.014	0.017	0.021
Ratio (3600 / 3400)	2.070	2.176	2.095

Finally, the changes in goethite structure as an influence of interacting with EE2 in the batch adsorption tests are illustrated in Figure 5.34 and Figure 5.35.

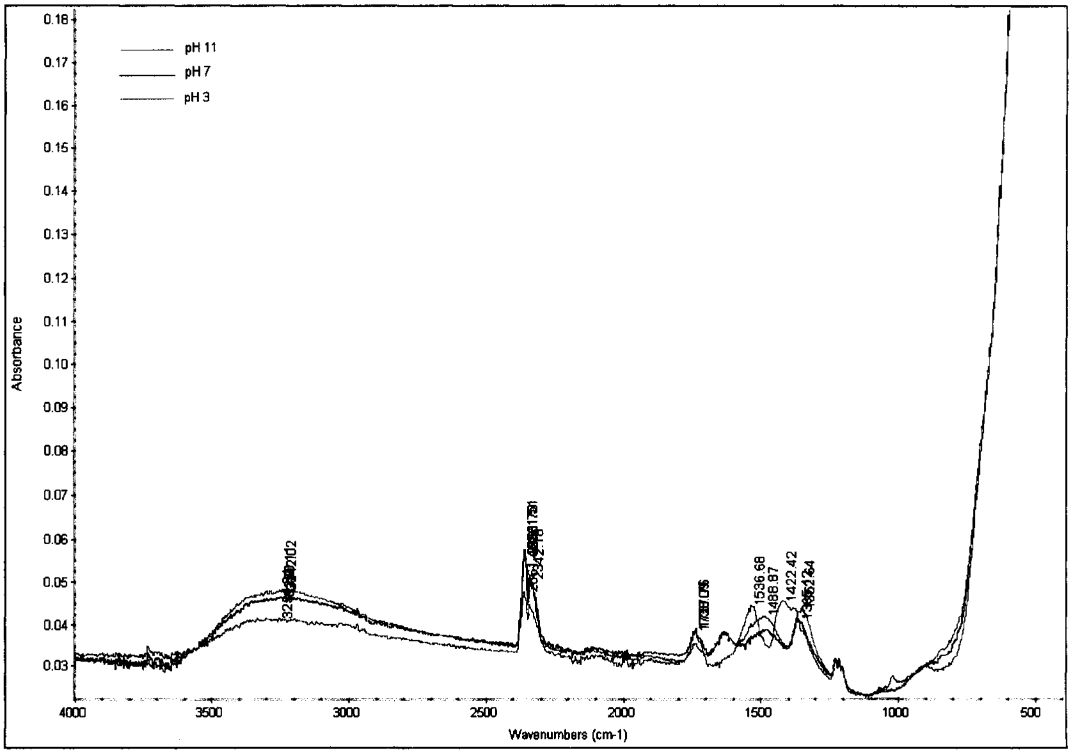


Figure 5. 34: FTIR Spectrum of Goethite Spiked with EE2 (wavenumbers 1300 – 1800 cm^{-1})

The ratio between absorption bands (range 1300 – 1800 cm^{-1}) of uncontaminated goethite samples were different than those obtained from goethite collected after the batch adsorption test with EE2. These differences are attributed to indirect influences of the interaction between EE2 and the mineral surfaces.

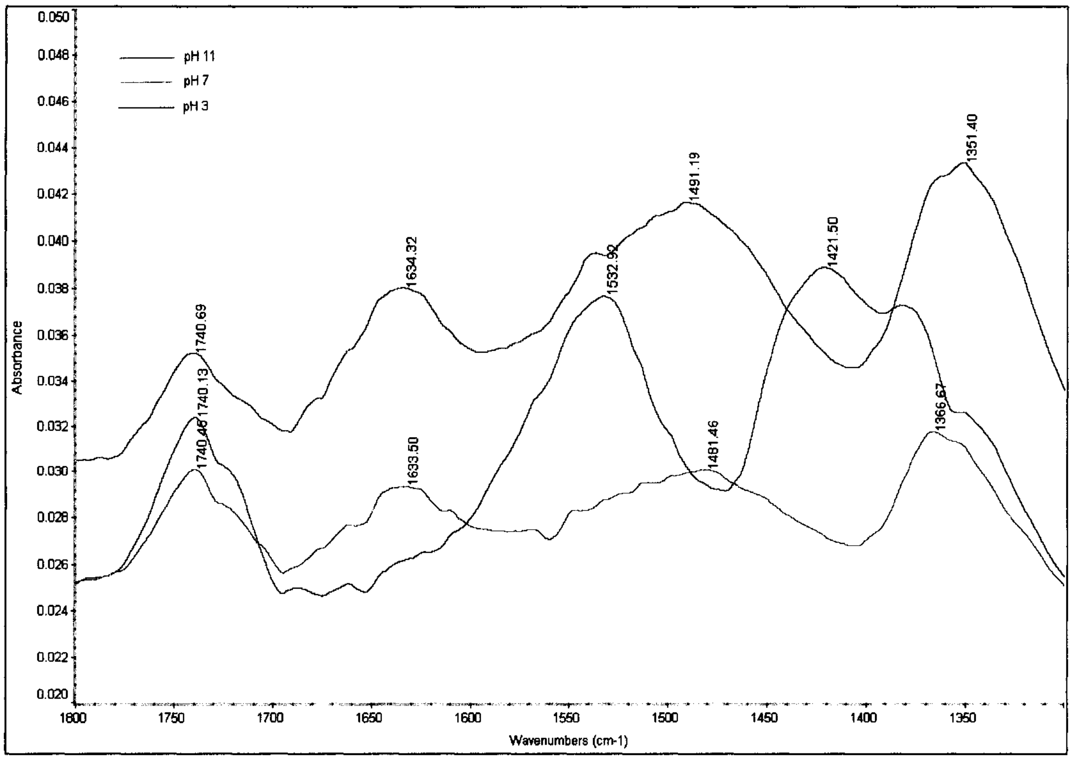


Figure 5. 35: FTIR Spectrum of Goethite Spiked with EE2 (wavenumbers 1800 – 1300 cm^{-1})

5.2.2 Stage 2: UV/Vis measurements of EE2 changes

As mentioned earlier in Chapter 4, UV/Vis analysis was performed to assess the behavior of dissolved EE2 in response to changes in pH. The UV spectra of EE2 at pH 7 showed three distinctive bands (275, 230, and 200 nm). These bands are representative of the phenolic OH group in the benzene ring of the hormone's structure. Results also indicated (data are not shown) that under aggressive acid conditions (pH 3), the OH group had undergone some changes; as was observed by the hyperchromic effect, i.e. the increase in the intensity of the spectral band at 200 nm. On the other hand, the spectra of EE2 at pH 11 exhibited a bathochromic effect (shift of a spectral band to lower frequencies, i.e. longer wavelengths), which was clearly visible as the former band at 275 nm under pH 7 has now been shifted to 290 nm under pH 11. This bathochromic shift is assumed to be the resultant of the formation of phenolate complex with sodium; Na was introduced by sodium hydroxide, NaOH, which was used to adjust the pH.

6.0 DISCUSSION

As shown in the results, the synthetic steroidal estrogen, 17α-ethynylestradiol, expressed different affinities of binding to different soil constituents. Partitioning onto clay minerals was greater than onto iron oxide or humic acid; the results are shown in Figure 5.1. Batch equilibration experiment showed that montmorillonite was able to achieve the utmost uptake rates. Only 20% of the solute remained in the aqueous phase after 24-hour equilibration interval. Goethite, on the other hand, displayed the lowest sorption capacity; merely 40% of the hormone concentration was uptaken by the end of the test period. Clay minerals, illite, and humic acid had intermediate capacities where almost 60% was partitioned into solid phases. These results suggest the correlation between EE2 partitioning and specific surface areas (Table 4.1) and the availability of adsorption sites. Casey et al. (2003) also reported a high correlation between the natural estrogen, 17β-estradiol, sorption and soil surface area although they performed investigations with higher concentrations.

Sorption Kinetics and Equilibration Time

Kinetic results demonstrated that EE2 uptake onto various soil constituents followed dissimilar approaches; a distinctive two-stage behavior for clay minerals as opposed to a single rapid mode for either goethite or humic acid. The two-stage approach, seen for montmorillonite and illite, could be characterized by an initial rapid uptake of EE2 onto external clay surfaces within the first hour, and a slower second-phase sorption, over 24 hours, attributed to diffusion of EE2 into intra-aggregate micropores and perhaps intercalation into interlayer regions of expanding clay, i.e. montmorillonite. Since the building layers of illite are held together through bonding potassium ions, EE2 has limited access to its interlayer sites, and thus, intercalation into interlayer regions of illite is not appreciable. This could account for the increased sorption capacities of montmorillonite beyond those of illite. In contrast, no further sorption was observed for goethite and humic acid beyond the rapid uptake within the first hour, which is attributed to interactions with external surfaces. Van Olphen (1977) has previously introduced the diffusion controlled sorption phenomenon. It was defined as the process where solute molecules diffuse into clay mineral structure and in some circumstances intercalation into interlayer regions, micropores, or into stacks or particle aggregates.

Similar trends were also observed in the experimental work of a number of researchers (Shareef et al., 2006; Van Emmerik et al., 2003). Van Emmerik et al. (2003) investigated the sorption of the natural estrogen, E2, onto clay minerals (kaolinite, illite and montmorillonite) and hydrous iron oxide (goethite). A two-stage sorption process was observed for the clay minerals that extended over several days, while uptake onto goethite was completed rapidly within seconds. However, Shareef et al. (2006) studied the sorption of EE2 from aqueous solutions to goethite, kaolinite and montmorillonite, and observed a rapid single-stage sorption to both goethite and kaolinite. In their results, the two-stage approach was only noticed for the uptake of EE2 onto montmorillonite.

An alternative explanation for this observed slow sorption is that surface catalyzed break down or biological transformation of EE2 could have taken place at the mineral surfaces

(Lee et al., 2003; Van Emmerik et al., 2003). This is not likely to be the case in this study as no evidence of break down products in the chromatography was found. Additionally, since the reported half-lives for EE2 under aerobic conditions in sea-water and marine sediments, river water, and in aquifer material were greater than 20, 46, and 81 days respectively, and no degradation under anaerobic conditions (Lai et al., 2000; Ying and Kookana, 2003; Ying et al., 2003), biodegradation is expected to be insignificant over the applied time scale of this study.

pH Effect

In an attempt to justify the uptake of EE2 onto external clay surfaces, several authors proposed hydrophobic interactions to be the dominant mechanism of non-polar organic compounds binding to solid phases (Jaynes and Boyd, 1991; Lai et al., 2000; Lee et al., 2003; Shareef et al., 2006; Van Emmerik et al., 2003; Yu et al., 2004). It was suggested that this hydrophobic interaction is associated with siloxane (–Si–O–Si–) bonds (Van Emmerik et al., 2003). Nevertheless, most of these studies also reported that sorption processes were found independent of pH, i.e., their interpretations were based on the fact that there was little or no correlation between sorption processes and layer charge or surface area. However, in the current results it is evident that EE2 sorption to clay minerals has a great dependency on pH, as can be seen in Figure 5.5 and Figure 5.6. This phenomenon could be explained by the resulting surface charge on clay surfaces. Soil constituents possess electrical charges originating from different sources. The sign and magnitude of these charges have a great influence on soil behavior. The flat basal surface of mineral soils carries a permanent negative charge due to isomorphic substitutions in the crystal lattice of clay minerals. However, due to imperfections and distortions of the clay minerals, their edge surfaces are characterized by the resultant variable charges (Jozefaciuk et al., 2002; Katari and Tauxe, 2000). At these edges, the broken bonds generate the amphoteric Al-OH and Si-OH surface function groups. The arising charge on these surface hydroxyls results from protonation and deprotonation processes depending on the pH of the solution. The charge build-up was assumed to follow a two-step process represented by Equation (6.1):

$$S-O^- \xleftarrow{+OH^-} S-OH^0 \xrightarrow{+H^+} S-OH_2^+ \qquad (6.1)$$

where S represents a single site for surface protonation (McBride, 1994; Sverjensky and Sahai, 1996). The net charge is thus determined by the equilibrium between the three chemical forms. For example, at pH below the point of zero charge for a specific mineral, the edge will be rich with $S-OH_2^+$ relative to $S-O^-$, resulting in a net positive charge. This assumption coincided with the results of the FTIR analysis (section 5.2.1), which showed that the occurrence and distribution of OH groups of clay minerals was significantly subject to pH values. However, a modified model (McBride, 1994) was proposed for charge development following Equation (6.2):

$$S-OH]^{-1/2} \underset{+OH^-}{\overset{+H^+}{\rightleftharpoons}} S-OH]^{+1/2} \qquad (6.2)$$

According to this latter scheme, the two chemical species coexist at the surface in relative populations determined by the solution pH. Consequently, it is evident that the amount of variable charge is directly related to the pH of the soil solution. Maximum sorption onto illite occurred around mid pH (ca. 7) while sorption onto montmorillonite was maximal at acid range pH (ca. 3). These pH values corresponded with isoelectric points of illite and montmorillonite of ca 7.5 and 2.5, respectively. Accordingly, non-polar EE2 primarily interacted with uncharged surface hydroxyl groups. The increased charge on clay surfaces at pH different from their point of zero charge resulted in decreased rates of adsorption. Moreover, at elevated pH levels, the dissociation of the phenolic group of EE2, forming a negative charge on the molecule, would give rise to electrostatic repulsion between EE2 and the surface. The effect of pH on the structure of EE2 was further examined by the UV analysis (section 5.2.2). The results endorsed that at very alkaline conditions, the chemical structure of EE2 was altered to form phenolate complexes, which agrees to the dissociation of the phenolic group. The resulting electrostatic repulsion would account for the more pronounced drop in sorption at higher pH. Consequently, it is believed that sorption mechanism of EE2 onto clay minerals was probably governed by a combination of hydrogen bonding with uncharged mineral surfaces and hydrophobic interaction with siloxane surface, attributed to nonspecific, short-range van der Waals interactions.

These results are different from earlier finding by Van Emmerik et al. (2003) and Shareef et al. (2006). Van Emmerik et al. (2003) observed no correlation between sorption of the natural estrogen (E2) to clay minerals and ambient pH, and indicated that van der Waals forces were principally responsible for the sorption process. They, as a result, concluded that sorption of E2 to clay minerals involved weak hydrophobic interaction with the siloxane face of the minerals. Similarly, Shareef et al. (2006) showed that EE2 sorption to kaolinite was not affected by pH, indicating that it was only weakly bound to external surfaces. Nonetheless, they demonstrated that sorption to montmorillonite was greatly influenced by pH, as noticed by the significant increase in sorption between pH 7 and pH 10. It was suggested that the montmorillonite flocculation state is closely associated with solution pH, i.e., at low pH montmorillonite has an edge-to-face arrangement which limits the access to its interlayer sites, whereas at higher pH, this arrangement is changed to form face-to-face structure that facilitates the accessibility of hydrophobic organic molecules to penetrate between the layers. On the basis of their results, they proposed that a combination of intercalation and flocculation could account for the increased sorption at high pH.

An alternative argument was proposed by Casey et al. (2003) while studying the sorption of the natural estrogen, 17β-estradiol. They hypothesized the association between sorption rates and cation exchange capacity (CEC). This hypothesis, however, is rather unlikely. 17β-estradiol possesses a phenolic group with a pK_a of 10.6 that will dissociate under very basic conditions, forming an organic anion. Thus, developing a positively charged molecule that is essential for cation exchange is certainly inconceivable. Accordingly, non-polar EE2, which also has a phenolic group within its structural arrangement, analogous to natural E2, will never be suitable for cation exchange. Furthermore, since EE2 ionization only occurs at high pH, a state at which soil particle surfaces are principally characterized by negative electric charges, electrostatic repulsion between EE2 and the surfaces is anticipated to prevail. Consequently, ion exchange was entirely disregarded as a potential sorption mechanism applicable to this current investigation.

Iron oxides are amphoteric surfaces characterized by their variable charges. The hydroxyl groups at the goethite surface will protonate at low pH providing a positive charge on the mineral surface, and will release protons at high pH rendering the surface negatively charged. Consequently, the increasing polarity of the goethite surface is responsible for the lower binding affinities of the non-polar molecule at both acidic and basic environments, as seen in Figure 5.7. This behavior indicates that EE2 mainly interacted with uncharged hydroxyl surfaces, as they are most abundant at the isoelectric point (ca. pH 7.3 - 8), plausibly by hydrogen bonding. These results are in full accordance with the results of Van Emmerik et al. (2003) who concluded that sorption onto goethite was weak, probably by hydrogen bonding between the natural estrogen (E2) and uncharged surface hydroxyl group. Additionally, the reduced sorption capacities of goethite at extreme pH conditions could be attributed to the transformation of goethite into different salts, as was shown in the results of the FTIR analysis (section 5.2.1). For example, under vigorous acidic condition (pH ~ 3) adjusted by the addition of acetic acid, the chemical structure of goethite was altered to form acetate of iron, demolishing the hydroxyl sites available for sorption.

Lai et al. (2000) studied the extent of EDCs sorption by goethite, and found that the iron oxide in the absence of organic matter was able to attain appreciable sorption. They suggested that the governing sorption mechanism is predominated by ion exchange between the surface hydroxyl group on the oxide and charged solute. As discussed earlier, this approach in highly unlikely since ion exchange sorption requires phenolic groups of EE2 to be positively charged. However, these groups are normally uncharged over a wide range of pH and will only be ionized at very high pH to give organic anions. Alternatively, Lee et al. (2003) gave another explanation for sorption of organic compounds on iron oxides. They suggested that the significant sorption might have, instead, been potentially lost in oxide-catalyzed transformation. They also proposed ligand exchange of phenolic molecules to iron and aluminum oxides. These assumptions, however, are not very applicable to the mechanisms involved in this study. There was no detection of any break-down products in the chromatographic analysis. Additionally, experiments done by other researchers confirmed that sorbed compounds were fully recovered and desorbed from the iron oxide, indicating no loss of the compound due to catalyzed reaction or any chemical sorption.

To understand the mechanism of interaction between EE2 and humic acid, it is important to examine the structure of humic material and its behavior under different pH states. Humic materials are derived from the degradation of natural organic matter. The major constituents of their structure are weakly condensed core of aromatic rings surrounded by a disordered network of aliphatic or alicyclic components. Humic materials incorporate a pool of functional groups such as carboxylates and phenolic hydroxyls (Hayes et al., 1989; Schlautman and Morgan, 1993). Sorption to organic matter was frequently reported to be predominantly controlled by hydrophobic interactions (Carter and Suffet 1982; Lai et al., 2000; Lee et al 2003; Schlautman and Morgan, 1993; Zhang and Zhou, 2005). Other researchers suggested that binding to humic material might follow different mechanisms rather than hydrophobic interaction (Yamamoto and Liljestradn, 2003; Yamamoto et al., 2003; Yu et al., 2004). They proposed the interaction between organic colloids and phenolic groups of estrogenic compounds. Additionally, a great contribution due to specific interaction via covalent or hydrogen bonding was also suggested.

Humic materials are highly sensitive to pH. At high pH, progressive deprotonation will take place, increasing the polarity of humic material. This appreciable deprotonation will alter the structure and decrease the ability of humic material to bind organic compounds. Furthermore, raising pH increases the solubility of humic materials, i.e. it facilitates dissolving more humic material. The increased solubility of humic material will, in turn, increase the concentration of EE2 in the extracted liquid phase indicating lesser sorption capacities of solid humic acid. On the other hand, at low pH, humic material will sorb hydrogen ions, lowering the negative charge on humic molecules and making it less hydrophilic. As a result, humic molecules will coagulate, increasing the size of the humic polymer and causing it to precipitate out of the solution. This charge decrease will also lead to increase the binding abilities of humic material to hydrophobic organic estrogens. This trend was clearly seen in Figure 5.9. The behavior observed in this study agrees with the findings of Carter and Suffet (1982) and Schlautman and Morgan (1993) as they investigated the binding of various PAHs to dissolved humic materials.

Generally, it was difficult to precisely determine the extent of EE2 partitioning to particular phases, since it fundamentally exists in the three phases simultaneously partitioned onto solid phase, truly dissolved in pore water, and finally partitioned into dissolved humic/colloidal material. This third phase, if not treated carefully and separately, will lead to increased EE2 concentrations in the extracted liquid phase, and therefore, being incorrectly considered as directly dissolved concentrations. For example, natural soil consisted of a mixture of soil minerals, metal content, and organic matter (Table 4.2). EE2 could be associated with mineral surfaces, solid organic matter, dissolved into aqueous phase, or partitioned into dissolved organic matter. However, the resultant sorbed concentrations (Figure 5.8) were comparable to those displayed by illite (Figure 5.5). This could be explained by the fact that illite accounts for 50% of the natural soil sample constituents.

In summary, it was found that the interaction between the synthetic steroid estrogen (EE2) and various soil constituents was governed by the combination of binding the non-polar molecule to uncharged soil surface hydroxyl groups through hydrogen bonding, in conjunction with hydrophobic interaction with siloxane surface, ascribed to van der Waals interactions. As such, altering the population of terminal hydroxyl groups accompanied with varying the electrical charge on the surfaces of soil constituents resulted, generally, in a lesser amount of EE2 being adsorbed to these charged surfaces. Thus, changing pH values from the corresponding isoelectric point of individual soil constituents would affect the chemical structure and the electrical charges of soil surfaces and hence influence the extent of their interaction with EE2. It is, consequently, hypothesized that shifting environmental conditions towards more pronounced acidic or alkaline conditions will have a great influence on the release and mobility of the estrogenic hormone within soil media. It was further found that clay minerals, i.e. montmorillonite and illite, might have a greater ability to adsorb and retain EE2 due to the potential of incorporating the molecule within their structures.

Effect of Anaerobic Conditions

The results of the set of experiments done under deprived oxygen conditions (Figures 5.10 to 5.13) did not differ much form those performed in the presence of excess oxygen (Figures 5.5 to 5.9), indicating that the presence or absence of oxygen had limited or no contribution towards the uptake of EE2 by various soil constituents. In other words, these data reinforces earlier speculations that surface chemical reactions (for example, oxidation) that might alter EE2 concentrations and lead to an overestimation of sorption capacities, are not of a significant role in this current study. This is also supported by the lack of any breakdown products in the chromatographic analysis under current experimental parameters and time scale. These findings also imply that the affinity of EE2 to partition to soil constituents within subsoil media is not anticipated to differ between aerated top soil regions and deep ground levels of air deficiency. Consequently, the release and mobility of EE2 is not expected to vary with depth into soil subsurface as a function of oxygen availability. The unique results shown by goethite (Figure 5.14), suggests the likelihood that EE2 affinity to goethite being different under aerobic versus nitrogen-controlled conditions, however, a reasonable explanation has not yet been proposed in the course of this study.

Case Studies: Effect of Biosolids Application

A conclusive insight into EE2 interactions with soil constituents, binding affinities, potential mobility, bioavailability and mechanisms controlling its fate within soil environment has been fairly established. Knowledge gained so far was based on interactions occurring within a background of pure Milli-Q water, however, it is essential for the scope of this research to verify the applicability of our observations and results under more realistic environmental circumstances where wastewaters and leachate from biosolids are being applied to a great extent over agricultural land and disposal sites. Such investigation has significantly enhanced the ability to assess and predict potential hazards of contaminating groundwater and surrounding water bodies.

Two case studies were performed, in which binding affinities and sorption capacities were investigated using leachate extracted from treated sludge (representative of a Canadian WWTP outlet). Principally, the application of leachate reduced the affinity of organic EE2 to partition into illite, natural soil, and goethite (Figures 5.15 to 5.17). In other words, the application of leachate would increase the apparent mobility of organic estrogens within soil environment. Very complex matrices of organic matter and dissolved organic carbon are integrated within investigated leachate. The presence of these matrices will compete for EE2 affinity providing available sorption sites and potential compartments favored for partitioning. As a result, EE2 will have a great tendency to be taken up and retained into these organic components, indicating a greater association with aqueous phases. Accordingly, adsorption coefficients were calculated based on Freundlich model, Equation (5.1), and were found to be less than those calculated earlier before the introduction of leachate to sorption batch tests. Results are shown in Table 6.1. Similar trend was observed for the effect of wastewater on affinities of organic compound (Zhang and Zhou, 2005). They also observed reduced sorption coefficient in the presence of wastewater compared to pure water. They suggested that organic and inorganic species in the effluents were responsible to adsorb onto granular activated carbon (GAC), limiting the availability of adsorption site for the organic compounds E1 and E2.

Table 6. 1: Parameters of Freundlich Isotherm, Milli-Q vs. Leachate

	Milli Q		**Leachate**	
Soil Material	K_f	n	K_f	n
Natural Soil	24.33	0.99	12.89	1.07
Illite	16.80	0.94	13.66	0.87
Goethite	6.32	1.08	0.002	3.66

A significantly different behavior was observed by montmorillonite. The application of leachate from biosolids increased EE2 affinity to partition into the solid phase. Greater sorption coefficients were obtained with leachate in comparison to Milli-Q water (K_f and n values were 738.2 and 0.95 with leachate compared to 45.87 and 0.90 with Milli-Q water, respectively). In an attempt to understand this behavior, two possible explanations are provided. It was thought that montmorillonite was able to sorb and retain, into its interlayer regions, organic molecules to which a great portion of EE2 was already partitioned. Alternatively, it was thought that the application of wastewater effluents, with its wide variety of organic and inorganic complexes, could have resulted in a greater swelling of the clay mineral. The extent of montmorillonite swelling is influenced by the properties of the clay mineral (e.g. layer charge), nature of interlayer cations and the chemistry of the water in the equilibrating solution. Montmorillonite layers principally carry permanent negative charge due to isomorphic substitutions. Exchangeable cations adsorbed in the interlayer region are responsible to compensate for the arising negative lattice charge. Once in contact with aqueous solution, water molecules penetrate the interlayer region and hydrate the exchangeable cations and clay surface oxygen groups. This hydration process creates repulsive hydration forces that overcome the weaker van der Waals attraction between layers resulting in interlayer swelling. Wastewater effluents contain complex mixture of suspended and dissolved solids, both organic and inorganic. The dissolved ions interact with water molecules leading to disturbance of their arrangement. Accordingly, this would affect the integrity and the extent of the hydration shells surrounding the clay particles. The overlap of these hydration shells is supposed to be responsible for the development of the swelling pressure. Several investigators hypothesized that these organic compounds can act as a dispersion agent in clay-water systems (Chappell et al., 2005; Chen et al., 1987; McFarlane et al., 2005; Mitchell, 1993; Tarchitzky et al., 1999; Tombácz and Szekeres, 2004). Consequently, EE2 molecules were granted a greater and more facilitated access to the interlayer regions, which resulted in higher sorption capacities. EE2 partitioning was also studied under different pH and redox conditions. As shown in the results, the effect of changing environmental conditions, e.g. pH, followed the same trend seen for experiments done with Milli-Q water. The difference, here again, was that the application of wastewater effluent resulted in a general decrease of EE2 partitioning to solid phases, except for montmorillonite where sorption capacity was significantly enhanced.

7.0 MODELING OF EE2 PARTITIONING TO SOIL

7.1 Model Development

This chapter shows an empirical model developed based on the results generated form the study. The model predicts quantitatively the partitioning of 17α-ethynylestradiol (EE2) within soil components. The developed model takes into consideration the effects arising from changes in the solution chemistry (pH), changes in levels of EE2, and effects due to the application of leachate from biosolids or reuse of wastewater effluents as common agricultural practices. Chapter 5 showed that the partitioning of the hydrophobic organic compounds, such as EE2, between solid and liquid phases was different for the soil constituents under study. Nevertheless, the correlation between sorbed amounts and aqueous concentrations, for all studied soil constituents, was found to follow a linear relationship when represented by a logarithmic scale, as shown in Figure 5.4. This correlation is defined by the general Formula (7.1):

$$Log C_s = Log\alpha + \beta * Log C_e \qquad (7.1)$$

This Formula (7.1) could be rewritten to give Equation (7.2):

$$C_s = \alpha * C_e^{\beta} \qquad (7.2)$$

Where C_s is EE2 concentration that is into the solid phase (μg/kg), C_e is EE2 equilibrium concentration that is into the aqueous phase (μg/L), α and β are empirical parameters that depend on adsorption characteristics. It is conceivable by mathematical manipulation that the parameter α will have the units [(μg/kg) / (μg/L)$^{\beta}$], while β is unitless. This Equation (7.2) is in full accordance with Freundlich sorption isotherm (7.3):

$$C_s = K_f * C_e^{\ n} \tag{7.3}$$

as described earlier in Chapter 2 and Chapter 5. The empirical parameters α and β are functions of soil material, and could be defined through their association with soil properties, such as cation exchange capacity (CEC) or specific surface area (SSA). However, in the results section, there was found no association between partitioning rates and CEC, therefore, the empirical parameters are more suitably defined based on soil minerals specific surface areas. Freundlich sorption coefficient and constant (Table 5.1) were plotted against specific surface areas of corresponding soil minerals (Table 4.1). Subsequently, formulas representing the correlation between parameters α or β versus soil specific surface areas are given in Equations (7.4) and (7.5) respectively:

$$\alpha = 0.35 * SSA - 11.15 \tag{7.4}$$

$$\beta = -0.002 * SSA + 1.17 \tag{7.5}$$

Where SSA is the respective soil mineral specific surface area (m^2/g).

Moreover, the results of this study (Figures 5.5 to 5.8) also showed that pH value of the solution has a great influence on the extent of sorption. It was noticed that most of the studied soil constituents responded in a similar manner to changes in solution pH; a sorption capacity maxima was achieved at the isoelectric point (pH_{iep}) and decreased steadily at pH levels below and above the pH_{iep}. The results are considered to follow a parabolic curve, which is described by the general formula (7.6):

$$(y-k) = -\gamma * (x-h)^2 \tag{7.6}$$

where h and k are the coordinates of the vertex of the parabola, and γ is a coefficient that characterizes the curvature of the parabola. The results of this research permit rewriting Equation (7.6) to give Equations (7.7) and (7.8):

$$(C_s - C_{s,iep}) = -\gamma * (pH - pH_{iep})^2 \tag{7.7}$$

Thus;

$$C_s = C_{s,iep} - \gamma * (pH - pH_{iep})^2 \tag{7.8}$$

In which, C_s is EE2 concentration that is partitioned into the solid phase (μg/kg), $C_{s,iep}$ is EE2 concentration (μg/kg) that is partitioned into the solid phase, at the isoelectric point, pH is the solution pH at which sorption concentrations are to be estimated, and pH_{iep} is the pH designating the isoelectric point. The parameter, γ, could be expressed as a function of soil characteristics. Equation (7.8) can rewrite to define γ as follows:

$$\gamma = \frac{C_{s,iep} - C_s}{\left(pH - pH_{iep}\right)^2} \qquad (7.9)$$

Utilizing the experimental data of this research, it was possible to identify dual values of γ for various soil constituents corresponding to two solution pH (acid range and alkaline range), however, it is assumed that the results are symmetrical around isoelectric point (pH_{iep}) and thus, a unique value of γ was calculated for each soil constituent. Furthermore, these γ values were plotted against the corresponding soil constituent's specific surface area, and the relationship between the both was defined to give the Formula (7.10):

$$\gamma = 0.03 * SSA - 0.62 \qquad (7.10)$$

Moreover, the term $C_{s,iep}$ in Equation (7.8) could be evaluated using Equation (7.2), since the parameters of the latter formula were derived from empirical data that were obtained under analogous experimental conditions. Accordingly, substituting Equation (7.2) into Equation (7.8) will give Equation (7.11):

$$C_s = \left(\alpha * C_e^{\beta}\right) - \gamma * \left(pH - pH_{iep}\right)^2 \tag{7.11}$$

Additionally, it is important to take into account the effect of the background solution, which represented the liquid phase in partitioning experiments. To do so, we must observe the effect that occurred when Milli-Q water was replaced by leachate from treated sludge in the case studies represented above in section 5.1.5. In both case studies, we may observe that each individual soil while applying leachate, behaved in a way analogous to their behavior resulted while using Milli-Q water, with the exception of a significant decrease in sorption capacities as a result of this replacement. This decrease could be modeled as a function of the organic matter present in liquid phase as follows:

$$K = 0.16 * OM \tag{7.12}$$

Where K represents the change in adsorption capacity due to leachate application, and OM is the percentage of organic matter present in the applied liquid phase.

Thus, the empirical model that is expected to estimate adsorbed concentration, accounting for all variable factors that were thought to be influential in this research could be offered in the following complete Equation (7.13):

$$C_s = \left(\alpha * C_e^{\beta}\right) - \gamma * \left(pH - pH_{iep}\right)^2 - K \tag{7.13}$$

Finally, the hormone-sorbed concentrations estimated in Equation (7.13) are presented in terms of equilibrium aqueous concentrations, which are usually obtained from monitoring studies. However, to model the distribution of EE2 after its direct introduction from point sources like wastewater effluents or other agricultural activities, this equation should be modified based on EE2 initial concentration, which is studied to a great extent in the literature. Consequently, it is essential to find the relationship between aqueous concentrations and hormone initial concentrations. For that, we assume that initial concentrations will partition between the liquid and the solid phases, without any losses, and hence, the mass balance could be given as:

$$C_i = C_e + C_s \tag{7.14}$$

Thus;

$$C_e = C_i - C_s \tag{7.15}$$

Where: C_i is EE2 initial concentration (μg/L), C_e is EE2 equilibrium concentration that is partitioned into the aqueous phase (μg/L), and C_s is EE2 concentration that is partitioned into the solid phase given in μg/L units. Furthermore, it is also imperative to find the correlation between the initial hormone concentration and adsorbed concentrations. Therefore, Equation (7.15) should be further manipulated to include a single variable that is EE2 initial concentration C_i. To achieve that, the relationship between initial and sorbed concentrations was derived for the experimental results, as shown in Figure 5.2, to give the Formula (7.16):

$$C_s = \frac{\delta}{100} * C_i \tag{7.16}$$

In which δ represents the percentage of initial EE2 that is partitioned into the solid phase. Accordingly, a value of the coefficient δ was found for each soil constituent. These values were further characterized as a function of soil property (specific surface area) to give Equation (7.17):

$$\delta = 0.44 * SSA + 29.49 \tag{7.17}$$

Consequently, by substituting Equation (7.16) into Equation (7.15) we can obtain Equations (7.18) and (7.19):

$$C_e = C_i - \left(\frac{\delta}{100} * C_i \right) \tag{7.18}$$

Hence,

$$C_e = C_i * \left(1 - \frac{\delta}{100} \right) \tag{7.19}$$

Finally, substituting Equation (7.19) into Equation (7.13), will give the final complete Equation (7.20) of the model in the form:

$$C_s = \alpha * \left(C_i * \left(1 - \frac{\delta}{100} \right) \right)^{\beta} - \gamma * \left(pH - pH_{iep} \right)^2 - K \tag{7.20}$$

It is worth mentioning that the developed model is applicable to low concentrations of EE2 (from 100 ng/L to 10 µg/L) used in this investigation. The coefficient in regards to generally low subsurface temperatures (30 °C was applied for all tests) might be also applied. Never the less, the application of this model could be further extended to predict the partitioning of other hormones and hydrophobic environmental pollutants. This model might be practical for engineers where based on typical geotechnical data (specific surface areas) they could predict sorption. The usefulness of proposed model is obvious for prediction of an impact of: a) the discharged water from WWTP, b) management of waste and biosolids, and for designing effective contaminant-containment barriers.

7.2 Model Validation

An empirical model was developed to estimate sorbed concentrations of the estrogenic steroidal hormone 17α-ethynyestradiol within soil components given its environmental initial concentration. The developed model takes into consideration the potential effects arising from changes in environmental conditions, precisely changes in solution chemistry (i.e. solution pH), changes in environmental levels of EE2, and probable effects due to the application of leachate from biosolids or reuse of wastewater effluents as common agricultural practices. The model was formulated in the following final Equation (7.20). In this model, the parameters α, β, δ, and γ are calculated by the general Formula (7.21):

$$\lambda_i = a_i * SSA + b_i \qquad (7.21)$$

In which λ_i represents the parameter to be calculated, SSA is the respective soil mineral specific surface area (m^2/g), a_i and b_i are constants given in Table 7.1. Finally, the term K can be calculated by Equation (7.12) given the percentage of organic matter present in the applied liquid phase.

Table 7. 1: List of Model Constants

Parameter λ_i	Constants	
	a_i	b_i
α	0.35	-11.15
β	-0.002	1.17
δ	0.44	29.49
γ	0.03	-0.62

To prove the validity of the model, with all the assumptions and simplification used during its development, this section will use the empirical model to regenerate some of the experimental data. This step will verify the applicability of the model to estimate the extent of EE2 sorption to various soil minerals, and consequently the affinity of the steroidal hormone EE2 to partition into these constituents under variable environmental conditions, such as changes in pH conditions and the potential impact of some agricultural activities, such as the reuse to treated wastewater or the leachate from biosolids. Multiple case studies will be studied and evaluated as follows: Case 1: partitioning of EE2 onto clay mineral (illite) using Milli-Q water, Case 2: partitioning of EE2 onto clay mineral (illite) using leachate, Case 3: partitioning of EE2 onto iron oxide (goethite) using Milli-Q water, and Case 4: partitioning of EE2 onto iron oxide (goethite) using leachate.

Case 1: Partitioning of EE2 onto clay mineral (illite) using Milli-Q water

The effect of pH on the behavior of partitioning of EE2 onto clay mineral (illite) was investigated in batch sorption experiments (section 4.4.3). The results of the test were displayed in Figure 5.5. The developed model (Equations 7.20 and 7.21) was employed (input values, Table 7.2) to mathematically predict these data and the results are shown in Figure 7.1. The figure clearly demonstrates the capability of the model to regenerate the results obtained in the laboratory within differences less than 20% and standard deviation < 3 (Appendix B).

Table 7. 2: Model Validation Case 1: Input Values

C_i	µg/L	10
pH_{iep}	unitless	7.5
K	%	0.0
α	µg/kg / (µg/L)n	16.80
β	unitless	0.98
γ	unitless	1.93
δ	%	63.33

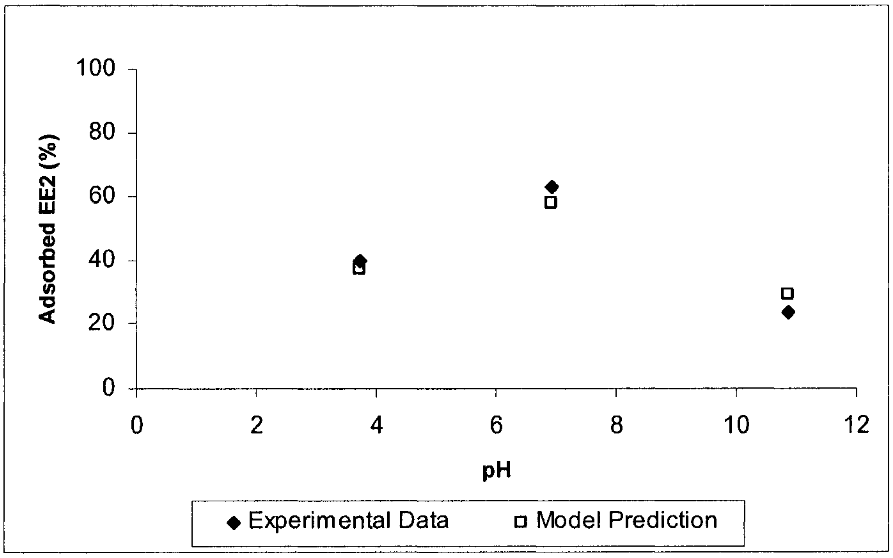


Figure 7. 1: Model Validation Case 1: Partitioning of EE2 to Illite using Milli-Q Water

Case 2: Partitioning of EE2 onto clay mineral (illite) using leachate

The effect of the introduction of wastewater effluents or leachate from treated sludge and biosolids was also studied (section 4.4.5). The results of introducing such media on the affinity of EE2 to adsorb to illite were shown in Figure 5.19. The developed model (input values, Table 7.3), here again, showed a great potential of its capability to reflect upon the synthetic estrogen distribution and behavior under the investigated conditions. The results are illustrated in Figure 7.2. The predicted results were in the vicinity of experimental data with standard deviations of < 6 (Appendix B).

Table 7. 3: Model Validation Case 2: Input Values

C_i	μg/L	10
pH_{iep}	unitless	7.5
K	%	11.03
α	μg/kg / (μg/L)n	16.80
β	unitless	0.98
γ	unitless	1.93
δ	%	63.33

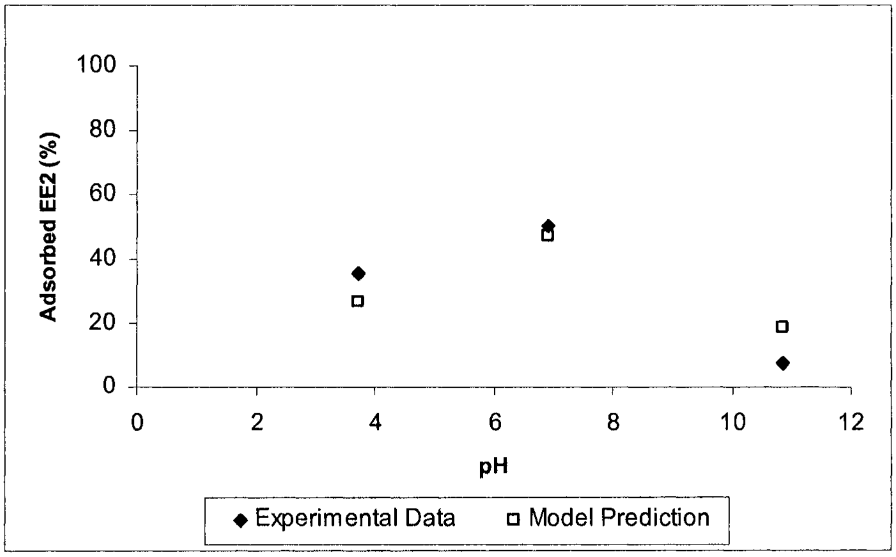


Figure 7. 2: Model Validation Case 2: Partitioning of EE2 to Illite using Leachate

Case 3: Partitioning of EE2 onto iron oxide (goethite) using Milli-Q water

The effect of pH on the interaction between EE2 and goethite (iron oxide) was examined in a laboratory configuration (section 4.4.3). Figure 5.7 demonstrated the results of these tests. These data were mathematically estimated using Equations (7.20) and (7.21) and input values (Table 7.4). The results were found to be very similar to the measured experimental results with standard deviation ranging from 0.24 to 3. A graphical illustration of these results is shown in Figure 7.3 (Appendix B).

Table 7. 4: Model Validation Case 3: Input Values

C_i	μg/L	10
pH_{iep}	unitless	7.3
K	%	0.0
α	μg/kg / (μg/L)n	6.32
β	unitless	1.05
γ	unitless	0.98
δ	%	51.27

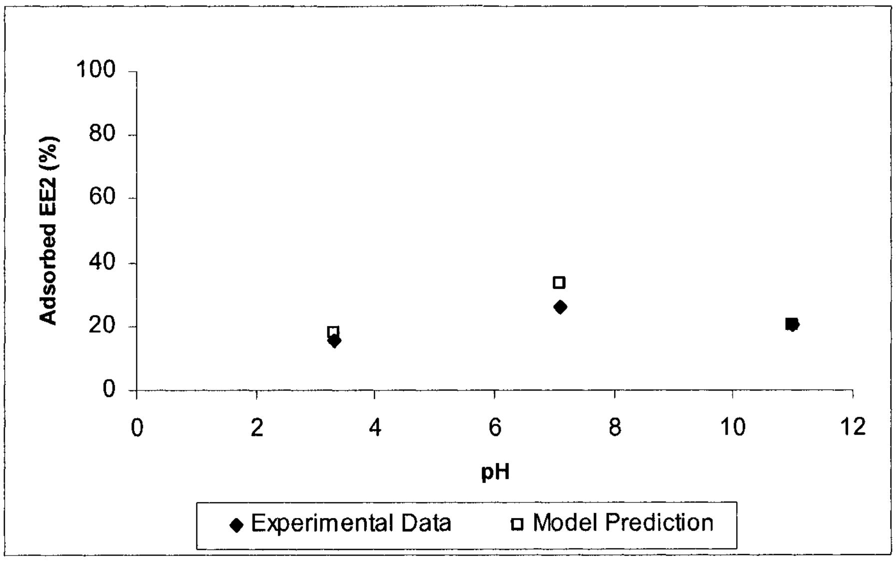


Figure 7. 3: Model Validation Case 3: Partitioning of EE2 to Goethite using Milli-Q Water

Case 4: Partitioning of EE2 onto iron oxide (goethite) using leachate

As described earlier (section 4.4.5) the potential hazard of some agricultural activities (reuse of wastewater or the use of biosolids as fertilizers) on the mobility of EE2 and its interaction with goethite was studied in batch sorption experiments. The developed model predictions, shown in Figure 7.4 (input values, Table 7.5), were nearly identical to data generated from laboratory analysis (Figure 5.21) with standard deviations as low as 1 (Appendix B).

Table 7. 5: Model Validation Case 4: Input Values

C_i	µg/L	10
pH_{iep}	unitless	7.3
K	%	11.03
α	µg/kg / (µg/L)n	6.32
β	unitless	1.05
γ	unitless	0.98
δ	%	51.27

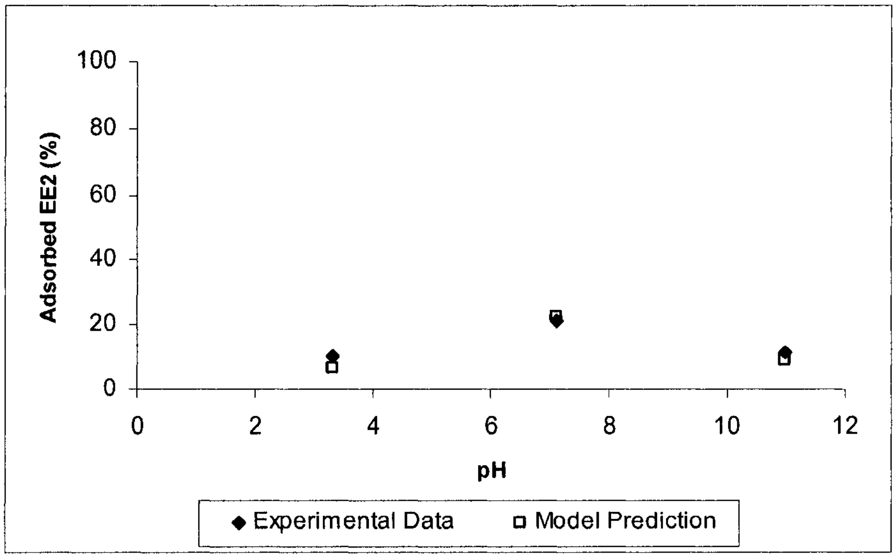


Figure 7. 4: Model Validation Case 4: Partitioning of EE2 to Goethite using Leachate

As can be seen from these results, the model has a great potential of regenerating experimental results, and could therefore be applicable to predict various environmental scenarios and estimate the extent to which the estrogenic hormone is expected to partition to solid phases, particularly into soil minerals. This model will, consequently, improve the ability to forecast environmental concentrations and to assess the hazard of organic estrogens to endanger groundwater and environmental ecosystems.

8.0 CONCLUSION AND CONTRIBUTION

This research gained a greater insight about the distribution and behavior of synthetic steroidal estrogens within subsurface soil media. Understanding the partitioning of these synthetic hormones between liquid and solid phases is crucial for the prediction and assessment of their behavior and fates in environmental systems. Most research on estrogens has been conducted on water samples, with solid samples largely being overlooked. Soils, in particular, have received very little attention, and data on these matrices are very scarce in the literature. The experimental work presented in this thesis permitted the investigation of the partitioning of 17α-ethynylestradiol to distinctive soil constituents such as humic acid, oxides (goethite) and clay minerals including illite and montmorillonite. The investigations were extended to the EE2 partitioning into a natural soil matrix from Montreal Island. The additional case studies simulated a complex situation when leachate from biosolids containing EE2 is partitioning to soil constituents in various environmental conditions.

Batch-equilibration methods were conducted at the nanogram level in an attempt to emulate current environmental concentrations. It was found that the synthetic steroidal estrogen, 17α-ethynylestradiol, expressed different affinities of binding to soil constituents. Partitioning onto clay minerals was greater than onto iron oxide or humic acid. Clay mineral, montmorillonite, exhibited highest sorption capacity (ca. 80%), while goethite showed the lowest sorption capacity of only 40%. Kinetic experiments also demonstrated that EE2 uptake onto various soil constituents followed dissimilar approaches; a distinctive two-stage behavior for clay minerals as opposed to a single rapid mode for either goethite or humic acid. The two-stage approach, seen for montmorillonite and illite, was characterized by an initial rapid uptake of EE2 onto external clay surfaces within the first hour, and a slower second-phase sorption, over 24 hours, attributed to diffusion of EE2 into intra-aggregate micropores and perhaps intercalation into interlayer regions of expanding clay. In contrast, no further sorption was observed for goethite and humic acid beyond the rapid uptake within the first hour, which is attributed to interactions with external surfaces. These results suggest the correlation between EE2 partitioning and specific surface areas and the availability of adsorption sites of various soil constituents.

In the current research it was evident that EE2 sorption to soil constituents had a great dependency on pH. This phenomenon was explained by the resulting electrical charge on their surfaces. Highest sorption values were achieved at the isoelectric points of individual soil constituent; where uncharged soil surfaces are most abundant. It was found that the interaction between the synthetic steroid estrogen (EE2) and various soil constituents was governed by the combination of binding the non-polar molecule to uncharged soil surface hydroxyl groups through hydrogen bonding, in conjunction with hydrophobic interaction with siloxane surface, ascribed to van der Waals interactions. As such, altering the population of terminal hydroxyl groups accompanied with varying the electrical charge on the surfaces of soil constituents resulted, generally, in a lesser amount of EE2 being adsorbed to these charged surfaces. It is, consequently, conceivable that shifting environmental conditions towards more pronounced acidic or alkaline conditions will have a great influence on the release and mobility of the estrogenic hormone within soil media.

The presence or absence of oxygen had limited or no contribution towards the uptake of EE2 by various soil constituents. These findings also imply that the affinity of EE2 to partition to soil constituents within subsoil media is not anticipated to differ between aerated topsoil regions and deep ground levels of air deficiency. Consequently, the release and mobility of EE2 is not expected to vary with depth into soil subsurface as a function of oxygen availability.

It was essential for the scope of this research to verify the implication of more realistic environmental circumstances where wastewaters and leachate from biosolids are being applied to a great extent over agricultural land and disposal sites. Such investigation has significantly enhanced the ability to assess and predict potential hazards of contaminating groundwater and surrounding water bodies. Principally, the application of leachate reduced the affinity of organic EE2 to partition into illite, natural soil, and goethite. In other words, the application of leachate would increase the apparent mobility of organic estrogens within soil environment. A significantly different behavior was exhibited by montmorillonite. The application of leachate from biosolids increased EE2 affinity to partition into the solid phase.

An empirical model was developed to estimate sorbed concentrations of the estrogenic steroidal hormone 17α-ethynyestradiol within soil components given its environmental initial concentration. The developed model takes into consideration the potential effects arising from changes in environmental conditions, precisely changes in solution chemistry (i.e. solution pH), changes in environmental levels of EE2, and probable effects due to the application of leachate from biosolids or reuse of wastewater effluents as common agricultural practices. This model could be very practical for engineers where based on typical geotechnical data they could predict sorption. Such a practice could be very useful for the discharge of waste and biosolids, or for designing contaminant-containment barriers.

Finally, this study results suggest that montmorillonite possessed the greatest sorption capacities under a wide range of environmental conditions. Additionally, it also showed a great potential to uptake the estrogenic hormone from applied wastewater effluents. Accordingly, is seems fair to recommend the use of montmorillonite as an effective amendment in building contaminant containment barriers, as they would efficiently enhance the retention of organic compounds and retard if not stop their migration towards groundwater. Other applications would be, for example, the use of clay minerals (especially montmorillonite) as useful materials into reactors of water and wastewater treatment plants. This will in turn promote the affinity of synthetic estrogens (i.e. EE2) to partition into the solid phase and consequently reduce their concentrations in the aqueous phase. Although the adsorbing EE2 to clay minerals is expected to decrease the hazard potential of leaching out to groundwater, surface water bodies are still in great danger of contamination through runoff. Moreover, the transport and translocation of these pollutants in lakes and rivers will greatly depend on their interaction with highly mobile suspended particles and colloidal matter, the hydrology of the ecosystem, and the subsequent settling, resuspension and sedimentation rates.

Potential Application of Results

The results of this research could be employed to serve some potential applications, such as:

(1) Prevention of runoff and/or infiltration to subsurface.

(2) Verification of the implication of common practices over agricultural land and disposal sites.

(3) Assessment and prediction of potential hazards of contaminating groundwater and surrounding water bodies.

(4) Enhancement of common engineering practices, such as the discharge of waste and biosolids, and the design of containment-contaminant barriers.

(5) Regulation of environmental legislations.

Contributions to Knowledge

(1) The research developed an analytical method for the extraction and measurement of 17α-ethynylestradiol in water and wastewater.

(2) It defined the behavior of 17α-ethynylestradiol in the presence of secondary minerals and natural soil.

(3) It defined the kinematics of sorption of low concentration sorbate (17α-ethynylestradiol) to different sorbents.

(4) It defined the partitioning of 17α-ethynylestradiol in the absence of oxygen.

(5) It defined the mechanisms of partitioning of 17α-ethynylestradiol to different charged surfaces.

(6) It developed an empirical model to predict the partitioning of estrogenic compounds.

(7) It assessed the impact of the presence of leachate from biosolids on the partitioning of 17α-ethynylestradiol.

(8) It suggested some practical research applications.

9.0 FUTURE WORK

This chapter provides recommendations for future work to be pursued in this field of research:

(1) Evaluation of the effect of temperature on the maximum achievable rates of the synthetic estrogen partitioning.

(2) Assessment of the mass balance based on analyzing and measuring the hormone concentration in both the liquid and the solid phases.

(3) Determination of the synergetic effect of a multi-component steroid system.

(4) Estimation of partitioning coefficient using soil leaching column methods.

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APPENDIX A: EXPERIMENTAL DATA

pH Effect

Table A. 1: pH effect on EE2 Adsorption to Illite, O_2

pH	Point #1	Point #2	Mean	Standard Deviation
3.72	41.02	39.61	40.31	0.7
6.93	62.84	63.32	63.08	0.24
10.86	25.66	22.00	23.83	1.83

Table A. 2: pH effect on EE2 Adsorption to Montmorillonite, O_2

pH	Point #1	Point #2	Mean	Standard Deviation
3.21	98.88	98.43	98.65	0.22
7.00	81.49	85.66	83.58	2.09
10.52	58.84	62.06	60.45	1.61

Table A. 3: pH effect on EE2 Adsorption to Goethite, O_2

pH	Point #1	Point #2	Mean	Standard Deviation
3.31	32.45	30.16	31.30	1.14
7.11	53.59	50.42	52.01	1.58
10.98	41.65	41.08	41.37	0.28

Table A. 4: pH effect on EE2 Adsorption to Humic Acid, O_2

pH	Point #1	Point #2	Mean	Standard Deviation
2.52	99.31	99.42	99.37	0.06
7.08	58.69	58.38	58.53	0.15
11.44	47.17	44.23	45.7	1.47

Table A. 5: pH effect on EE2 Adsorption to Natural Soil, O_2

pH	Point #1	Point #2	Mean	Standard Deviation
3.35	53.13	54.29	53.71	0.58
6.73	67.93	68.96	68.44	0.52
11.18	28.64	25.97	27.31	1.34

Effect of Anaerobic Conditions

Table A. 6: pH effect on EE2 Adsorption to Illite, N_2

pH	Point #1	Point #2	Mean	Standard Deviation
3.54	41.08	43.41	42.25	1.16
7.01	74.66	83.19	78.92	4.26
11.18	23.81	13.97	18.89	4.92

Table A. 7: pH effect on EE2 Adsorption to Montmorillonite, N_2

pH	Point #1	Point #2	Mean	Standard Deviation
3.16	86.34	90.00	88.17	1.83
6.55	89.39	81.08	85.24	4.16
10.75	78.69	77.00	77.85	0.85

Table A. 8: pH effect on EE2 Adsorption to Goethite, N_2

pH	Point #1	Point #2	Mean	Standard Deviation
3.50	92.78	95.63	94.20	1.43
7.06	35.35	37.24	36.29	0.94
10.9	16.67	7.48	12.07	4.59

Table A. 9: pH effect on EE2 Adsorption to Humic Acid, N_2

pH	Point #1	Point #2	Mean	Standard Deviation
2.27	99.53	99.31	99.42	0.11
6.93	44.76	46.27	45.51	0.75
11.71	53.31	31.15	42.23	11.08

Table A. 10: pH effect on EE2 Adsorption to Natural Soil, N_2

pH	Point #1	Point #2	Mean	Standard Deviation
3.54	56.55	50.95	53.75	2.8
7.03	67.66	66.94	67.3	0.36
10.5	20.70	19.15	19.92	0.78

APPENDIX B: MODEL EVALUATION

Table B. 1: Model Evaluation Case 1: Partitioning of EE2 to Illite using Milli-Q Water

pH	Experimental Data*	Model Prediction*	Standard Deviation
3.72	40.31	37.62	1.35
6.93	63.08	58.42	2.33
10.86	23.83	29.61	2.89

* Adsorbed EE2 (%)

Table B. 2: Model Evaluation Case 2: Partitioning of EE2 to Illite using Leachate

pH	Experimental Data*	Model Prediction*	Standard Deviation
3.72	35.44	26.58	4.43
6.93	50.11	47.38	1.36
10.86	7.18	18.57	5.70

* Adsorbed EE2 (%)

Table B. 3: Model Evaluation Case 3: Partitioning of EE2 to Goethite using Milli-Q Water

pH	Experimental Data*	Model Prediction*	Standard Deviation
3.31	15.65	17.89	1.12
7.11	26.01	33.41	3.70
10.98	20.69	20.21	0.24

* Adsorbed EE2 (%)

Table B. 4: Model Evaluation Case 4: Partitioning of EE2 to Goethite using Leachate

pH	Experimental Data*	Model Prediction*	Standard Deviation
3.31	10.08	6.86	1.61
7.11	21.33	22.38	0.52
10.98	11.56	9.18	1.19

* Adsorbed EE2 (%)

APPENDIX C: GLOSSARY

APEO : Alkylphenol Polyethoxylates

CEC : Cation Exchange Capacity

D4 : Internal standard (17a-Ethynylestradiol-2,4,16,16-d4)

EDC : Endocrine Disrupting Chemicals

E1 : Estrone

E2 : Estradiol

E3 : Estriol

EE2 : 17α-Ethynylestradiol

FTIR : Fourier Transform Infrared Spectroscopy

HPLC : High Pressure Liquid Chromatography

K_{oc} : Normalized Organic Carbon Partitioning Coefficient

K_{ow} : Octanol Water Partitioning Coefficient

MeEE2: Mestranol

MS : Mass Spectrometer

PAH : Polycyclic Aromatic Hydrocarbons

PBDE : Polybrominated Diphenyl Ethers

PCB : Polychlorinated Biphenyls

pK_a : Dissociation Coefficient

SSA : Specific Surface Area

STP : Sewage Treatment Plant

STW : Sewage Treatment Work

UV/Vis: UV/Visible Spectrophotometer

WWTP: Wastewater Treatment Plant