The Efficiencies of Subsoils for on-site wastewater disposal with respect to Endocrine Disrupting Chemicals

Small Scale Study prepared for the Environment Protection Agency

L.W. Gill & C. Ó Súilleabháin

Department of Civil, Structural and Environmental Engineering Trinity College Dublin

Dr F. Regan & A. Moran

School of Chemical Sciences Dublin City University

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1.0 Introduction

The endocrine system controls the basic functions of reproduction, growth and maintenance by a system of chemical messengers (hormones) in animals. It is well known that certain chemicals can interfere with the endocrine system in many ways to produce an undesired response or disruption, affecting the health, growth and reproduction of a wide range of organisms. This phenomenon of Endocrine Disrupting Chemicals (EDCs) has emerged as a major environmental and human health issue and has attracted much research attention worldwide in the last decade.

In the Irish environment, there has been little research to date on this issue, apart from an ongoing EPA funded project entitled, "Endocrine Disrupting Substances in the Irish aquatic environment" (2000-MS-2-M1). This project has primarily been looking at surface freshwater resources and in particular the effects of EDCs from Wastewater Treatment Works. However, there has been scant research both in Ireland and worldwide on the fate of EDCs and removal processes from on-site wastewater treatment systems and more particularly the effluent which percolates through the subsoil en route to the groundwater resource.

In Ireland, wastewater from over one third of the population is treated in small-scale independent systems where connection to a sewer is deemed to be unfeasible, usually in rural areas. The most prevalent treatment application is the conventional septic tank system with over 350 000 systems currently installed in Ireland (EPA, 2000). In situations where a septic tank installation is not suitable, additional treatment in the form of mechanically aerated systems or filter systems can be used which include rotating biological contactors (RBC), biologically aerated flooded filters (BAFF) and sequencing batch reactors (SBR). The effluent then flows from the treatment system into a suitable soil percolation area where further physical, chemical and biological secondary treatment processes occur.

1.1 Background

Groundwater is an important resource in Ireland which is under increasing risk from human activities with contamination arising from both 'diffuse' (generally agricultural)

and 'point sources', the latter being exemplified by farmyards (manure and silage storage) and septic tank systems (Daly, 1993). The main aquifers occur in fissured bedrock formations, overlain by superficial deposits (referred to as subsoils in Ireland) of variable thickness and permeability. In areas where the subsoil permeability is too low to allow sufficient soakage there is a risk to watercourses from effluent ponding. Groundwater, on the other hand, is especially at risk in areas where bedrock is close to the surface, where subsoils of high permeability underlie the site and where the water table is close to the surface. With an estimated 200,000 wells and springs in use in Ireland (Wright, 1999) the prevention of groundwater contamination from on-site domestic sewage effluent is of critical importance as, once contaminated, the consequences are usually longer lasting than for surface water owing to longer residence times; moreover, groundwater remediation is usually expensive and often impossible.

The national groundwater protection scheme is based on the concepts of risk and risk management. A hazard-pathway-target model is used for the scheme, in which the hazard is the potentially polluting activity, the pathway is the groundwater vulnerability and the target is the groundwater resource, generally assumed to be the aquifer, or source such as a water supply well or spring (Misstear *et al*., 1998). For protection scheme purposes, the groundwater vulnerability is subdivided into extreme, high, moderate or low vulnerability categories. The division of groundwater into these vulnerability categories is dependent on various factors, the principal ones being the permeability and thickness of the subsoil. The permeability governs the transmission rate of a fluid through the subsoil, and hence the period in which attenuation of contaminants can take place by physical, chemical and/or biological processes. Therefore the permeability of a given subsoil thickness provides a measure of the amount of amelioration that can occur.

A recommended septic tank treatment process involves domestic wastewater (excluding roof / road drainage) flowing into a two chambered tank in which primary sedimentation occurs and also some anaerobic digestion. The effluent then overflows into a suitable soil percolation area where further physical, chemical and biological treatment processes occur. The subsoil percolation area performs a straining and filtration function, in

conjunction with sorption and ion exchange and acts as an attached growth medium for aerobic biodegradation due to the unsaturated nature of the soil. Hence, the hydraulic and attenuation properties of the natural ground downstream of the treatment system are important for the further removal of pollutants and the protection of water resources and are viewed as an inherent part of the treatment system.

The publication of the guidance manual for Treatment Systems for Single Houses (EPA, 2000) is aimed at protecting groundwater resources from contamination by domestic wastewater effluent by defining acceptable site suitability criteria. Key to this approach is an intensive site assessment procedure. This assessment, comprised of a desk study followed by an on-site visual trial hole inspection and percolation test, determines the vulnerability of local groundwater resources and identifies receptors potentially at risk. The percolation test is also required to determine the assimilation capacity of the subsoil. It is recommended that the percolation rate obtained from the standard percolation test (the so-called T-value) for subsoils receiving septic tank wastewater effluent must fall within the specified range of 1min per 25mm to 50min per 25mm of water level fall (i.e. 1 to 50). In addition, a minimum unsaturated subsoil depth of 1.2m below septic tank percolation fields should exist before the site may be deemed suitable for on-site treatment of domestic wastewater effluent from a conventional septic tank design. Where subsoil T-values fall outside the range 1 to 50, or the minimum unsaturated subsoil depth of 1.2m does not exist, a second percolation test (the P-test) should be carried out from ground level. If the resulting P value is in the range 1 to 50 then the guidance manual suggests installing some form of secondary treatment process, providing that there is 0.6m of unsaturated subsoil for percolation of the secondary effluent. These small-scale treatment systems (RBC, SBR etc) provide secondary treatment aerobic degradation either by fixed film or suspended growth microbial processes. Another option promoted in the guidelines is the use of an intermittent stratified sand filter either as a secondary treatment unit or as a polishing filter in place of the percolation area. A stratified sand filter normally comprises of three layers of sand which decrease in coarseness with depth. Effluent is dosed uniformly onto the filter surface at regular intervals by a pump from a collection chamber.

This small scale project presents the results of a series of samples taken and analysed specifically for Endocrine Disrupting Chemicals from four sites which were being monitored as part of the three year research study funded by the Environmental Protection Agency (2000-MS-15-M1) into the effectiveness of both septic tank and secondary treatment on-site wastewater systems designed according to the Treatment Systems for Single Houses guidelines (EPA, 2000).

1.2 Project Aims and Objectives

This small scale project aims to provide an insight into the treatment effectiveness of both the on-site systems and the in-situ subsoils with respect to certain EDCs by dovetailing into the ongoing research project (2000-MS-15-M1), thus gaining the benefit of the extensive experimental infrastructure whilst it was established. The existing sites were being sampled regularly for a range chemical and biological determinants and further benefit from the project is yielded by this additional study into EDCs from on-site wastewater disposal systems, an area which has received little attention to date. Although only one set of samples were taken from each site due to the financial constraints, the study aims to provide a brief insight as to the extent of any potential groundwater pollution by such EDCs and indicate whether further long-term studies are deemed strategically necessary.

The aim of the small-scale project was to determine the fate of five known EDCs typically found in domestic wastewater effluent: three natural steroids (oestrone, 17β oestradiol and oestriol), one surfactant (nonylphenol) and one organic oxygen compound (bisphenol A). The sites were designed so that comparisons could be made with respect to their degradation in septic tanks, in a secondary treatment peat filter and then finally down through the depth of different subsoil types.

The main MS-15 project was divided into two phases of on-site field trials on a total of four sites. The sites were fundamentally chosen according to the site assessment procedure as set out in Treatment Systems for Single Houses (EPA, 2000). One set of

EDC samples were taken from a representative percolation trench on each site at end of each respective trial period which ensured that the maximum time had been allowed for the site to become established. It should be noted that no EDC samples were taken from the stratified sand filters since it was outside the scope of this project.

A summary of the project is presented as follows,

July 02-July03 Two parallel on-site trials at sites in County Kildare of 12 months duration where effluent was discharged into a standard percolation.

- Site 1 : septic tank effluent into subsoil with T-value of 15
- Site 2 : secondary treated effluent into subsoil with T-value of 29

July03-March 04 Two parallel on-site trials at sites in County Wicklow of 9 months duration where effluent was evenly split between a standard percolation area and a stratified sand filter.

- Site 3 : septic tank effluent into subsoil with T-value of 33
- Site 4 : secondary treated effluent into subsoil with T-value of 52

2.0 Background

Recent years have seen heightened interest into the investigation of a number of compounds that interfere with the normal action of the endocrine system, collectively known as Endocrine Disrupting Compounds (EDCs). An EDC has been defined as '*an exogenous substance or mixture of substances that alters the function(s) of the endocrine system and consequently causes adverse health effects in an intact organism or it's progeny or (sub) population'*. Compounds that interfere with the action of endogenous oestrogen hormones are known as environmental oestrogens, oestrogen mimics, oestrogenic chemicals or xenoestrogens.

Numerous studies on a range of species have indicated possible endocrine disrupting effects from a host of anthropogenic chemicals. Exposure to elevated levels of environmental oestrogens has been associated with sexual disruption of fish populations, eggshell thinning in bird populations and gonad modification of reptiles. For example, a major research effort was carried out on the effects of wastewater treatment effluent on British fish which demonstrated estrogenic effects (Purdom *et al.*, 1994). Despite early evidence of the phenomenon of endocrine disruption, it has only become a focussed topic of debate and research activity since the early 1990s. This is mainly due to the potential problems with reproductive abnormalities for human health in the form of reduced sperm quality and counts, testicular cancer and breast cancer but also because of certain wildlife experiencing endocrine disruption to their reproductive systems including egg shell thinning due to pesticides and the feminising effects of estrogens and surfactants on fish (Colborn *et al.*, 1996).

Compounds identified as priority EDCs include various members of distinct chemical groups such as the bioflavanoids, synthetic oestrogens, phenols, alkylphenols, polychlorinated biphenyls, phthalates, brominated flame retardants, organochlorine pesticides, dioxans and furans. The diverse chemical nature of these compounds is reflected in the diversity of their application, with their widespread use leading to their distribution in various environmental and biological matrices. In order to appreciate the

consequences of exposures to EDCs, it is important to highlight the significance of the endocrine system and of the disruption of its normal function in biological systems.

2.1 The Endocrine System

The body has two important communication systems: a) the nervous system and b) the endocrine system, which regulate and co-ordinate the activity of the body's muscles and internal organs. The endocrine system in both plants and animals is responsible for regulating and integrating the function of different cells and is responsible for growth, reproduction, maintenance, homeostasis and metabolism. In animals the endocrine system consists of several glands in different areas of the body which produce hormones with different functions. These hormones are transported by the bloodstream to target organs where they invoke as natural response. The mechanism of action of the steroid hormones is based on a lock-and-key receptor binding procedure. The target cells are comprised of a bonding site (receptor) and effector site. Hormones attach to the receptor and the effector site is altered which produces the desired response. The receptor sites have a very high affinity for a specific hormone meaning that only very low concentrations are required to achieve the response. There are, however, other chemical compounds that can interact with the hormone-binding site of the receptor and such EDCs can elicit a response at low concentrations.

2.1.1 Mode of Endocrine Disruption

The proposed mode of action of an EDC is to bind to the receptor site in place of the natural hormone. If an EDC succeeds in binding to the hormone receptor it has the potential to activate or block the cellular response characteristic of the hormone it has replaced. Compounds possessing remarkably different structures have been found to compete for the traditional oestrogen binding sites on the oestrogen receptor and elicit varying degrees of response.

The EDC can interact with hormone receptors in agonist, antagonist or partial agonist manner. Agonists work to mimic the effect of normal hormones through binding to the

receptor site and inducing the expected response from the cell. Antagonists block the normal response by binding to the receptor in place of the natural hormone. If the binding is sufficient to prevent binding of the natural hormones then no response will occur. Partial agonists that bind to receptor sites will not induce the full response from the cell but some effect will be observed. There can also be more complicated effects such as disruptions of the synthesis and removal of hormones and their receptors and interactions with multiple hormone systems (Birkett and Lester, 2002). While the molecular structure of EDCs binding to specific receptors can vary greatly, there are often similarities between these compounds; many of the oestrogen mimicking EDCs share the common structural motif of a phenol or a functional equivalent. The natural hormones are normally very short-lived due to a metabolic clearance mechanism within the body. However, certain EDCs tend to be very persistent to such metabolic clearance and can also bio-accumulate within the body, obviously rendering them of concern even in very slight concentrations.

2.2 Sources of Endocrine Disrupting Chemicals

Many different types of compounds have been identified as EDCs, all having very different applications. The broad nature of application has resulted in the distribution of these compounds in various environmental and biological matrices and leads to the risk of repeated exposures with compounds originating from different sources. The main sources of exposure to these compounds include the environment, dietary components and medical and cosmetic products.

The aquatic environment acts as a sink for many compounds discharged via the wastewater treatment system. The main input of EDCs to the wider environment is as a result of direct point discharge from both domestic and industrial wastewater treatment facilities. EDCs have been detected in sewage and wastewaters, surface and groundwater as well as soils and sediments. Conventional wastewater treatment facilities, i.e. those employing primary and secondary treatment technologies, are proving inefficient for the complete removal of all EDCs prior to the discharge of ‘treated effluents’. While a significant proportion of the EDCs are retained in the sludge, the stable chemical nature

of many of these compounds results in their persistence in the final effluent at low concentrations.

Aside from possible exposure through drinking water sources, dietary exposure to EDCs results from components of diet known as bioflavanoids, phytohormones or phytoestrogens. These natural plant hormones are less potent than the oestrogens found in mammals and in terms of a mass balance approach it has been suggested that the action of such weak or anti-oestrogens may work to combat the action of elevated environmental oestrogen exposure. Some flavones and isoflavones may play an important role in cancer prevention as they are found in plants that are associated with reduced cancer rates.

Several physico-chemical and biological processes determine the concentration, behaviour and ultimate fate, of chemical species in the wider environment. Such processes include sorption-desorption, volatilisation and chemical and biological transformation. The chemical characteristics of the compound, including solubility, vapour pressure and its partition coefficient, will impact upon its residence time in the wider environment.

2.3 Endocrine Disrupting Chemicals in wastewater effluent

Knowledge of endocrine disruption is constantly increasing as does the list of chemicals involved. Such chemicals have been the subject of much research activity in the past decade and are identified by either *in vivo* or *in vitro* assays (or preferably by a combination of both). The compounds can be lumped into the following generic categories: steroid compounds, surfactants, pesticides, polyaromatic hydrocarbons and other organic oxygen compounds, which are to a greater or lesser degree associated with domestic sewage effluent, the subject of this study.

2.3.1 Steroid compounds

Natural steroid oestrogens can be found in sewage effluent generated by women of reproductive age. There are at least six distinct oestrogen hormones but only three are

present in significant amounts in the female body; 17β-oestradiol, oestrone and oestriol. All three molecules have a 17-carbon system, which is the steroid with a methyl group at carbon-13 and an aromatic ring with a hydroxyl group at carbon-3 (Figure 2.1). 17β-oestradiol is the most biologically active oestrogen produced by the ovaries, and is synthesised from androgens by the aromatase complex of enzymes. Natural oestrogens can be considered EDCs after deconjugation restores biological potency.

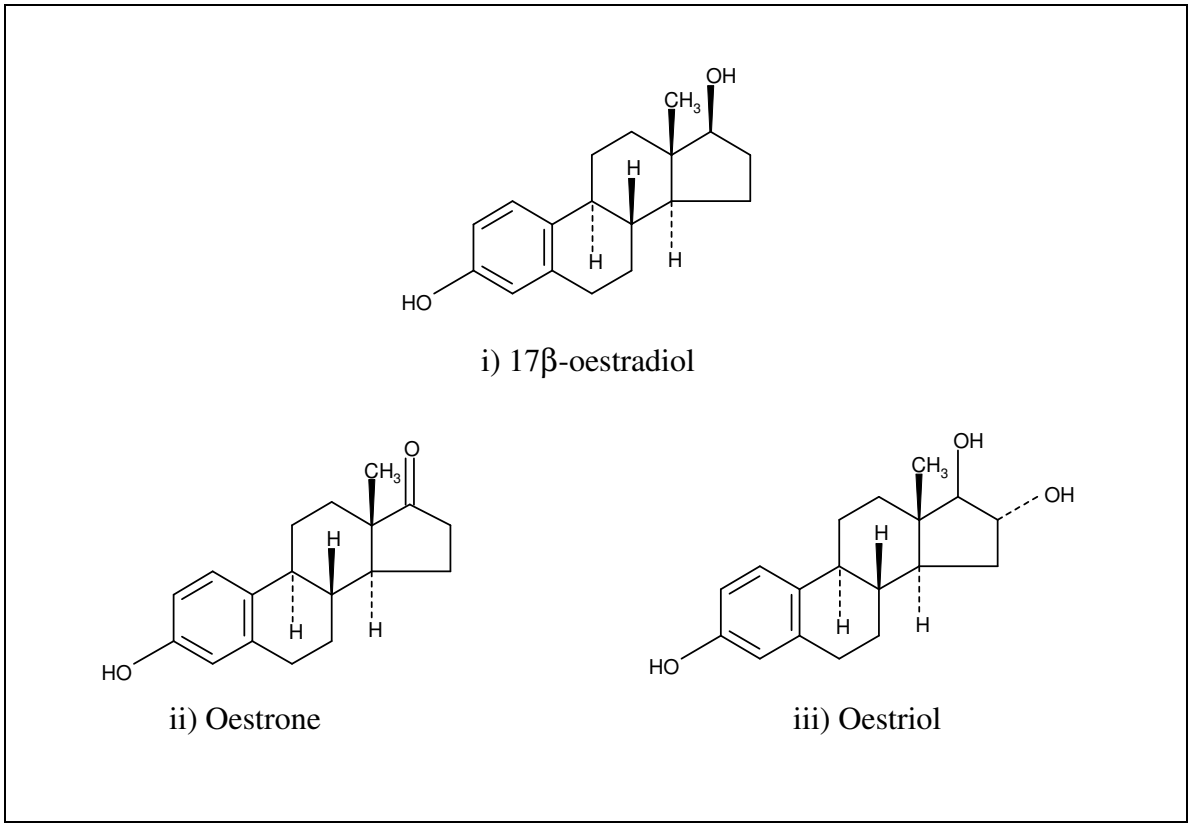
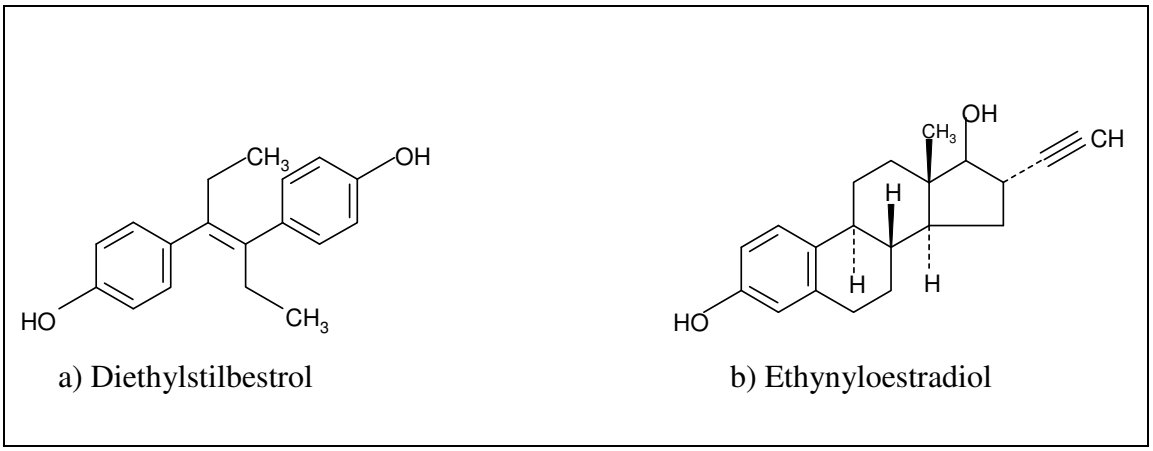


Figure 2.1 Chemical structure of three natural estrogens

Synthetic oestrogens are also found in wastewater effluent originating from the contraceptive pill or Hormone Replacement Therapy (for example, diethylstilbestrol and ethynyloestradiol, as shown in Figure 2.2).



a) Diethylstilbestrol b) Ethynyloestradiol

Figure 2.2 Chemical structures of synthetic estrogens: diethylstilbestrol and ethynyloestradiol.

Testosterone is the principal hormone of the androgen group of steroids. Secretion of testosterone increases during puberty and is responsible for the development of male secondary sexual characteristics. In women androgens are present in the form of androstenedione, which can be converted to testosterone and dihydrotestosterone as needed. Suppression of androgen activity has been seen to improve the survival rate of patients diagnosed with prostate cancer. Anti-androgen treatments are being investigated as therapeutic agents. The synthesis, transport and metabolism of androgens may be disrupted, by both xenoestrogenic and anti-androgenic substances. Progesterone and testosterone can be found in lower concentrations than oestrogens in wastewater and mainly originate from food, in particular meat products, where the hormones have been used as growth enhancers in the livestock beforehand.

Steroid compounds tend to be lipophilic which makes them sparingly soluble in water and hence most likely to sorb onto particulates but natural oestrogenic steroids have a higher solubility than synthetic steroids (Ying *et al.*, 2002).

2.3.2 Phytoestrogens

These are natural compounds present in herbs, grains, vegetables and fruit which have a much weaker endocrine disrupting capability than the above endogenous oestrogenic compounds. They are also commonly found in sewage effluent as a result of food consumption. The two main groups that have been studied are isoflavones (found in

soybeans and legumes) and lignans (produced from the breakdown of fruits and vegetables in the gut).

2.3.3 Organic oxygen compounds

This group of EDCs includes dioxins, bisphenols and phthalates. The main compounds of concern in domestic sewage effluent are bisphenol A (Figure 2.3) which is generated from the production of polycarbonate and epoxy resins and certain phthalates which are involved in the manufacture of PVC and other resins. These plastics are used in food packaging, tooth fillings, dental materials (and other domestic products) and can leach into the product thereby finding their way into the sewage effluent. Bisphenol A has been shown to be 10 000 times less potent than 17β-oestradiol (Perez *et al.*, 1996) and was identified as an oestrogen mimic after tests with yeast conditioned media.

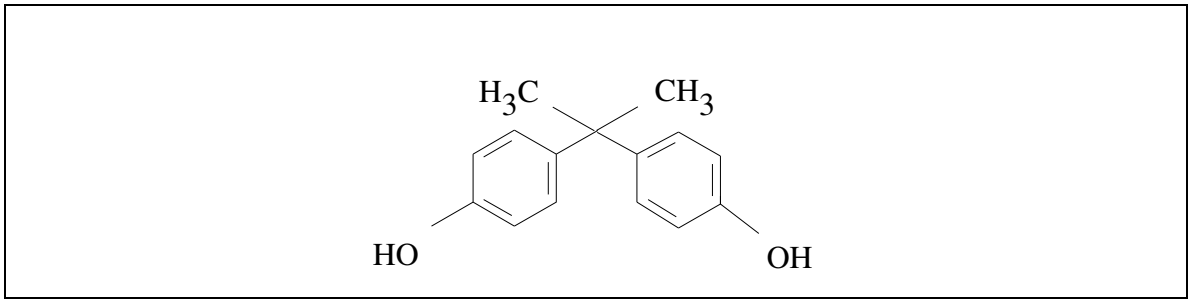


Figure 2.3 Chemical structure of bisphenol-A.

2.3.4 Surfactants

Surfactants exhibit varying degrees of oestrogenicity and are common in sewage effluent primarily from their use in detergents. The main EDCs of interest are a large number of chemicals known collectively as alkylphenols and their ethoxylates, particularly the nonylphenol compounds which are used in the household detergents and also in other cleaning products, cosmetics and paints. The alkylphenols, octylphenol and nonylphenol, are also used as antioxidants in plastics. Both nonylphenol and octylphenol (Figure 2.4) have been identified as oestrogen mimics using vitellogenin gene expression in trout hepatocytes, gene transcription in transfected cells and by the growth of breast cancer cell lines.

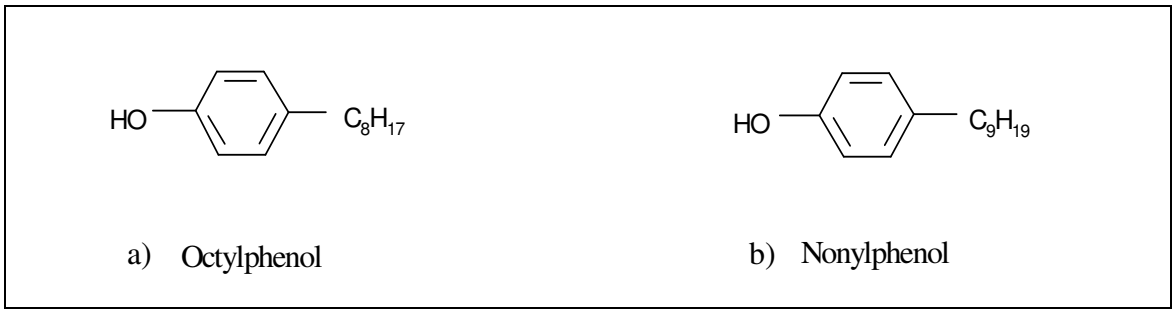


Figure 2.4 Chemical structures of octylphenol and nonylphenol

Both octylphenol and nonylphenol are found in the wider environment as a result of the biodegradation of the alkylphenolic polyethoxylates (APEOs) and are themselves recalcitrant breakdown products. The non-ionic alkylphenol ethoxylated surfactants, namely octylphenol ethoxylate and nonylphenol ethoxylate, are found in domestic and industrial products with some of the applications including shampoos, some detergent-containing petrols and pesticide formulations. Their structure consists of an alkylphenol with a side chain of several ethoxylate groups, ranging from 2 to 20 units. The biotransformation of the APEOs to the parent alkylphenols involves hydrophilic attack of the ethoxylate chain, which is progressively shortened by one ethoxylate group at a time.

There are no known natural sources of nonylphenol and so any presence detected in the environment must have originated from an industrially manufactured source. For example, alkylphenols and alkylphenol ethoxylates were found in the River Aire due to wool scouring detergents used in the local industry which promoted hermaphroditism in male trout (Walker, 2000). Most studies have shown that alkylphenols have a lower potency than bisphenol A (Fawell and Chipman, 2001).

2.3.5 Pesticides

Pesticides (including insecticides, herbicides and fungicides) comprise by far the largest group of endocrine disrupting chemicals, particularly chlorinated pesticides such as DDT and methoxychlor. Although these have serious implications both groundwater and surface water sources due to their land application and agricultural runoff, they are not generally significant in domestic wastewater effluent. However, they can enter the human

food chain via herbivores which have consumed plants which have absorbed these chemicals and also from polluted water sources. Indeed, some Water Treatment Works have installed monitoring systems upstream of their river intakes to warn of high pesticide concentrations, prevalent at certain times of year, which trigger an emergency treatment response such as the addition of powdered activated carbon into the flow.

2.3.6 Polychlorinated compounds

This group of chemicals includes polyaromatic hydrocarbons (PAH) which are usually generated from the incomplete combustion of organic materials. It also includes brominated flame retardants and polychlorinated biphenyls (PCB) which are mainly used in industrial applications. Hence, none of these polychlorinated compounds are usually found in significant concentrations in domestic effluent. PAHs, however, have been found in significant concentrations in Wastewater Treatment Works effluent from urban areas with combined sewer systems (which take surface runoff water) and also areas with appreciable industrial activity.

2.3.7 Organotin Compounds

These compounds are used predominately in the plastics industry and in particular during the processing of PVC. One of the most common organotin product is tributyltin (TBT) which has been used as an antifouling agent supplied to boat hulls. However, again these compounds are not normally a concern with respect to domestic on-site wastewater generation although there can be some traces used in household products which have been shown to leach into any food and water contain within.

2.4 Fate of EDCs in wastewater treatment plants and subsoil

Any EDC discharged into the wastewater from individual domestic premises will either experience a single stage treatment in the form of a septic tank before discharge to the percolation area or an additional level of secondary treatment, again before discharge to the subsoil. The fate and behaviour of an EDC is mainly influenced by its physiochemical properties and the majority of EDCs tend to favour adsorption onto solid surfaces or into

biota. The tendency for the EDC to adsorb onto particulates is often correlated against its octanol/water partition coefficient (K_{ow}). A log K_{ow} value of less than 2.5 indicates a low sorption potential and log K_{ow} of greater than 4 shows a high sorption potential. For example, natural oestrogens oestrone, 17β-oestradiol and oestriol have log K_{ow} values of 3.43, 3.94 and 2.81 respectively (Lai *et al.*, 2000). If log $K_{ow} \geq 4$ (eg 17β-oestradiol) then the main removal mechanism will be by adsorption due to the hydrophobic properties whilst if log K_{ow} is in the range 1.5 to 4 then there is a moderate affinity for sorption to biosolids and also biodegradation seems to be more influential (Birkett and Lester, 2003). Once through the septic tank and /or secondary treatment system, EDCs with a high solubility in water are more likely to be transported down through the subsoil and into the groundwater regime due to their greater mobility.

2.4.1 On-site wastewater treatment

Wastewater effluent produced in the house initially passes into the septic tank where sedimentation of the solids occurs. Here the most significant mechanism of EDC removal is adsorption to the solids which settle as the sludge. Numerous studies have shown that significant EDC removal occurs in the sludge at Wastewater Treatment Works (Ying *et al.*, 2002) but few studies have looked at on-site systems. One study on the night soil process in Japan (which is similar to a septic tank), showed that the majority of steroid oestrogens were accumulated in the sludge and not passed forward in the aqueous phase (Takigami *et al.*, 2000). Concentrations of 17β-oestradiol for example were 5000 times more concentrated in the sludge than in the effluent from the treatment plant. However, research of alkylphenols in on-site wastewater disposal in Cape Cod, Massachusetts identified bispehnol A concentrations in the septic tank effluent as high as 1µg/l (Rudel *et al.*, 1998).

Once in the sludge, anaerobic degradation will take place. Previous studies have looked at EDC removal in sludge but have concentrated on full-scale treatment works where the sludge is constantly removed from the unit treatment processes and sent to anaerobic digesters. These digesters are optimised in terms of temperature (normally at 35^{o}C), with the solids residence time in the region of 15 days. In septic tanks, however, the sludge

commonly spends at least one year and often much longer in the tank and the temperature in the tanks is not regulated, (being about 12 ^{o}C on average in Ireland) which produces a much slower rate of digestion. There have not been many reported studies of the breakdown of EDCs within septic tank sludge processes although it is known that steroid oestrogens are reduced (Takigami *et al.*, 2000).

Many on-site treatment systems now involve using packaged secondary treatment systems which are based on the same processes used in the large-scale municipal treatment works. In secondary treatment processes there are high concentrations of aerobic bacteria and other micro-organisms. Here EDCs can be adsorbed onto the solids but also can be degraded biologically or chemically and in some cases volatised due to the aeration required. Studies have also shown that removal efficiency of hydrophobic compounds is strongly related to solids retention time (SRT) in biological treatment processes indicating that accumulation of the compound on the biosolids (Strenn *et al.*, 2003). Several studies have indicated that both biodegradation and sorption are important in the removal of EDCs in wastewater treatment plants and also indicated that oestrone often remained the most persistent steroid oestrogen in the final effluent with concentrations up to 70 ng/l (Ternes *et al.*, 1999). This was also concluded by another study (D'Ascenzo *et al.*, 2003) that found oestrone to be the most resistant to decomposition in wastewater treatment processes compared to the other natural oestrogens. Levels of oestrone (E1), 17β-oestradiol (E2) and oestriol (E3) were measured in urine, septic tank effluent and also activated sludge effluent. The women on average excreted 32, 14 and 106 μg/day of E1, E2 and E3 respectively whereas in the septic tank effluent it showed that most of the oestrogens had been deconjugated to free oestrogens which was presumed to be by enzyme activity by the faecal bacteria. A study on steroids EE2 (17α-ethinylestradiol), E2 and E1 in secondary treated effluent in Berlin detected steroids in the ng/l range using a solid phase extraction technique with subsequent analysis performed by liquid chromatography and detection by mass spectrometry (Zuehlke *et al.*, 2004). Again it was shown that E1 persisted in the effluent at average levels of 157 ng/l which was of the order 10 times greater than E2 and EE2.

In terms of surfactants, the alkylphenols are mainly removed by bacterial degradation in secondary processes which also indicates that such EDCs would be expected to pass through the septic tank phase relatively unchanged, which is of relevance to on-site systems which only use a septic tank. Many of the alkylphenols are degraded to more hydrophobic compounds which will then adsorb to particulates thereby finding their way into the sludge. The organic oxygen compounds, phthalates and bisphenol A have been shown to be easily removed in aerobic secondary treatment processes (Staples *et al.*, 1998).

Although natural sewage effluent has been shown to be faintly oestrogenic, some studies have dosed higher concentrations of steroids into a works to assess different treatment strategies (Walker, 2000) finding that a combination of UV and activated carbon (or ozone and activated carbon) proved very successful in removal if the water is used for recycling.

2.4.2 Treatment within the subsoil

The subsoil is considered to be the point at which further removal or degradation of organic, chemical and biological pollutants can occur on substances which have passed through the on-site process units. This is the same for the EDCs, most of which tend to be lipophilic and hence more likely to sorb onto the soil particulates. In the unsaturated soil there will also be further aerobic microbiological and chemical degradation of the compounds. In the groundwater environment itself photolysis and volatisation can not occur and degradation processes will not be so evident due to the low dissolved oxygen concentrations which mitigate against microbiological activity.

Few studies have looked directly at domestic effluent being discharged within the subsoil, as occurs in the percolation area until recently due to the ever growing applications of Soil Aquifer Treatment (SAT) and Aquifer Storage and Recovery (ASR) projects particularly for reuse of grey water or the direct injection of wastewater (Masters *et al.*, 2004). The effect of soil-aquifer treatment of secondary treated effluent was assessed on 13 year old percolation fields using 17β-oestradiol, oestriol and testosterone

which revealed that only 17β-oestradiol was picked up at concentrations < 1.8 ng/l in the shallow lysimeters 1.5m depth in the soil (Mansell and Drewes, 2004). Oestriol and testosterone were not detected at the detection limit of 0.6 ng/l which was put down to the adsorption and bioactivity. Another recent study looked at biological degradation of E1, E2, E3 and EE2 in soil on 3 sites using lysimeters at depths from 0.15m to 1.05m and wells (Synder *et al.*, 2004). Only one sample in the soil at 1.05m depth on one site picked up a concentration of oestriol (175 ng/l) but for all the others nothing was detected above the limit of 10ng/l.

Experiments carried out on aquifer materials in the laboratory (Ying *et al.*, 2003, 2004) have shown that alkylphenols bound strongly to the sediment whilst bisphenol A and 17β-oestradiol only demonstrated a weak affinity. Equally, aerobic degradation experiments showed that nonylphenol and 17β-oestradiol degraded quickly with half lives of 2 and 7 days respectively whilst 17α-ethynylestradiol was much slower with a half life of 81 days. Finally, bisphenol A and octylphenol showed absolutely no degradation. Laboratory experiments have also been carried out to mimic such soil aquifer treatment whereby secondary treated effluent was passed through 2.4m of sandy loam (Cordy *et al.*, 2004). There were no concentrations of 17β estradiol, estrone or estriol in the secondary treated effluent (limit 5 ng/l) but bisphenol A was found in concentrations 110-180 ng/l. No bisphenol A, however, was detected in the effluent after passing through the soil matrix.

The study on secondary treated wastewaters in Berlin (Zuehlke *et al.*, 2004) also showed that after the effluent had passed through bank filtration only in a few samples were any levels of steroids detected above the detection limit of 0.1ng/l. However, some studies have found positive detections of oestrogens in the groundwater (Kuch and Ballschmiter, 2001). Equally shallow wells picked up oestrogenic activity in reclaimed water in shallow wells from surface water which downstream of a municipal secondary treatment plant in Arizona (Quanrud *et al.*, 2004).

Studies have also been undertaken on sewage sludge application to land which, as indicated above, will contain significant concentrations of a range of EDCs. There are

many guidelines in Europe with regards to the addition of sludge to land which aim to maintain pathogens, nitrate and metals within acceptable limits but little has been done to set guidelines with respect to organic micropollutants. Certain EDCs have been shown *theoretically* (based as a function of water solubility, vapour pressure and octanol water partition coefficient K_{ow}) to be more susceptible to leach out of the solids and then percolate down into the groundwater regime but not enough experimental field data have been collected to verify such hypotheses (Wilson *et al.*, 1996). Studies have been undertaken in the laboratory using lysimeters of soil with sewage sludge applied to the top. These have indicated a degree of EDC leaching from the sludge which then tends to adsorb to the soil particles lower down the column (PRENDISENSOR 2000).

2.5 Acceptable levels of EDCs for human exposure

Not enough research has been carried out to date to determine specific levels of EDCs that would be of concern to humans in drinking water. The previous sections show that there is a possibility that low concentrations of certain EDCs could reach water sources (groundwater or surface water) and therefore could be present in drinking water from which the effects would be cumulative. However, more research into the cumulative effects of real water mixtures is required before the risk to humans of specific EDCs can be defined. Drinking water standards, for example, do not currently include endocrine disruption as a risk on which to base limits. Several EDCs do have legislation which concerns their production, use or concentration in water sources - especially pesticides - but this has not been developed on account of their endocrine disrupting properties. For example, the EC Drinking Water Directive (98/83/EC) set a maximum admissible concentration of 0.1µg/l for any individual pesticide in drinking water and 0.5µg/l for the total presence of pesticides including toxic metabolites. Several EDCs are also listed in the Dangerous Substances Directive (76/464/EEC) such as List I substances, lindane, PCP, dieldrin and endrin and thus must be considered as part of the recent umbrella Water Framework Directive (2000/60/EC).

3.0 Sites and Instrumentation

3.1 Introduction

As discussed in Chapter 1, the objective of this small scale study was to analyse a series of samples from the four existing project sites set up for the EPA MS-15 project in order to enhance the understanding of the processes involved and performance of different on-site treatment systems and subsoils in the removal of Endocrine Disrupting Compounds. This chapter briefly describes the sites used and instrumentation employed to obtain the samples. Much more detailed information on the sites, however, can be found in the Final Report from the EPA MS-15 project.

A number of essential criteria had to be satisfied during the site selection process in order for the four sites eventually selected to be deemed suitable. T-values of between 1 and 25, 25 and 50, and greater than 50 had to be identified, site location and trial hole inspections had to satisfy EPA (2000) guidelines and there had to be a sufficient number of residents in each dwelling to ensure that at least four, 20m long percolation trenches could be used. Hence, rigorous site assessments were carried out according to the EPA guidelines (EPA, 2000) to identify the following four sites used for the project.

3.2 Sites

Sites 1 and 2 were located in County Kildare and were sampled simultaneously for a 12 month period from July 2002 to July 2003. Subsequently, Sites 3 and 4 were identified in County Wicklow and constructed which were then in operation for a 9 month period from July 2003 until March 2004. The sites had the following characteristics due to the nature of the existing treatment system and the percolation test value (T-value) for the subsoils.

Site 1: septic tank effluent into subsoil with T-value of 15
Site 2: secondary treated effluent into subsoil with T-value of 29
Site 3: septic tank effluent into subsoil with T-value of 33

Site 4: secondary treated effluent into subsoil with T-value of 52

3.2.1 Site 1 The Curragh (Co. Kildare)

This site consisted of 4 adults (1 male and 3 females), although the number fluctuated throughout the year. There was also a livery stable attached to the house in which three people were employed. While the employees did not partake in the daily routine of the house they did have access to an external toilet that was connected to the wastewater system of the main house. The average wastewater production for the 4 adults was 105 lcd over the project period. All flows entered a septic tank from where the effluent passed into 4 x 20m percolation trenches.

The subsoil characteristics were assessed from the trial hole (Figure 3.1) and are given in Table 3.1. The percolation T-tests on site returned a T-value of 15 which shows relatively freely draining subsoil which was a somewhat surprising result due to the amount of clay shown in the classification.

Figure 3.1 Trial hole on Site 1.

	Soil / subsoil Texture & Classification	Soil Structure	Density	Colour	Preferential Flowpaths
0.1m	A Horizon	Crumb	Medium	Dark brown	Roots
0.2m					
0.3m					
0.4m	SILT/CLAY	Structureless-massive	Low	Brown	Some root ends and macropores present
0.5m					
0.6m					
0.7m					
0.8m	sandy CLAY (w/silt) interspersed with rounded cobbles	Structureless-massive	Medium	Reddish Brown	Macropores, cracks & voids around some cobbles
0.9m					
1.0m					
1.1m					
1.2m	sandy CLAY (w/silt) interspersed with rounded cobbles	Structureless-massive	Medium	Brown	Macropores, cracks & voids around some cobble
1.3m					
1.4m					
1.5m					
1.6m					
1.7m					
1.8m					
1.9m					
2.0m					
2.1m					
2.2m					
2.3m	Base of hole				

Table 3.1 Characterisation of subsoil profile at Site 1.

3.2.2 Site 2 Rochestown (Co. Kildare)

Five people were resident at Site 2 throughout the duration of the project; a husband and wife, their daughter and two sons. All the children were students at primary and secondary schools, whilst the father ran a business from home and the mother worked in the nearby town. The average wastewater production for the 5 people was 56 lcd over the project period. All flows entered a septic tank from where the effluent was pumped to a Puraflo® secondary treatment system. The secondary effluent then discharged by gravity into 4 x 20m percolation trenches (as for Site 1).

The subsoil characteristics were assessed from the trial hole (Figure 3.2) and are given in Table 3.2. The percolation T-tests on site returned a average T-value of 29.

Figure 3.2 Trial hole on Site 2.

	Soil / subsoil Texture & Classification	Soil Structure	Density	Colour	Preferential Flowpaths
0.1m	A Horizon	Crumb	Medium	Dark brown	Roots
0.2m					
0.3m					
0.4m	B Horizon sandy CLAY (w/silt)	Structureless – massive	Medium	Reddish brown	Some roots and macropores
0.5m					
0.6m					
0.7m					
0.8m					
0.9m					
1.0m	C Horizon sandy SILT (w/clay)	Structureless – massive	Medium	Brown	None evident
1.1m					
1.2m					
1.3m					
1.4m					
1.5m					
1.6m					
1.7m					
1.8m					
1.9m					
2.0m					
2.1m					
2.2m	Base of hole				

Table 3.2 Characterisation of subsoil profile at Site 2.

3.2.3 Site 3 Aughrim (Co. Wicklow)

There were four people resident at Site 3, a mother and her three children, living in a three bedroom house. Two of the children were in school, while the youngest had yet to start school and was cared for at home by his mother. The average wastewater production for the 4 people was 83 lcd over the project period. All flows entered a septic tank and the effluent was then equally split whereby half passed by gravity into 2 x 20m percolation trenches and the other half pumped to a stratified sand filter.

The subsoil characteristics were assessed from the trial hole (Figure 3.3) and are given in Table 3.3. The percolation T-tests on site returned a T-value of 33.

Figure 3.3 Trial Hole on Site 3.

	Soil / subsoil Texture & Classification	Soil Structure	Density	Colour	Preferential Flowpaths
0.1m	A Horizon	Crumb	Medium	Dark brown	Roots, some evidence of macropores
0.2m					
0.3m					
0.4m	sandy SILT (w/clay)	Structureless - single grain	Medium	Reddish brown	Some root ends
0.5m					
0.6m	Very gravely clayey SAND interspersed striated cobbles	Structureless – single grain	Medium	Dark brown	Some macropores evident in pockets of gravel and around cobbles
0.7m					
0.8m					
0.9m					
1.0m					
1.1m					
1.2m					
1.3m					
1.4m					
1.5m					
1.6m					
1.7m					
1.8m					
1.9m					
2.0m					
2.1m					
2.2m					
2.3m	Base of Hole				

Table 3.3 Characterisation of subsoil profile on Site 3.

3.2.4 Site 4 Killaveny (Co. Wicklow)

There were four people resident at Site 4, a mother, father and their two children. The two children were in school, the mother was a housewife and the father worked locally and returning home for lunch most days. Another daughter was in college and returned home occasionally some weekends. The average wastewater production for the 4 people was 123 lcd over the project period. All flows entered a septic tank from where the effluent was pumped to a Puraflo® secondary treatment system. The secondary effluent was then equally split, whereby half passed by gravity into 2x 20m percolation trenches and the other half pumped to a stratified sand filter.

The subsoil characteristics were assessed from the trial hole (Figure 3.4) and are given in Table 3.4. The percolation T-tests on site returned a T-value of 52.

Figure 3.4 Trial hole on Site 4

	Soil / subsoil Texture & Classification	Soil Structure	Density	Colour	Preferential Flowpaths
0.1m	A horizon	Crumb	Medium	Dark brown	Roots, some evidence of macropores
0.2m					
0.3m					
0.4m	clayey SAND with some rounded cobbles	Structureless - single grain	Dense	Light brown	Some root ends
0.5m					
0.6m	gravely clayey SAND interspersed with gravel and rounded cobbles	Structureless - single grain	Dense	Dark brown	None obvious although pockets of cobbles create macropores
0.7m					
0.8m					
0.9m					
1.0m					
1.1m					
1.2m					
1.3m					
1.4m					
1.5m					
1.6m					
1.7m					
1.8m					
1.9m					
2.0m					
2.1m					
2.2m					
2.3m	Base of hole				

Table 3.4 Characterisation of subsoil profile on Site 4.

3.3 Site Construction

To comply with the project specifications a septic tank was installed at Sites 1and 3, with respective T-values in the range 1 to 25 and 25 to 50. A secondary treatment system, preceded by a septic tank, was installed on the other two Sites 2 and 4. The secondary treatment system installed on Sites 2 and 4 was a Puraflo® system produced by Bord na Móna. Puraflo® is a peat based biofiltration system for the treatment of septic tank effluent. Septic tank effluent enters a sump from where it is pumped to a fibrous peat media (Figure 3.5) which is contained in moulded polyethylene modules.

Figure 3.5 Open Puraflo® module containing fibrous peat media.

3.3.1 Construction of Percolation Trenches

On Sites 1 and 2 the effluent from each system was split equally (via a distribution box) between four 20m long parallel percolation trenches (Figure 3.6) on each site which were built to the EPA specifications (EPA, 2000). On Sites 3 and 4 the effluent was split at the distribution box whereby half was sent to two 20m long parallel percolation trenches, again built to the EPA specifications, and the other half diverted to a stratified sand filter constructed at the site (Figure 3.7). There was generally 450mm cover above the

percolation pipes (i.e 800mm cover above the invert of the trench) along its full length, and the pipes were laid at a 1:200 gradient.

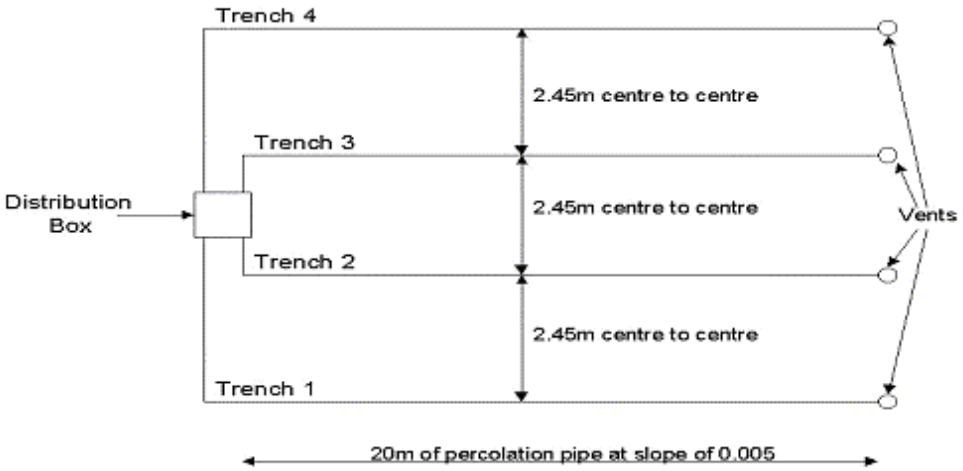


Figure 3.6 Plan of percolation area on Sites 1 and 2 (EPA, 2000).

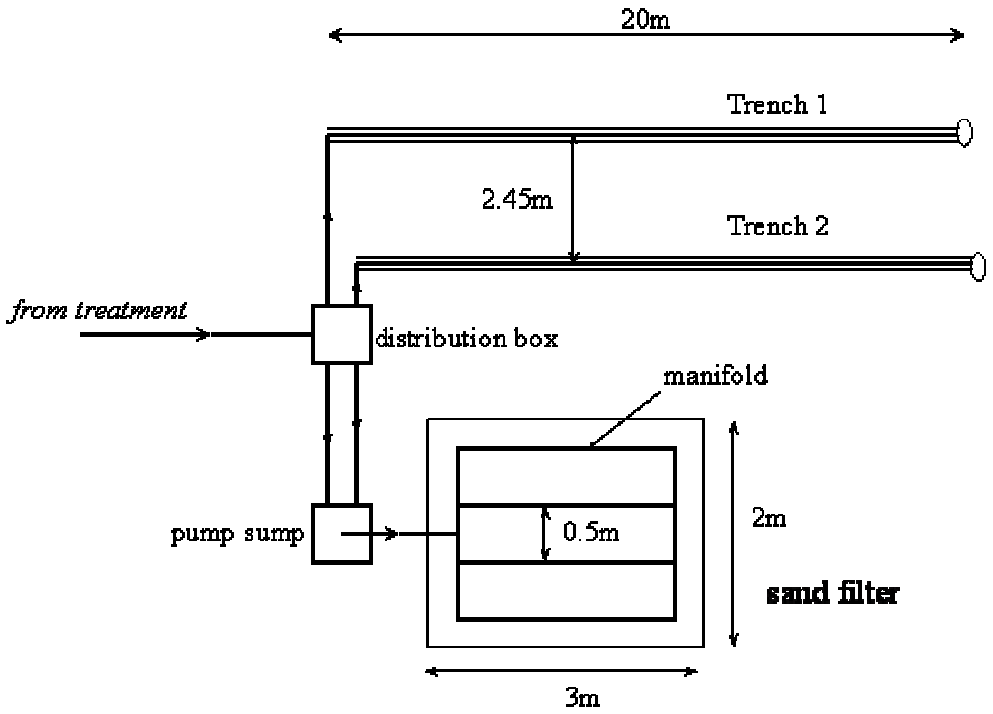


Figure 3.7 Plan view of trenches and stratified sand filter on Sites 3 and 4.

Figure 3.8 Percolation pipe on 250mm gravel bed.

The gravel was placed in the trench in two phases, an initial layer of 250 mm thickness as a distribution layer below the percolation pipe and a subsequent 250 mm thick layer to protect the pipe Figure 3.8). Prior to backfilling, a geotextile, Terram® in this case, was placed over the second gravel layer to prevent fines being washed into the distribution gravel and causing clogging.

3.4 Instrumentation

The successful installation of instrumentation to record a number of parameters over the research period was an essential component of the research (Figure 3.9). Automatic samplers and flow monitors were installed downstream of the septic tanks, and secondary treatment system to obtain a profile of the effluent entering the percolation trenches. Suction lysimeters (referred to as lysimeters) and zero-tension samplers were installed along the length of the percolation trenches to obtain soil moisture samples for analysis for some of the characteristic constituents of domestic wastewater effluent (COD, NO_3, NO_2, NH_4, PO_4, Cl and enteric bacteria). Rainfall volume, evapotranspiration and

chloride concentration were also analysed to determine the effect of dilution on the system. Tensiometers were installed to monitor the soil moisture pressure below the percolation area.

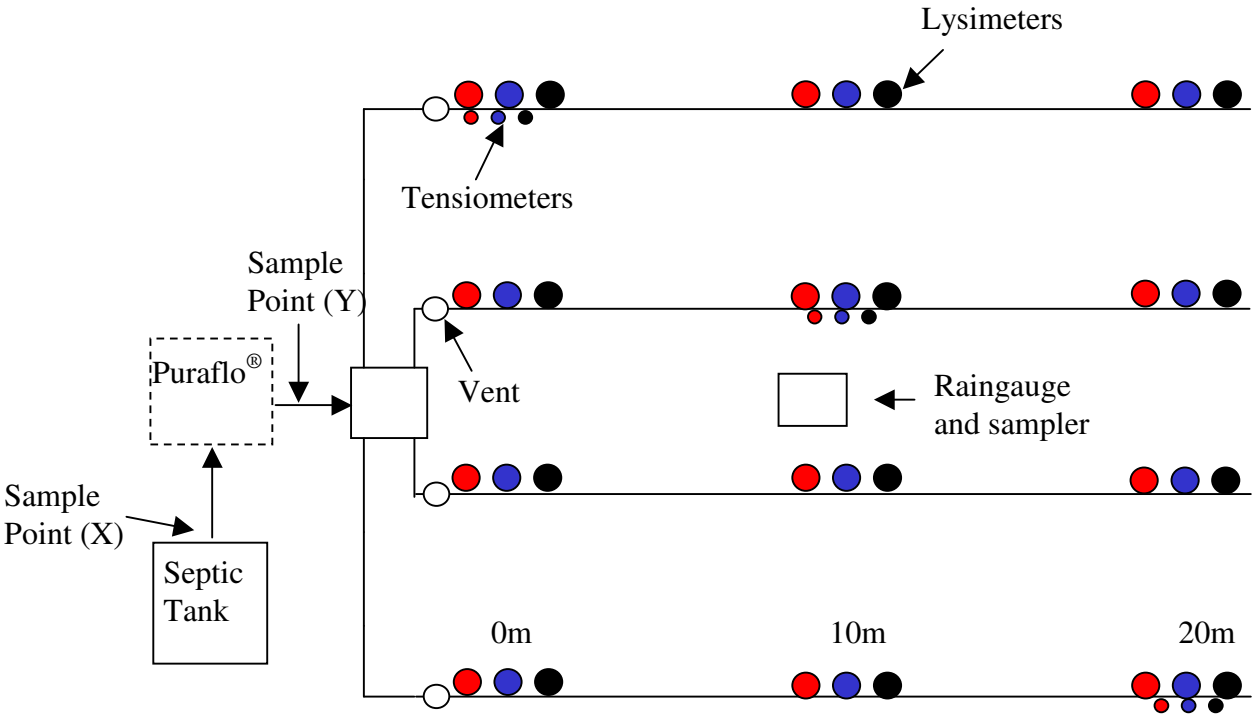


Figure 3.9 Schematic of instrumentation layout.

Bühler Montec xian 1000 automatic samplers were installed on all sites, two on Sites 2 and 4 (downstream of the septic tank and Puraflo® respectively) and one on Sites 1 and 3 (downstream of the septic tank). The installation of samplers upstream and downstream of the Puraflo® system enabled the changes in effluent quality across the secondary treatment system to be assessed. During the course of the project they were programmed to take hourly samples over the 24 hours preceding lysimeter sampling, thus providing a diurnal profile of the influent entering the percolation trenches. The samples were then mixed to provide a composite sample.

Nine Soilmoisture Equipment Corporation suction lysimeters were installed into each percolation trench during construction. When installed, each trio consisted of different length lysimeters, 1.3 m (red), 1.6 m (blue) and 1.9 m (black), installed to different depths below the trench invert (Figure 3.10).

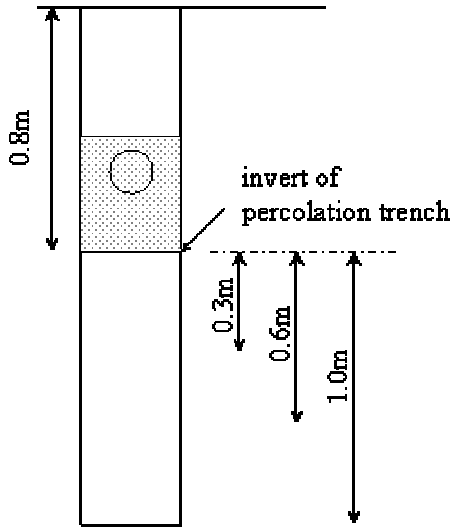


Figure 3.10 Cross-section and typical instrumentation depths

3.5 Sampling for EDCs

In total 42 samples were taken for EDC analyses representing one trench per site. The EDC samples were taken towards the end of each respective trial thereby ensuring that the system had reached equilibrium and the biomat has formed. Hence, Sites 1 and 2 was sampled at the beginning of July 2003 whilst Sites 3 and 4 were sampled in March 2004, as indicated in the sampling schedule below (see Table 3.5).

	Septic tank effluent	*Secondary treated eff.*	*Percolation area*	*Total no. samples*	**Sample date**
Site 1	1	-	9	10	July 15th 2003
Site 2	1	1	9	11	July 22nd 2003
Site 3	1	-	9	10	March 18th 2004
Site 4	1	1	9	11	March 18th 2004

Table 3.5 Schedule of EDC sampling

One percolation trench was chosen as representative of the percolation area on each site (from the previous sampling results) and samples at all three locations and all three depths in the subsoil were taken. The samples from the subsoil are taken by suction lysimeters which are put under pressure the night before (under a suction of 50 cbar) and

samples collected the next day. The EDC samples were collected in 1 litre glass jar and stored in the fridge before being couriered down to Limerick Institute of Technology for analysis. In a few cases samples had to be collected over 2-3 consecutive days in order to obtain the sufficient sample volume.

Samples of the septic tank and Puraflo® effluent were formed of discrete hourly samples discharged into glass containers taken over the 24 hours preceding lysimeter sampling which were then mixed to provide a composite sample.

The EDC samples were given the following codes prefix according to the Site.

Site 1 - RL Site 2 - JK Site 3 - NK Site 4 - JH

These prefixes were then followed by a number for the subsoil samples according to the sampling positions along the trench as shown schematically for Site 1 in Figure 3.11. The effluent from the septic tank was marked X and effluent from the Puraflo® was marked Y, after the site prefix.

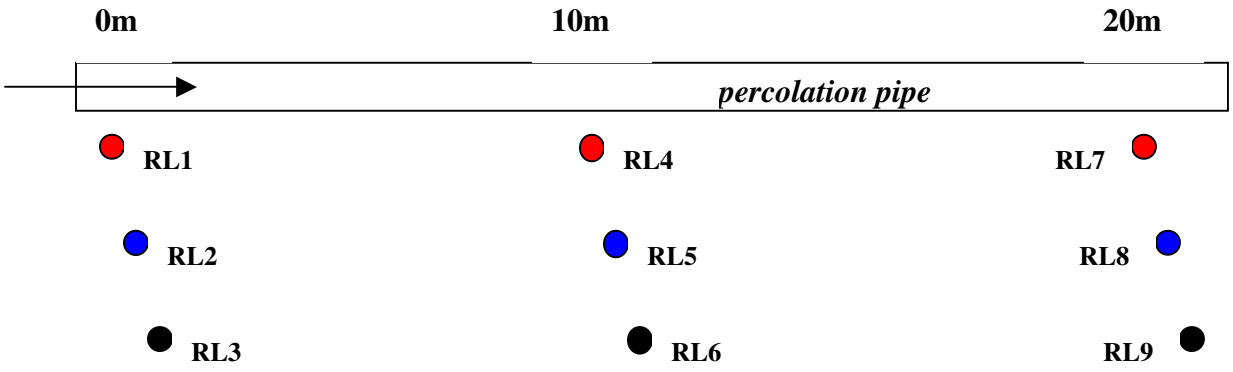


Figure 3.11 Schematic cross-section through trench showing sampling positions

4.0 Summary of MS-15 project results

The overall results from the EPA MS-15 project are presented in this Chapter since they provide a background to the fate to the various contaminants in the on-site wastewater effluents discharged into the subsoil. More importantly, from the point of view of the EDC samples, they indicate the extent to which the effluent had spread across the percolation area on each site which was primarily a function of the biomat growth. In general it was found that the percolation areas which received secondary treated effluent (Sites 2 and 4) did not develop a significant biomat due to the low organic loading and hence the effluent was concentrated over a relatively small area.

In all the following results the red blue and black depth planes refer to 0.3m, 0.6m and 1.0m depths respectively in the subsoil below the percolation trench (as shown on Figure 3.10).

4.1 Site 1 (septic tank effluent)

Analyses of the chemical and microbiological parameters on Site 1 revealed that effluent had reached all parts of the percolation area with the exception of the 20m position on Trench 4. The EDC samples were all taken from Trench 2 (code RL1 – RL9) which received effluent at all points along the 20m length.

The average strength of the septic tank effluent (STE) over the 12 month period is shown in Table 4.1.

	Concentration (mg/l)						
	COD	**NO_3-N**	**NO_2-N**	**NH_4-N**	**PO_4-P**	**Cl**	**pH**
Maximum	638.0	3.4	0.23	72.8	54.8	93.0	8.33
Minimum	188.0	0.0	0.08	20.3	5.2	27.0	7.32
Average	**383.4**	**0.7**	**0.16**	**53.0**	**14.2**	**56.6**	**7.73**

Table 4.1 Summary of chemical analysis of STE on Site 1.

The COD, nitrogen and ortho-phosphate concentrations and loads down through the different subsoil depth planes are shown on Tables 4.2, 4.3 and 4.4. These results give the average across all four trenches and across the whole percolation area which show that the main reduction of occurs above the red depth plane in the distribution gravel and first 500mm of subsoil.

	Concentration (mg/l)	Load (g/d)	Removal (g/d)
STE	383.4	160.6	-
Red Depth Plane	78.0	40.8	119.8
Blue Depth Plane	77.7	42.9	-2.1
Black Depth Plane	56.5	32.7	10.2

Table 4.2 Reduction in COD load attributed to the specific treatment steps on Site 1.

	NO_3-N		NO_2-N		NH_4-N		Total N	
	Conc. (mg/l)	Load (g/d)	Conc. (mg/l)	Load (g/d)	Conc. (mg/l)	Load (g/d)	Conc. (mg/l)	Load (g/d)
STE	0.7	0.3	0.2	0.1	53.0	22.2	54.0	22.6
Red	4.1	2.1	0.2	0.1	9.9	6.5	14.5	8.8
Blue	6.8	3.8	0.3	0.2	5.1	2.8	12.0	6.6
Black	6.7	3.9	0.2	0.1	5.8	3.4	12.8	7.4

Table 4.3 Average concentration and loading rate of NO_3-N, NO_2-N, NH_4-N and Total inorganic N measured in the STE and at the three depth planes on Site 1.

	Concentration (mg/l)	Load (g/d)	Removal (g/d)
STE	12.2	5.9	-
Red Depth Plane	2.2	1.2	4.7
Blue Depth Plane	1.2	0.7	0.5
Black Depth Plane	1.0	0.6	0.1

Table 4.4 Reduction in ortho-PO_4-P attributed to the specific treatment steps on Site 1.

The specific results that were obtained on day of sampling for EDCs (July 15th 2003) are presented in Table 4.5. These results show that lysimeters RL10-15 were receiving effluent that had percolated down through the subsoil matrix but lysimeters RL16, RL 17 and RL18 seemed to be receiving direct STE due to poor installation of the lysimeters creating preferential flow paths down the outside.

	COD	NH_3-N	NO_2-N	NO_3-N	*Total N*	Ortho-PO_4-P	Cl	pH
RLX	465	60.8	0.21	0.0	*61.01*	9.6	56	7.65
RL10	54.7	7.7	0.48	2.0	*10.18*	0.59	45	8.05
RL11	48.1	1.8	0.18	0.7	*2.68*	0.18	50	7.72
RL12	50.1	2.5	0.02	2.2	*4.72*	0.10	34	8.20
RL13	32.0	10.3	0.3	1.2	*12.8*	0.24	37	7.51
RL14	18.5	0.3	0.06	10.6	*10.96*	0.65	40	7.54
RL15	15.0	0.2	0.3	20.3	*20.8*	1.22	38	7.26
RL16	50.9	28.1	0.02	0.0	*28.12*	5.9	35	7.43
RL17	45.2	34.1	0.05	0.0	*34.15*	4.8	40	7.47
RL18	49.0	33.5	0.02	0.0	*33.52*	6.5	35	7.46

Table 4.5 Results on 15th July 2003 for Site 1.

4.2 Site 2 (secondary treated effluent)

Analyses of the chemical and microbiological parameters on Site 2 revealed that effluent had only reached the 0m position on all Trenches. The EDC samples on this site were all taken from Trench 1 (code JK1 – JK9).

The average strength of the septic tank effluent (STE) and secondary effluent (SE) over the 12 month period is shown in Tables 4.6 and 4.7.

	Concentration (mg/l)						
	COD	**NO_3-N**	**NO_2-N**	**NH_4-N**	**PO_4-P**	**Cl**	**pH**
Maximum	1630.0	5.3	0.42	161.5	61.9	290.0	8.39
Minimum	484.0	0.0	0.00	98.8	19.3	74.1	7.3
Average	**791.6**	**1.4**	**0.28**	**131.0**	**32.3**	**117.8**	**7.9**

Table 4.6 Summary of chemical analysis of STE on Site 2.

	Concentration (mg/l)						
	COD	**NO_3-N**	**NO_2-N**	**NH_4-N**	**PO_4-P**	**Cl**	**pH**
Maximum	316.0	60.5	13.6	45.2	85.6	185.0	7.61
Minimum	98.0	15.3	0.8	6.9	16.8	51.3	6.07
Average	**188.1**	**36.9**	**7.4**	**19.7**	**33.6**	**92.6**	**6.42**

Table 4.7 Summary of the results of chemical analysis of SE on Site 2.

The COD, nitrogen and ortho-phosphate concentrations and loads down through the different subsoil depth planes are shown on Tables 4.8, 4.9 and 4.10. These results give the average across all four trenches *at 0m plane only* which again shows that the main reduction of COD occurs above the red depth plane. However, there is much less removal of the nitrogen load and phosphorus loads down through the subsoil compared to Site 1.

	Concentration (mg/l)	**Load**	
		(g/d)	**Removal (g/d)**
STE	791.6	223.0	-
SE	188.1	55.5	167.5
Red Depth Plane	107.5	30.9	24.6
Blue Depth Plane	76.2	21.9	9.0

Table 4.8 Reduction in COD load attributed to the specific treatment steps on Site 2.

Depth Plane	**NO_3-N**		**NO_2-N**		**NH_4-N**		**Total N**	
	Conc. (mg/l)	**Load (g/d)**	**Conc. (mg/l)**	**Load (g/d)**	**Conc. (mg/l)**	**Load (g/d)**	**Conc. (mg/l)**	**Load (g/d)**
SE	37.1	10.5	7.4	2.1	20.5	5.8	64.0	18.0
Red	52.0	14.9	3.3	0.9	5.8	1.7	59.2	17.0
Blue	56.2	16.1	0.6	0.2	1.5	0.4	57.9	16.6

Table 4.9 Average concentration and loading rate of NO_3-N, NO_2-N, NH_4-N and Total inorganic N measured on the red and blue depth planes on Site 2.

	Conc. (mg/l)	Loading (g/d)	pH
SE	33.6	9.5	6.42
Red Depth Plane	23.9	6.9	6.30
Blue Depth Plane	20.3	5.8	7.22

Table 4.10 Average ortho-PO_4-P concentration and loading rates measured over project duration on Site 2.

The specific results that were obtained on day of sampling for EDCs on Site 2 (July 22nd 2003) are presented in Table 4.11. These results clearly show that only lysimeters JK1, JK2 and JK3 were receiving the treated effluent (ie the 0m position along the trench) and the rest of the lysimeters, JK4-JK9 were just picking normal soil moisture.

	COD	NH_3-N	NO_2-N	NO_3-N	*Total N*	Ortho-PO_4-P	Cl	pH
JKX	1329	109	0.4	1.6	*111.0*	27.3	90	7.46
JKY	283	18.6	0.18	15.3	*34.08*	32.8	62	7.49
JK1	155	1.0	0.3	33.5	*34.80*	17.6	65	5.84
JK2	59	0.0	2.0	32.6	*34.6*	19.6	63	7.21
JK3	42	0.0	0.2	42.4	*42.6*	8.2	62	7.57
JK4	19	0.0	0.04	1.0	*1.04*	0.1	0	7.47
JK5	12	0.0	0.0	2.6	*2.6*	0.0	0	7.12
JK6	12	0.2	0.03	1.2	*1.43*	0.0	0	7.25
JK7	18	0.0	0.0	0.0	*0.0*	0.06	0	7.14
JK8	11	0.0	0.0	0.2	*0.2*	0.0	0	6.84
JK9	18	0.0	0.0	0.8	*0.8*	0.0	0	7.20

Table 4.11 Results on 22nd July 2003 for Site 2.

4.3 Site 3 (septic tank effluent)

Analyses of the chemical and microbiological parameters on Site 3 revealed that effluent had only seemed to have reached the 0m position on both Trenches although there was a suspicion that some of the lysimeters may have been installed slightly out of the effluent plume. There was also the hypothesis on this site preferential flowpaths may have been present in the subsoil due to the large cobbles found in the matrix. The EDC samples were all taken from Trench 1 (code NK1 – NK9).

The average strength of the septic tank effluent (STE) over the 9 month period is shown in Tables 4.12.

	Concentration (mg/l)						
	COD	**NO_3-N**	**NO_2-N**	**NH_4-N**	**PO_4-P**	**Cl**	**pH**
Maximum	2703.0	71.1	0.7	4.3	16.7	135	8.53
Minimum	540.0	19.8	0.22	0.5	3.0	56	6.96
Average	***1307.8***	***41.7***	***0.45***	***2.0***	***7.4***	***88.4***	**7.68**

Table 4.12 Summary of chemical analysis of STE on Site 3.

The COD, nitrogen and ortho-phosphate concentrations and loads down through the different subsoil depth planes are shown on Tables 4.13, 4.14 and 4.15. These results give the average across all four trenches *at 0m plane only* which again shows (as for Site 1) significant removal of COD, total nitrogen and ortho-phosphate above the red depth plane. The total nitrogen removal continues with depth in the subsoil due to nitrification of the ammonia and then subsequent denitrification of the nitrate particularly between the blue and the black plane.

		Load	
	Concentration (mg/l)	**(g/d)**	**Removal (g/d)**
STE	1307.2	215.1	-
Red Depth Plane	128.5	23.9	191.2
Blue Depth Plane	102.7	19.6	4.3
Black Depth Plane	84.7	16.7	2.9

Table 4.13 Average reduction in COD on Site 3.

Depth Plane	**NO_3-N**		**NO_2-N**		**NH_4-N**		**Total inorg. N**		**pH**
	Conc. (mg/l)	**Load (g/d)**	**Conc. (mg/l)**	**Load (g/d)**	**Conc. (mg/l)**	**Load (g/d)**	**Conc. (mg/l)**	**Load (g/d)**	
STE	2.0	0.4	0.5	0.09	41.7	8.08	48.4	8.57	7.68
Red	22.0	3.6	0.3	0.05	5.1	0.9	30.1	4.55	6.80
Blue	20.9	3.4	0.7	0.12	7.2	1.2	31.3	4.72	6.78
Black	6.1	1.0	0.4	0.07	3.8	0.6	11.7	1.67	6.97

Table 4.14 Average NO_3-N, NO_2-N, NH_4-N and Total inorganic N concentration measured on Site 3.

	Concentration (mg/l)	Load	
		(g/d)	Removal (g/d)
STE	7.4	1.22	-
Red Depth Plane	0.4	0.07	1.15
Blue Depth Plane	0.2	0.04	0.03
Black Depth Plane	0.1	0.02	0.02

Table 4.15 Average reduction in ortho-PO_4-P on Site 3.

The specific results that were obtained on day of sampling for EDCs on Site 3 (March 15th 2004) are presented in Table 4.16. These results clearly show that lysimeters NK1, NK2 and NK3 were receiving the treated effluent (ie the 0m position along the trench) but also that by the end of the trial period lysimeter NK4 also seemed to be receiving effluent indicating the growth of the biomat with time. The rest of the lysimeters, NK5-JK9 were just picking up normal soil moisture.

	COD	NH_3-N	NO_2-N	NO_3-N	*Total N*	Ortho-PO_4-P	Cl	pH
NKX	940	54.1	0.0	1.95	*56.05*	7.17	68	7.60
NK1	200	2.0	0.05	51.2	*53.25*	1.18	56	7.34
NK2	188	4.2	0.2	25.3	*29.7*	0.90	48	7.22
NK3	161	2.7	0.95	17.6	*21.20*	0.85	29	7.59
NK4	106	1.4	0.02	23.75	*25.17*	0.54	23	7.44
NK5	99	1.4	0.02	10.31	*11.71*	0.36	17	7.53
NK6	121	0.6	0.03	9.45	*10.08*	0.51	14	7.33
NK7	113	1.6	0.03	17.05	*18.68*	0.72	14	7.31
NK8	89	1.1	0.03	8.85	*9.98*	0.68	14	7.31
NK9	146	1.3	0.09	4.50	*5.89*	0.24	14	7.16

Table 4.16 Results on 18th March 2004 for Site 3.

4.4 Site 4 (secondary treated effluent)

Analyses of the chemical and microbiological parameters on Site 4 revealed that effluent had only reached the 0m position on all Trenches, as for Site 2. The EDC samples on this site were all taken from Trench 1 (code JH1 – JH9).

The average strength of the septic tank effluent (STE) and secondary effluent (SE) over the 9 month period is shown in Tables 4.17 and 4.18.

	Concentration (mg/l)					
	COD	**NH_4-N**	**Total-N**	**PO_4-P**	**Cl**	**pH**
Maximum	1393.0	83.0	85.2	13.9	116	8.27
Minimum	446.0	27.8	29.13	3.8	33.3	6.08
Average	**812.6**	**56.6**	**57.85**	**7.9**	**68.8**	**7.31**

Table 4.17 Summary of chemical analysis of STE on Site 4.

	Concentration (mg/l)					
	COD	**NO_3-N**	**Total-N**	**PO_4-P**	**Cl**	**pH**
Maximum	370.0	63.4	66.3	11.8	85.0	7.74
Minimum	68.0	23.6	31.8	5.1	20.1	5.57
Average	**215.8**	**42.0**	**48.7**	**8.1**	**55.4**	**6.34**

Table 4.18 Summary of the results of chemical analysis of SE on Site 4.

The COD, nitrogen and ortho-phosphate concentrations and loads down through the different subsoil depth planes are shown on Tables 4.19, 4.20 and 4.21. These results give the average across all four trenches *at 0m plane only* which again shows that the main reduction of COD occurs above the red depth plane. However, as for Site 2, there is much less removal of the nitrogen load down through the subsoil compared to Sites 1 and 3. There is, however, a large ortho-phosphate removal between the blue and black depth planes presumably due to a change in the mineralogy of the subsoil at that depth.

		Load	
	Concentration (mg/l)	**(g/d)**	**Removal (g/d)**
STE	812.6	200.0	-
SE	215.8	53.1	146.9
Red Depth Plane	109.3	28.0	25.1
Blue Depth Plane	89.5	22.0	6.0
Black Depth Plane	89.7	22.0	0.0

Table 4.19 Average reduction in COD on Site 4.

Depth Plane	NO_3-N		NO_2-N		NH_4-N		Total inorg. N		pH
	Conc. (mg/l)	Load (g/d)	Conc. (mg/l)	Load (g/d)	Conc. (mg/l)	Load (g/d)	Conc. (mg/l)	Load (g/d)	
STE	42.0	10.3	0.2	0.06	6.5	1.6	48.7	12.0	6.4
Red	50.6	12.9	0.4	0.10	3.8	1.0	53.7	13.7	6.0
Blue	48.5	12.7	0.3	0.08	3.0	0.8	50.5	13.2	6.2
Black	45.6	12.1	0.1	0.03	2.1	0.6	46.9	12.5	6.6

Table 4.20 Average NO_3-N, NO_2-N, NH_4-N and Total inorganic N concentration measured on Site 4.

	Concentration (mg/l)	Load	
		(g/d)	Removal (g/d)
SE	8.1	2.0	-
Red Depth Plane	6.8	1.7	0.3
Blue Depth Plane	4.8	1.3	0.4
Black Depth Plane	0.6	0.2	1.1

Table 4.21 Average reduction in ortho-PO_4-P on Site 4.

The specific results that were obtained on day of sampling for EDCs on Site 4 (March 18th 2004) are presented in Table 4.22. These results clearly show that only lysimeters JH1, JH2 and JH3 were receiving the treated effluent (ie the 0m position along the trench) and the rest of the lysimeters, JH4-JH9 were just picking normal soil moisture.

	COD	NH_3-N	NO_2-N	NO_3-N	*Total N*	Ortho-PO_4-P	Cl	pH
JHX	892	56.3	0.01	1.6	*57.91*	8.2	80	7.86
JHY	370	2.8	0.06	62.7	*65.56*	8.9	85	6.8
JH1	147	3.7	0.20	49.0	*52.9*	7.53	71	6.7
JH2	120	2.8	0.00	47.7	*50.5*	5.04	74	6.4
JH3	171	0.8	0.0	31.5	*32.3*	0.01	39	6.8
JH4	178	2.0	0.01	0.01	*2.02*	0.35	1	7.41
JH5	114	1.5	0.01	0.02	*1.53*	0.01	8	7.14
JH6	174	0.9	0.01	0.01	*0.92*	0.01	14	7.21
JH7	111	1.9	0.05	0.06	*2.01*	0.01	2	7.13
JH8	155	2.6	0.05	0.02	*2.67*	0.01	3	7.21
JH9	170	1.4	0.03	0.35	*1.78*	0.01	5	7.22

Table 4.22 Results on 18th March 2004 for Site 4.

5.0 EDC Analysis, Procedures and Methods

5.1 Determination of EDCs in the aquatic environment

The analysis and identification of oestrogen mimicking EDCs is generally achieved using a combination of biological assays and analytical separation techniques. Biological potency of a compound can be assessed using *in vivo* or *in vitro* assays and activity is usually expressed relative to that of oestradiol, which is the most common endogenous oestrogen. Chromatographic methods are routinely used for the analysis of environmental oestrogens due to their tailored selectivity and high resolving power resulting in efficient analyses. Coupling of chromatography-based separation techniques to sensitive detection methods has allowed the qualitative and quantitative measurement of target analytes in complex matrices. The automation of many devices also allows for continuous analyses and increased sample through-put while the modular design of some systems allows on-line coupling to pre-separation preparatory units. Due to the significant interest in the development of analytical techniques for the determination of EDCs in a variety of matrices, several reviews of analytical methodologies used have been published recently.

The following five EDCs were selected for analysis based on their prevalence in domestic wastewater, variety in terms of source and also their likely persistence through the unit treatment processes and thus levels in the effluent.

- **Natural oestrogens** (oestrone, 17β-oestradiol, oestriol)
- **Organic oxygen compound** (bisphenol A)
- **Surfactants** (nonylphenol)

However, due to the analytical technique used several other compounds were also analysed for at the same time, as described in Section 5.3.

5.2 Sample preparation techniques

EDCs in the aquatic environment are often present in complex matrices such as effluent samples. For this reason, wide ranges of sample preparation and preconcentration

techniques have been employed in conjunction with analytical separations. Preconcentration is necessary due to sensitivity limitations of the instrumental techniques.

5.3 Experimental

Figure 5.1 illustrates a separation of the key EDCs using GC-MS. The instrumental conditions etc. are noted below.

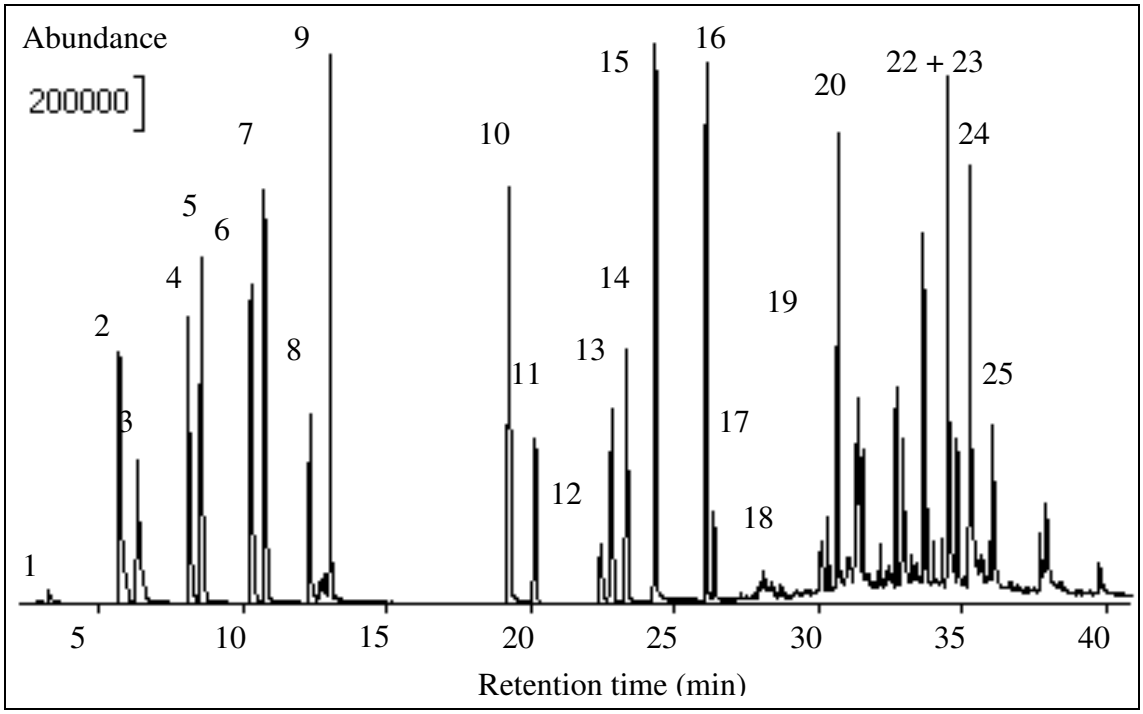


Figure 5.1 Determination of priority EDCs using GC-MS in scan mode.

Temperature ramp conditions = 80°c/1 min; ramp - 8°c to 150; 150 for 8 min; ramp - 11 to 280; 280 for 5 min. Peak identification: 1. Phenol, 2. ethylphenol, 3. Dichlorophenol, 4. 4- Propylphenol, 5. 2-sec Butylphenol, 6. 2,4,5 Trichlorophenol, 7. Biphenyl, 8. Methylparaben, 9. 2,3 tertbutyl-4-hydroxyanisol 10. 4-hexloxyphenol,11. Hexachlorobenzene, 12. Pentaclorophenol, 13. Lindane, 14. Octylphenol, 15. 4-heptyloxylphenol, 16. Octylphenol 2 Polyethoxylate, 17. Nonylphenol, 18. Nonylphenol 2 Polyethoxylate, 19. Bisphenol A, 20. Delidrin, 21. DES, 22. Oestrone, 23. 17β-oestradiol, 24. Ethynyl Oestradiol, 25. NP 12 polyethoxylate. Unlabelled peaks due to column bleed.

Taking the analytes of interest from that separation limits of detection (without preconcentration) (Table 5.1) were calculated as between 20 and 0.05 mg/l. However, the limits achieved in this study were 1000 times (i.e. 0.02 – 0.00005 mg/l) less than the values in Table 5.1 since preconcentration (1000 ml) was carried out.

EDC (In order of migration)	**Resolution (GC/MS)**	**LOD (mg/l)**
Biphenyl	1.1	0.05
Nonylphenol	-	0.02
Bisphenol A	16.4	0.02
17β-Oestradiol	2.1	2.0
Oestrone	0.6	2.0
Ethynyloestradiol	2.0	5.0
Oestriol	5.0	20.0

Table 5.1 GC-MS method limit of detection and analyte resolution

5.3.1 Instrumentation

The GC/MS separation was carried out using a 5890 Hewlett Packard series II Gas Chromatograph coupled with a 5971 Hewlett Packard Mass Spectrometer with a 5973A autosampler (With a capacity of 100 ampoules). The carrier gas used was helium of GC grade, and was supplied by BOC gases, Dock Rd. Limerick. A HP-5MS capillary column (30 m length x 0.25 nm diameter x 0.25 µm internal diameter) coated with a 5% biphenyl siloxane stationary phase, was used for the analysis. The injection volume used was 1µl and the flow rate of the carrier gas was approximately 1 ml/min. The detector was set to detect mass ions with an m/z in the range of 50-550 and the Emv averaged at approximately 2000 V for the duration of this experiment. Data was captured and stored using a Dell optiplex with Hewlett Packard software. For identification of the endocrine disrupting compounds present in the injected sample the Wiley library on the HP chem. Station software was used.

A system tune was run prior to each sequence to ensure effective detection of the mass ions. The tuning system functions by detecting a known substance, which has 3 distinctive peaks at 69m/z, 219m/z and 502m/z and their concentrations, should be at a ratio of 1:4:10. The tuning system adjusts the Emv (through the range of 1800-3000) until it reach's an Emv which produces the corresponding ratio's of the three peaks at the desired 1:4:10 ratio, for this reason the Emv stated above is only an approximate value.

5.3.2 Reagents

All compounds investigated in the separations were purchased from Sigma-Aldrich, Tallaght, Dublin, Ireland. The compounds included phenol, ethylphenol, dichlorophenol, propylphenol, butylphenol, trichlorophenol, biphenyl (internal standard) methylparaben, methyloxylphenol, hexyloxylphenol, hexachlorobenzene (HCB), pentachlorophenol, lindane, octylphenol, nonylphenol, octylphenol 2 polyethoxylate heptyloxylphenol, nonylphenol 2 polyethoxylate, bisphenol A, dieldrin, diethylstilbestrol, oestrone, 17β-oestradiol, ethynyloestradiol, nonylphenol 12 polyethoxylate and oestriol. These compounds had a purity of equal or greater than 97% with the exception of technical grade 4-Nonylphenol ~85% content of *p*-isomers. A pure form of nonylphenol was also purchase from Sigma-Aldrich. Acetonitrile and methanol were HPLC grade and were purchased from Sigma-Aldrich, Dublin, Ireland. All compounds were used without further purification. The alkylphenol ethoxylates (nonylphenol ethoxylate and octylphenol ethoxylate) were purchased under the trade name Igepal from Sigma-Aldrich, Dublin, Ireland. All compounds were used without further purification.

5.3.3 Standard preparation

Stock solutions of the compounds with the exception of oestriol and oestrone were prepared on 100% acetonitrile. Oestriol and oestrone were prepared in 100% methanol. The corresponding working standards were obtained by dilution of the stock solution with acetonitrile.

5.3.4 Sample preparation

Alkylphenols

The solid phase extraction method entailed a pre-rinse of 5ml acetone, 5ml acetonitrile and 5 ml de-ionised water the solid phase extraction packing was C18 and the Eluting solvent is 0.5 ml acetonitrile and 1.5 ml of acetone. Prior to testing the sample was adjusted to pH 2 and was filtered to remove particulate matter. The method involved adjusting the sample to pH 10-12, the addition of 30 ml of acetonitrile, and sonication for 30 min. The sample was then rotary evaporate to reduce the volume of sample (10 ml) and increase the preconcentration factor.

Oestrogens

Only liquid samples were tested for the presence of oestrogens. The method employed was taken from the literature (Ingrand *et al*., 2003) as no solid phase extraction technique was developed for the oestrogens in the current study. For oestrogen determination the SPE cartridge were C18. The conditioning method involved the addition of 5 ml hexane, 5 ml methanol, 5 ml of dichloromethane and 5 ml of de-ionised water. When the sample was applied to the cartridge. The SPE cartridge was then dried by purging air over it for 10 min. The analytes were then eluted with 6 ml of 1:6 methanol: dichloromethane. The samples were evaporated to 2 ml to achieve preconcentration.

5.3.5 GC/MS separation conditions

During the chromatographic method development the GC temperatures were altered but the following parameters remained constant:

- Injection port temperature – 240^0C;
- Detector temperature – 280^0C;
- Flow rate – 1ml /min

6.0 Results

Two different preconcentration techniques were employed on the samples: one for the preconcentration of oestrogens and the second for the preconcentration of alkylphenols. GC-MS-MS analysis was then carried out on the extracts and the results from the four sites are shown in Tables 6.1 to 6.4. Mass spectrometric determination of all chromatographic peaks (of EDCs) was carried out in addition to those corresponding to the five target species.

- oestrone
- 17β-oestradiol
- oestriol
- bisphenol A
- nonylphenol

The effective limit of detection for the above five target species EDCs after preconcentration was as follows: oestrone (2 μg/l), 17β-oestradiol (2 μg/l), oestriol (20 μg/l), bisphenol A (20 ng/l) and nonylphenol (20 ng/l).

In the following results tables the symbol "?" is used where mass spectral results highlighted the presence of a particular analyte, but the concentrations seen were well below limits of detection (following preconcentration) and therefore could not be confirmed to be present. The symbol "O" is used to highlight samples that were treated to the preconcentration step described in Section 5.3.4 that show the presence of the analytes highlighted. It is not possible to provide exact concentrations for these analytes at this stage (i.e. greater volumes of samples would be required). The evidence from this study shows that they occur at or just below their limit of detection (taking into account a factor of 1000 preconcentration), where LOD is defined as a signal that is three times the background noise. As the signals are so low, while it is evident from chromatographic and mass spectral data that the analyte is present, they are below limit of quantitation for analysis. For example, in sample JHX the target species oestrone and 17-β oestradiol were detected at concentrations of low μg/l. However, it should be noted that due to the limitation of sample volumes obtained from each sample it was not possible to preconcentrate enough sample in replicates to determine the reproducibility of the analysis.

The sample points which are shaded grey on the results Tables 6.2, 6.3 and 6.4 are those which were known not to have been receiving effluent in the percolation area from the analyses of several other parameters measured throughout the MS-15 project, as described in Sections 4.1 to 4.4.

6.1 EDC results from Site 1

Table 6.1 shows the results of the analysis on samples taken from Site 1, where septic tank effluent was discharged directly into the percolation trenches. Effluent was known to have reached all positions on the trenches due to the extensive development of the biomat.

sample name	m.w. ION	r.t. Min	**RLX**	**RL10**	**RL11**	**RL12**	**RL13**	**RL14**	**RL15**	**RL16**	**RL17**	**RL18**
Phenol	94.00	3.60	X	X	X	X	X	X	X	X	X	X
Ethylphenol	122.00	5.64	?	X	X	X	X	X	X	X	X	X
Dichlorophenol	162.00	6.17	X	X	X	X	X	X	X	X	X	X
Propylphenol	107.00	7.31	X	X	X	X	X	X	X	X	X	X
Butylphenol	121.00	7.50	X	X	X	X	X	X	X	X	X	X
Trichlorophenol	196.00	8.79	X	X	X	X	X	X	X	X	X	X
Hexyloxylphenol	194+110	17.90	X	X	X	X	X	X	X	X	X	X
Lindane	183.00	19.40	X	X	X	X	X	X	X	X	X	X
Octylphenol	206+107	19.50	?	O	O	O	O	O	O	O	O	O
Hexyloxylphenol	208+110	19.90	X	X	X	X	X	X	X	X	X	X
Nonylphenol	107+220	21.00	X	X	X	X	X	X	X	X	X	X
Dieldrin	263+345	2440	X	X	X	X	X	X	X	X	X	X
Bisphenol A	213.00	24.50	X	X	X	X	X	X	X	X	X	X
Diethylstilbestrol	268.00	25.49	X	X	X	X	X	X	X	X	X	X
Oestrone	270.00	27.70	X	X	X	X	X	X	X	X	X	X
17β-Oestradiol	272.00	27.90	X	X	X	X	X	X	X	X	X	X
Ethynyloestradiol	296.00	28.50	X	X	X	X	X	X	X	X	X	X
Oestriol	288.00	30.22	X	X	X	X	X	X	X	X	X	X

Table 6.1 Results set from Site 1

The three points at the 20m position (RL16, RL17 and RL18) had been shown to be receiving the effluent directly without significant percolation through the subsoil matrix due to the occurrence of preferential flowpaths. All other sample positions, however, were receiving effluent which was representative of having percolated down through the subsoil matrix at each respective depth. The subsoil at such depths was characterised as a sandy-clay and thus contained a relatively high proportion of clay compared to the other sites.

The lack of oestrogens detected in either the effluent or out in the percolation area is maybe not that surprising considering that the majority of inhabitants and workers in the stables at the time were male. The only EDC that was detected both in the effluent and at all sample positions was octylphenol at levels of ~50 ng/l. Octylphenol is a constituent in household detergents and other cleaning products which would be used in relatively high quantities on this site since the business of the premises is a livery stable. The relatively high COD values for Site 1 across the percolation area may also explain the occurrence of octylphenol in practically all samples for that site.

6.2 EDC results from Site 2

Table 6.2 illustrates the results of the EDC samples obtained for Site 2. The percolation area was receiving secondary treated effluent from the peat filter and the subsoil was characterised as a sandy-silt with slower percolation characteristics than Site 1.

No oestrogens were found to occur at the detectable level in either the septic tank effluent JKX or the secondary treated effluent JKY. However, surprisingly, levels of oestrogens (~2.0 μg/L) were found out in the percolation area at sampling points both where the effluent was known to have reached (JK2) and also at the 20m point (JK7, JK8 and JK9) where effluent was known not to have reached. There could be two explanations for this. The effluent that was sampled at point JK2 may have been discharged from the secondary treatment unit several days before the sampling date (since it would take some time to percolate down to the sampling depths) compared to those samples collected at the septic tank and secondary treated samples. Hence, this may be representative of conditions several days earlier at that time the production of oestrogens was higher. This highlights the fact that a much longer and more frequent

sampling study would be required to gain a full picture of the processes. The results obtained at the 20m sampling points however, must have been generated from a source at the surface rather than from the treatment system. Several animals (dogs, pigs and wildfowl) had continual access to the area and could have urinated on the surface which would have percolated down into the subsoil. If this is the case then it indicates the relative efficiency of the septic tank in removing such EDCs in a highly organic environment compared to the removal capability of the subsoil on its own. The occurrence of ethynloestradiol at these sampling positions, however, is confusing since it is a synthetic oestrogen. Re-analysis of the tandem MS data showed that this could have been confused with another oestrogen or even a phytoestrogen (see Section 2.3.2).

sample name	m.w. ION	r.t. Min	**JKX**	**JKY**	**JK1**	**JK2**	**JK3**	**JK4**	**JK5**	**JK6**	**JK7**	**JK8**	**JK9**
Phenol	94.00	3.60	X	X	X	X	X	X	X	X	X	X	X
Ethylphenol	122.00	5.64	?	?	?	X	X	X	X	X	X	X	X
Dichlorophenol	162.00	6.17	X	X	X	X	X	X	X	X	X	X	X
Propylphenol	107.00	7.31	X	X	X	X	X	X	X	X	X	X	X
Butylphenol	121.00	7.50	X	X	X	X	X	O	X	X	X	X	X
Trichlorophenol	196.00	8.79	X	X	X	X	X	X	X	X	X	X	X
Hexyloxylphenol	194+110	17.90	X	X	X	X	X	X	X	X	X	X	X
Lindane	183.00	19.40	X	X	X	X	X	X	X	X	X	X	X
Octylphenol	206+107	19.50	?	?	X	X	X	X	X	X	X	X	X
Hexyloxylphenol	208+110	19.90	X	X	X	X	X	X	X	X	X	X	X
Nonylphenol	107+220	21.00	X	X	X	X	X	X	X	X	X	X	X
Dieldrin	263+345	2440	X	X	X	X	X	X	X	X	X	X	X
Bisphenol A	213.00	24.50	X	X	X	X	X	X	X	X	X	X	X
Diethylstilbestrol	268.00	25.49	X	X	O	X	X	X	X	X	O	X	X
Oestrone	270.00	27.70	X	X	O	X	X	X	X	X	O	O	O
17β-Oestradiol	272.00	27.90	X	X	O	X	X	X	X	X	O	O	O
Ethynyloestradiol	296.00	28.50	X	X	O	X	X	X	X	X	O	O	O
Oestriol	288.00	30.22	X	X	X	X	X	X	X	X	X	X	X

Table 6.2 Results set from Site 2

There is also evidence of traces of octylphenol (< 20 ng/l) in the effluent but no other traces were discovered out in the percolation area indicating the further degradation of the EDC probably in the biomat. An isolated incidence of butylphenol at JK4 is likely to be due to some analytical error / contamination. In this instance where only one sample of 1000 mL is available from each site it is not possible to eliminate errors like these. Traces of ethylphenol were discovered in the septic tank, secondary treated and the shallowest depth in the subsoil. Phenol and its derivatives are basic functional units of a wide variety of synthetic organic pollutants including pesticides. And thus their presence can indicate sources associated with chemicals of everyday household or outdoor use.

6.3 EDC results from Site 3

Site 3 was receiving septic tank effluent which was discharged into subsoil characterised as gravely-clayey-sand with slower percolation characteristics than either Sites 1 or 2. The results for Site 3 (shown in Table 6.3) showed no detectable EDCs in the septic tank effluent and neither was any firm detection of EDCs achieved out in the percolation area. This may be due to the placement of the lysimeters at the edge of the plume as described in Section 4.3, which could have acted to reduce any concentration due to the rainfall recharge. However, there is slight evidence of ethynyloestradiol (component of the contraceptive pill) at the 10m sampling point which, at that time, would have been receiving at the largest volume of effluent due to the progressive development of the biomat.

There is also evidence of octylphenol in sub-detection limit concentrations (<20 ng/l), again possible evidence of cleaning products. The analyte was found by mass spectral library comparison of the chromatographic peak at the retention time of octylphenol. For confirmation of results in these samples, larger sample volumes would be required (i.e. up to 5 litres) from each site would be needed for preconcentration and sample clean-up. For Site 3 the COD levels remain high throughout the sample set and this may explain the appearance of phenolic compounds (from mass spectral evidence) in many of the samples.

sample name	m.w. ION	r.t. Min	NKX	NK1	NK2	NK3	NK4	NK5	NK6	NK7	NK8	NK9
Phenol	94.00	3.60	X	X	X	X	X	X	X	X	X	X
Ethylphenol	122.00	5.64	X	X	X	X	X	X	X	X	X	X
Dichlorophenol	162.00	6.17	X	X	X	X	X	X	X	X	X	X
Propylphenol	107.00	7.31	X	X	X	X	X	X	X	X	X	X
Butylphenol	121.00	7.50	X	?	?	X	X	X	X	X	X	X
Trichlorophenol	196.00	8.79	X	X	X	X	X	X	X	X	X	X
Hexyloxylphenol	194+110	17.90	X	X	X	X	X	X	X	X	X	X
Lindane	183.00	19.40	X	X	X	X	X	X	X	X	X	X
Octylphenol	206+107	19.50	X	?	?	?	?	X	X	X	X	X
Hexyloxylphenol	208+110	19.90	X	X	X	X	X	X	X	X	X	X
Nonylphenol	107+220	21.00	X	X	X	X	X	X	X	X	?	X
Dieldrin	263+345	2440	X	X	X	X	X	X	X	X	X	X
Bisphenol A	213.00	24.50	X	X	X	X	X	X	X	X	X	X
Diethylstilbestrol	268.00	25.49	X	X	X	X	X	?	X	X	X	X
Oestrone	270.00	27.70	X	X	X	X	X	X	X	X	X	X
17β-Oestradiol	272.00	27.90	X	X	X	X	X	X	X	X	X	X
Ethynyloestradiol	296.00	28.50	X	X	X	X	?	X	X	X	X	X
Oestriol	288.00	30.22	X	X	X	X	X	X	X	X	X	X

Table 6.3 Results set from Site 3

6.4 EDC results from Site 4

The subsoil was characterised as gravely-clayey-sand with the slowest percolation characteristics of all four sites. The EDC results for Site 3 are shown on Table 6.4. The septic tank effluent was shown to contain detectable concentrations of oestrogens oestrone and 17-β oestradiol at concentrations of low µg/l. Oestradiol and oestrone (metabolite of oestradiol) were identified in the subsoil for samples JH2 and JH3. These oestrogens were only picked up at the 0m sampling point which corroborates the evidence from the ongoing MS-15 project that effluent only reached these points due to restricted development of the biomat due to the lower organic concentrations in the secondary treated load. It is perhaps surprising that such concentrations are passing through both septic tank and the peat filter, although Site 4 did have the highest number of female residents compared with all the other sites studied.

sample name	m.w. ION	r.t. Min	JHX	JHY	JH1	JH2	JH3	JH4	JH5	JH6	JH7	JH8	JH9
Phenol	94.00	3.60	X	X	X	X	X	X	X	X	X	X	X
Ethylphenol	122.00	5.64	X	?	X	X	X	X	X	X	X	X	X
Dichlorophenol	162.00	6.17	X	X	X	X	X	X	X	X	X	X	X
Propylphenol	107.00	7.31	X	X	X	X	X	X	X	X	X	X	X
Butylphenol	121.00	7.50	X	X	X	O	O	X	X	X	X	X	X
Trichlorophenol	196.00	8.79	X	X	X	X	X	X	X	X	X	X	X
Hexyloxylphenol	194+110	17.90	X	X	X	X	X	X	X	X	X	X	X
Lindane	183.00	19.40	X	X	X	X	X	X	X	X	X	O	X
Octylphenol	206+107	19.50	X	?	X	X	X	X	X	X	X	X	X
Hexyloxylphenol	208+110	19.90	X	X	X	X	X	X	X	X	X	X	X
Nonylphenol	107+220	21.00	X	X	X	?	X	X	X	X	X	X	X
Dieldrin	263+345	24.40	X	X	X	X	X	X	X	X	X	X	X
Bisphenol A	213.00	24.50	X	X	X	X	X	?	X	X	X	X	X
Diethylstilbestrol	268.00	25.49	O	X	X	X	?	X	X	X	X	X	X
Oestrone	270.00	27.70	O	X	X	X	O	X	X	X	X	X	X
17β-Oestradiol	272.00	27.90	O	X	X	O	0	X	X	X	X	X	X
Ethynyloestradiol	296.00	28.50	X	X	X	X	X	X	X	X	X	X	X
Oestriol	288.00	30.22	X	X	X	X	X	X	X	X	X	X	X

Table 6.4 Results set from Site 4

The alkylphenol results were inconclusive due to the limit of detection restrictions because of small sample volumes available for analysis with traces of nonylphenol and octylphenol occurring in areas where the effluent was known to be percolating. There were, however, concentrations of butylphenol detected in the subsoil down to 2m depth. Butylphenol has been shown to have oestrogen like properties and is widely used as an intermediate in the manufacture of varnish and lacquer resins, as an antioxidant and as a motor oil additive, and therefore can enter the environment through a number of routes.

The only slight indication of bisphenol A on any of the sites was detected at a position on this site where the effluent was known not to have percolated and so the validity of this result has to be questioned.

Finally, an isolated incident of lindane was found in the subsoil at the 20m point, again an area where the effluent was known not to have reached. However, this field had been used for crops prior to the construction of the percolation area and so the occurrence of lindane, a commonly used pesticide, must have been due to a previous application.

6.5 Discussion of results

From the results obtained, it is clear that the EDCs selected for this study are absent at the detection levels achieved for the majority of samples tested. This makes it difficult in general to make valid comparisons between sites, treatment systems and soil types especially due to the one-off nature of each sampling event and also the level of detection achievable with volumes of only one litre.

There appeared to be no discernable difference to the quality of the effluent according to the length of time the sites had been established; Sites 1 and 2 had been established for 12 months compared to 9 months for Sites 3 and 4. There was also no particular evidence of any degradation of EDCs with depth in the subsoil, as detected by the sets of three lysimeters at 0.3m, 0.6m and 1.0m depth below the percolation pipe at each sampling position, with the exception possibly the oestrogens on Site 2. In fact the results for both oestrogens and alkylphenols often indicate the opposite scenario whereby EDCs were found at deeper depths but not in the shallower subsoil above, indicating perhaps the sporadic nature of production and disposal of such EDCs (particularly oestrogens) which would need to be captured by a more prolonged sampling regime. Equally, it is not possible to compare the different subsoils from the results of these trials due to the lack of conformity between raw effluent samples in terms of EDCs since all sites were not receiving the same concentration of effluent.

The processes in the septic tank and subsequent breakdown in the secondary treatment peat filter can not be confidently gained from the results, although Site 4 did seem to

indicate that oestrone and 17β-oestradiol had been reduced to below the limit of detection by the secondary treatment process. However, this result must be tempered by the fact that the oestrogens were found out in the subsoil at detectable concentrations which must have previously passed through the peat filter. However, there does seem to be a correlation between the particular composition of the different households in terms of EDCs in the effluent, for example the high level of surfactants on Site 1 which is the livery stable and the higher level of oestrogens in Site 4 with the largest number of female inhabitants.

The fate and behaviour of EDCs has been shown (Chapter 2) to be mainly influenced by its physiochemical properties and the majority of EDCs tend to favour adsorption onto solid surfaces or into biota. Hence, in general it would be expected that all of the target analytes would be immediately trapped in the humic material or natural organic matter in the sample as shown by the COD results in Chapter 4 which illustrates that there is a large concentration of oxidisable organic matter.

Oestrogens

A surprising result from the study was that oestriol was not detected in any of the sites even though its octanol/water partition coefficient (K_{ow}) is lower than both oestrone and 17β-oestradiol making it less susceptible to adsorption to organic materials in the septic tanks and secondary processes. The reason for this may be due to its higher detection limit at 20 μg/l in the analysis compared to the other oestrogens. However, this may also indicate that it is more susceptible to aerobic degradation as shown in other studies whereby oestrone is commonly the most persistent of the oestrogens after secondary treatment which also concurs with some evidence in this study. In most of the reported studies on secondary treated effluent the highest levels to which oestrone has been detected is of the order of 100-200 ng/l, a concentration 10 times lower than this study was able to detect. It should also be noted that the other oestrogens are normally at even lower levels (10 ng/l, for example) and that out in the subsoil, previous studies have only detected samples (if detected at all) generally in the range <10 ng/l. Where oestrogens have been detected on these sites they are of the relatively high concentrations with oestrone (~2 μg/l) and 17β-oestradiol (~2 μg/l).

Ethynylestradiol, a component of the contraceptive pill, was shown to be present in Sites 2 and 3 and is known to display a much slower degradation half-life than the natural oestrogens and so would be expected to still be present in the subsoil even if the natural oestrogens had been long degraded.

There is evidence that the oestrogens have only been detected in relatively high concentrations in the subsoil on the two sites receiving secondary treated effluent which may be a function of the higher hydraulic loading on the subsoil and low organic concentrations in such effluent compared to the septic tank effluent which has been shown to develop a much more extensive biomat. It should be appreciated, however that the oestrogenic mass spectral selection of oestrogen compounds may be due to the presence of flavanoids that have similar structural and molecular weight characteristics. While these samples are taken at a distance away from the direct effluent, there may be some re-conjugated species in later samples. This can occur where microbial action is higher. Alternatively, these results may be due to naturally occurring oestrogens providing the same mass spectral information.

Organic oxygen compounds

Bisphenol A is generated from the production of polycarbonate and epoxy resins and certain phthalates which are involved in the manufacture of plastics used in food packaging and other domestic products. It was used in this study since it known to leach into products and thereby find its way into domestic sewage effluent. Previous studies have measured concentrations in the septic tank effluent as high as 1 µg/l, although bisphenol A is much less potent as an EDC compared to the oestrogens. Secondary treated effluent has showed bisphenol A was found in concentrations 110-180 ng/l. In this study, however, no bisphenol A was detected in any of the effluents or after passing through the soil matrix on the sites (down to a detection limit of 20 ng/l) indicating that it is possibly not of concern for typical on-site type effluents produced in Ireland. The organic oxygen compounds, phthalates and bisphenol A have been shown to be easily removed in aerobic secondary treatment processes but this was not possible to verify since none were detected in any septic tank effluent.

Surfactants

In terms of surfactants, the alkylphenols have been shown to be mainly removed by bacterial degradation in secondary processes which also indicates that such EDCs would be expected to pass through the septic tank relatively unchanged. This seems to have been corroborated by these results. On the sites the only surfactant product being picked up (down to levels 20 ng/l) seemed to be octylphenol and then only in the septic tank effluents and not in the secondary treated effluents indicating its removal more by aerobic bacterial degradation in the secondary processes than adsorption to organic particulate matter in the septic tank. Many of the alkylphenols are degraded to more hydrophobic compounds which will then adsorb to particulates and then find their way into the sludge. Alkylphenols, however, were not found conclusively in most samples, although octylphenol was being selected in some samples by the mass spectrometer but larger sample volumes would be needed to confirm these results.

Previous aerobic degradation experiments have shown that nonylphenol degraded quickly whereas octylphenol showed absolutely no degradation. Again there seems to be some evidence of this in these studies with octylphenol being commonly found on all sites out in the percolation area but only one possible trace of nonylphenol (well below the limits of detection) on Site 4.

7.0 Conclusions

The results obtained from the four sites have indicated the presence of four of the five target analytes in some of the samples tested. The analytes are only present at very low concentrations often below detection limit (with preconcentration) and generally only in samples known to be percolating effluent. The results do not indicate that the analytes in question, where present in the original waste, are removed while passing through the sub-soil and as such the study cannot make any definitive conclusions about the ability of the subsoil to reduce these EDCs.

There is evidence that some of the oestrogens have been detected in relatively high concentrations in the subsoil compared to other studies, particularly on the two sites with secondary treatment. However, the levels of the other targeted EDCs (organic oxygen compounds and surfactants) from on-site treatment systems are very low, particularly for bisphenol A (down to limits of 20 ng/l) and as such probably pose little threat to groundwater sources. This small study has also provided some evidence that the surfactants are passing through the septic tanks but get removed in secondary treatment processes although this would need to be confirmed by a much more rigorous sampling schedule.

It should be remembered that most studies have shown that alkylphenols have a lower endocrine disruption potency than bisphenol A and equally that bisphenol A has been shown to be 10 000 times less potent than the oestrogen, 17β-oestradiol. Hence, it would appear that the main target for future research should be the oestrogens which seem to be present in higher concentrations than the other compounds and also have a higher potency. As an initial study this proves a useful guide to show both the levels of endocrine disrupting chemicals that should be expected from such on-site effluent in Irish households and also some of the removal processes as they pass through typical treatment units and down through the subsoil towards the groundwater. However, a more thorough study involving longer sampling times, to allow for larger sample volumes would aid the study and would confirm the presence and removal of some other potential endocrine disrupters.

7.1 Recommendations for further research

Further study on the fate of endocrine disrupters in on-site wastewater effluent is required, particularly concentrating on oestrogens which, as discussed previously, have the highest endocrine disruption potency and have been found in the highest concentrations in this study. Such a study should take regular samples over the periods of several months in order to catch variations in concentration profiles and also the lag in such peaks as they move through the treatment systems and down into the subsoil. The studies should be carried out on both sites receiving secondary effluent and septic tank effluent and sample sizes from the lysimeters should be at least 5 to 10 litres (taken over the period of several days if needs be) to enable analysis of the oestrogen to be made down to detection levels of single ng/l.

Studies should also be particularly focussed on sites with more sandy subsoils, with fast percolation (T-values 1-5) to see what the effect more freely draining soil has on the degradation of such EDCs.

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