DOCTOR OF PHILOSOPHY

Behaviour of endocrine disrupting chemicals and nitrate in the environment

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Award date:
2008

Awarding institution:
Bangor University

Behaviour of endocrine disrupting chemicals and nitrate in the environment

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July 2008

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Summary

The work in this thesis is divided into two discrete sections: the first describes the behaviour of estrogenic pollutants in soil, whilst the second describes the development of a nitrate and turbidity sonde for use in monitoring pollutants in groundwater boreholes.

Endocrine disrupting chemicals are of increasing concern due to their effects on aquatic organisms especially the feminisation of male fish. Some of the chemicals of concern are naturally occurring steroid hormones such as estrogens (reviewed in Chapter 2). Investigations into the biodegradation of the estrogens: estrone and 17 β-estradiol (published in the journal *Soil Biology and Biochemistry*; Chapter 3) were undertaken, alongside investigations into the sorption of the estrogens to soil (Chapters 3 and 4) and their ability to be leached through the soil profile during rainfall events (Chapter 4). Throughout these investigations ^{14}C-labelled estrogens was applied to different soils in various animal wastes. It was concluded that the estrogens were mineralised rapidly in soil and they behave differently when applied to soil in urine rather than water, which has implications for future work. Groundwater is an important resource for water supplies in the UK. The most common pollutant is nitrate, as reviewed in Chapter 5. UV spectrophotometry was investigated in the laboratory to develop a standard nitrate analysis method (Chapter 6). A major interference in nitrate measurement with UV in natural waters is dissolved organic carbon (DOC), which has an absorbance in the same range as nitrate (Chapter 7). Following laboratory development and calibration with humic acids to overcome the complications arising from DOC a field sonde was developed using UV spectrophotometry (Chapters 8 & 9). A separate sonde was developed to evaluate turbidity in natural waters. Chapter 10 shows the laboratory and field tests involved with the turbidity sonde, the work that goes into developing a new product and the results obtained.

There are many further experiments that could be developed from the work in this thesis. In the estrogen section experiments need to be carried out with intact soil cores and in the field with conditions that cannot be achieved in a laboratory. To put the work into a more global context different animals, agricultural practices, climate and geography would need to be considered. In the nitrate section future work includes the continued testing of the nitrate and turbidity sondes. There is also the possibility of developing a DOC sonde and the potential of phosphorus measurement could be explored.

Acknowledgements

I would like to thank:

My supervisor Professor Davey Jones for all his patience, help and guidance and also Professor Brian Reynolds for helpful suggestions.

Martin Payne at GeoVista without whom much of the work in this thesis would not have been possible. Thanks also to Simon, Seghir and Tony at GeoVista for all their help.

Jonathan Roberts for his help in the laboratory and field, Gareth Edwards for his help with the radioactive work in this thesis and Annie Brittain for help with ion chromatography.

I am grateful to Dave Buckley at the British Geological Survey for allowing me to accompany him on a field trip and Alexander Gallagher for keeping me informed of the progress of the sonde and Peter Neve, Mark Kyriacou and the Monitoring and Data (Chemical) Team at Environment Agency Wales for allowing me to accompany them on two days to collect groundwater samples.

My friends and colleagues in the lab especially Dr. Prysor Williams and Dr. Paula Roberts for help with collecting animal faeces and Dr. Liz Boddy for her help and support and cakes.

My parents, grandparents and Sarah for their interest and support.

Finally Alex for always being there to support me and little Dora for always wagging her tail when I come home.

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CHAPTER 1

Introduction

1.1 General introduction and layout of the thesis

The work in this thesis was supported by the European Social Fund (ESF) with the aim of developing research skills in an Objective One area of Europe, namely North West Wales. The work in the thesis is divided into two discrete sections: the first describes the behaviour of estrogenic pollutants in soil, whilst the second describes the development of a nitrate and turbidity sonde for use in monitoring pollutants in groundwater boreholes.

Endocrine disrupting chemicals are of increasing concern due to their effects on aquatic organisms especially the feminisation of male fish. Some of the chemicals of concern are naturally occurring steroid hormones such as estrogens. Chapter 2 reviews the current literature concerning the behaviour of endocrine disrupting chemicals in the environment, especially the behaviour of estrogens. Chapter 3 investigates the biodegradation of the estrogens, estrone and 17 β-estradiol, when applied to agricultural land in animal wastes. Radiolabelled hormone was applied to different soils in various animal wastes and the mineralisation of the hormone measured. Also the sorption of the hormones to soil was investigated to help explain the results found. Chapter 4 describes the results of experiments to investigate the potential of the hormones to move though soil during rainfall events. This was achieved using radiolabelled hormones and leaching columns.

Groundwater is an important resource for water supplies in the UK. The most common pollutant of this resource is nitrate. Chapter 5 reviews the current literature concerning nitrate in groundwater including methods of measurement. During the work carried out in this thesis it became apparent that it would be possible to construct both a nitrate and turbidity sonde, and consequently turbidity is also considered in Chapter 5. Chapter 6 describes the initial development of the nitrate analysis method using laboratory equipment and nitrate standards. The major interference in the nitrate measurement in natural water samples is from dissolved organic carbon (DOC), which has an absorbance in the same region as nitrate. Chapter 7 describes the investigation into the effect of DOC on the measurement of nitrate using a range of different humic acids from the International Humic Substances Society. Chapter 8 shows the development of the nitrate sonde, including some of the problems that were encountered and how they were overcome. In chapter 9 the results from testing the sonde using natural groundwater samples are shown and also the results from a sonde being run down the

borehole. The development of the turbidity sonde in the laboratory, initial testing and testing in the field is shown in Chapter 10.

The general discussion in Chapter 11 examines the significance of the results from the previous experimental chapters in a global context. Conclusions are drawn and areas of further work identified. Finally, the appendix includes photographic illustrations of the experiments.

1.2 Aims of the thesis

The aims of the thesis were partly determined by the ESF funding that had been obtained and also partly by the needs of the industrial partners, GeoVista Ltd. The overall aims of the thesis are as follows:

- Investigate the rate of degradation of naturally occurring estrogens applied to land in animal manures and urine including the effect of application to different soils.
- Investigate the potential for leaching of estrogens to occur during rainfall and so the potential for the estrogens to pollute aquatic ecosystems.
- Develop and test a novel method to enable the *in-situ* measurement of nitrate within groundwater boreholes and to investigate any potential interferences with the method.
- Develop and test a method to enable the *in-situ* measurement of turbidity within groundwater boreholes and to investigate any potential interferences.
- Produce working nitrate and turbidity sondes in conjunction with GeoVista that can be sold to customers worldwide.

CHAPTER 2

Literature Review – Behaviour of the endocrine disrupting chemicals, estrogens, in the environment

2.1 Introduction

There are different definitions describing the action of endocrine disruptors in biological organisms, however, essentially they are either naturally occurring or synthetic substances that interfere with the functioning of endocrine systems in organisms resulting in unnatural responses (Hainsworth, 2000). The endocrine system is an interconnected network of chemical communication in organisms. It includes hormones, the ductless glands that secrete hormones and the molecular receptors on or in target cells that respond to hormones. It functions with the nervous system to mediate internal regulation and maintain homeostasis, which is the steady state physiological condition of the body (Campbell et al., 1999). The hormones that are involved in the endocrine system are manufactured and secreted in small quantities into the bloodstream by an endocrine gland or a specialised nerve cell and regulate growth or functioning of a specific tissue or organ in a distant part of the body (Daindith, 2000). The concentrations of hormones required to control most metabolic and endocrine functions are small. Concentrations of hormones in the blood range from 1 ng l^{-1} to a few mg l^{-1} (Guyton and Hall, 2000).

The primary natural female steroid hormones are estrogens, which are produced in the ovary and corpus luteum. Estrogens stimulate the development of the female reproductive system and secondary sex characteristics (Campbell et al., 1999). There are also androgens which are the principal male hormones, such as testosterone, which stimulate the development and maintenance of the male reproductive system and secondary sex characteristics (Campbell et al., 1999).

The sources of endocrine disrupting chemicals are varied but they enter the environment in three main ways (Hainsworth, 2000),

- Releases to air, land or water as by-products from industrial processes or waste disposal,
- Aquatic discharges from domestic or industrial wastewater treatment plants and sewage systems,
- Intentional use e.g. pesticides.

These compounds are believed to cause many different effects in animals. Some general ones include deformities, embryo mortality, impaired reproduction and development, depression of thyroid and immune functions and feminisation of organisms e.g. fish (van der Kraak, 1998).

2.2 Types of endocrine disruptor

Endocrine disruptors can be divided into four main groups (Ying et al., 2002a);

- Natural hormones - from any animal, such as human hormones released from sewage effluent and animal hormones from the spreading of manure on land.
- Natural chemicals – produced by certain plants and fungi.
- Synthetically produced pharmaceutical hormones such as the contraceptive pill and treatments for hormone responsive cancers released from sewage effluent.
- Man-made chemicals – certain pesticides, chemicals in consumer and medical products and industrial chemicals.

2.1.2 Natural and synthetic hormones

These include 17 β-estradiol (E2) (Figure 2.1), estrone (E1) (Figure 2.2) and estriol (E3) (Figure 2.3). Also of concern are the pharmaceutical contraceptives ethynyl estradiol (EE2) (Figure 2.4) and mestranol (MeEE2) (Figure 2.5) (Ying et al., 2002a). 17 β-estradiol has been found to cause feminisation of male fish at levels as low as 1 ng l^{-1} in male trout (Hansen et al., 1998). There is also concern over the increasing use of steroid drugs in farming where they are used to control the oestrus cycle, treat reproductive disorders and induce abortion (Prezoisi, 1998). Steroid hormones are also fed to animals as growth promoters. These may contain endogenous hormones like estradiol, testosterone and progesterone or synthetic substances like zeranol, trenbolone acetate or melengestrol acetate (Lange et al., 2002). When released, estrogens will interact with the environment, for example, 17 β-estradiol can undergo sorption and degradation reactions in soils and sediments. It has been estimated that 17 β-

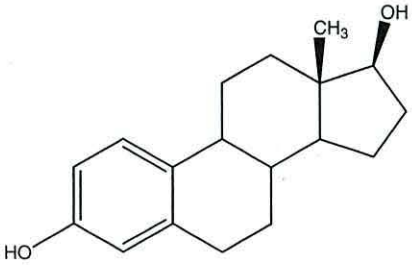

Figure 2.1: 17 β-estradiol

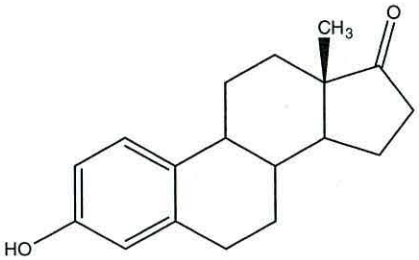

Figure 2.2: Estrone

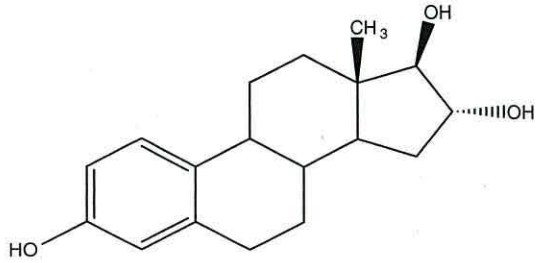

Figure 2.3: Estriol

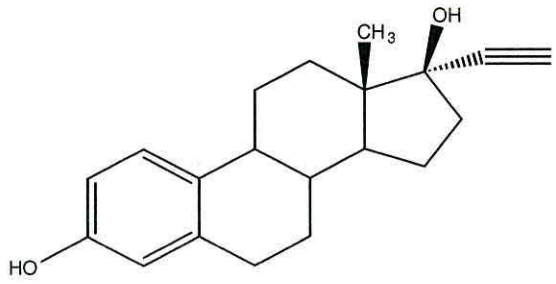

Figure 2.4: Ethynyl estradiol

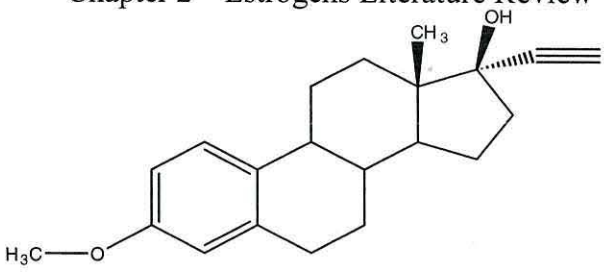

Figure 2.5: Mestranol

estradiol and estrone have half lives of 2 to 6 days in water and sediment (Ying et al., 2002a). Estradiol and estrone also have several oxidation steps in their metabolic pathways and as they are released in small quantities it would be expected that environmental exposure would be low. However 17 β-estradiol has been detected in sewage treatment effluents at levels of 3 - 48 ng l^{-1} in the UK (Ying et al, 2002a). Synthetic hormones are generally expected to have a lower rate of biodegradation than natural ones but the available data is contradictory. For example, for the synthetic hormones ethynyl estradiol and mestranol both biodegradability and prolonged persistence have been reported (Ying et al., 2002a). The behaviour of estradiol and estrone in soil will be studied in this project and so estrogens will be discussed in more detail later in this review.

2.2.2 Pesticides

The pesticide o-p'-DDT (dichlorodiphenyltrichloroethane) (Figure 2.6) has been shown to be estrogenic and cause feminisation in birds, turtles and frogs (Prezoisi, 1998). Other pesticides, such as chlordecone, dieldrin, aldrin, endosulfan, toxaphene and linuron all display estrogenic and anti-androgenic properties. The o-p' isomer of DDT is estrogenic at a potency of 1 mg kg^{-1} (Miyamoto and Klein, 1998). Even though it is no longer in use it is still detectable in many regions of the world as chlorinated pesticides such as DDT can persist in soils for 3 to 20 years (Brady and Weil, 2002).

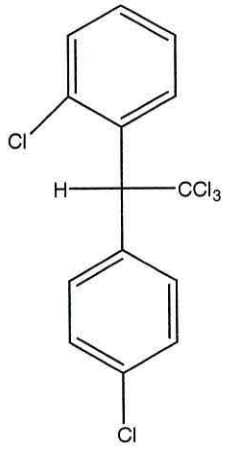

Figure 2.6: o-p-DDT

2.2.3 Polychlorinatedbiphenyls

PCBs are used in transformers, capacitors and hydraulic fluids. Several isomers, hydroxylated metabolites and commercial PCB mixtures are estrogenic and others have been found to alter the function of the thyroid, which can affect the development of the gonads (Prezoisi, 1998). There is a wide range of isomers with different degrees of chlorination. The more chlorinated molecules have higher lipophilicity and lower water solubility and biodegradation potential. This means that bioaccumulation and environmental persistence increases as the amount of chlorination increases (Miyamoto and Klein, 1998).

2.2.4 Alkylphenolic chemicals

Figure 2.7: Nonylphenol

Alkylphenolics contain an alkyl chain bonded to phenol. Nonylphenol (NP) (Figure 2.7) is used as an intermediate for phenol resin production, in antioxidants and in polymerisation accelerators. A decreasing amount is used for production of nonylphenol ethoxylates, which can be used as surfactants in detergents, pesticide formulations and as additives in textile and leather manufacturing (Miyamoto and Klein, 1998). Environmental degradation can lead to corresponding alkylphenols. Nonylphenol enters sewage treatment works where the amount biodegraded is very uncertain as it is reported in a review by Miyamoto and Klein (1998) to be between 0 and 100%! They quote the most likely value to be about 70%. The half-life of NP in the environment is reported to be 30 to 58 days (Miyamoto and Klein, 1998). It is also known that 4-nonylphenol binds to estrogen, progestin and androgen receptors (Prezoisi, 1998). Alkylphenolics and their degradation products are not produced naturally and so their presence is likely to be solely due to anthropogenic sources (Ying et al., 2002b).

2.2.5 Tributyl tin oxide (TBT)

TBT is different to the other chemicals previously mentioned as it is androgenic instead of estrogenic. This means that it mimics male hormones such as testosterone. It can cause the development of imposex females in organisms such as whelks. This involves the development of a penis and vas deferens tissues in females, which prevents breeding (Miyamoto and Klein, 1998). TBT is found in fungicides and biocides in water purification

and desalination processes and in wood protection. TBT was also used as an antifouling agent on boats but this has now been banned. Typical levels in water in river and marine environments are 5 ng l^{-1} and in sewage sludge 10 mg kg^{-1} (Miyamoto and Klein, 1998).

2.2.6 Phytoestrogens

These include flavenoids, isoflavones (found in soy proteins), coumestans, lignans and myco-estrogens (Prezoisi, 1998). B-sitisterol can cause infertility in animals such as sheep that graze on plants rich in phytoestrogens. It is assumed that phytoestrogens do not persist or bioaccumulate in the environment and exposure will be restricted to wildlife eating certain plants (Miyamoto and Klein, 1998). This could be a problem where there are large monocultures of certain crops which wildlife has to eat due to feed limitation (Miyamoto and Klein, 1998).

2.3 Environmental effects of endocrine disrupting chemicals

When assessing the environmental effects of endocrine disrupting chemicals there are different factors to consider. Just because a chemical affects endocrine systems *in vitro* or *in vivo,* it does not mean that it is necessarily an environmental endocrine disruptor when actually released into the environment (Miyamoto and Klein, 1998). Also the potency of each individual endocrine disruptor needs to be considered as do fluctuations in the levels of the chemicals. The concentrations present in the environment can vary throughout the year with the changing seasons as they can become more diluted in environments such as rivers when there is more rainfall and more concentrated during the summer months (Miyamoto and Klein, 1998). The amount of rainfall and the temperature will also affect the concentrations present in soil (Miyamoto and Klein, 1998). As no effect can occur to an organism without exposure to the disruptor, the releases and environmental fate of the chemicals also need to be understood which includes the interactions of the chemicals with water, soil, sediment and air. To complicate this there can be simultaneous exposure of organisms to several contaminants (Miyamoto and Klein, 1998). This can lead to synergistic effects (one chemical increases the effects of another) or antagonistic effects (one chemical reduces the effects of another) (Clark, 2002). When looking at natural populations of organisms it is important to remember that there could be other factors present which could be affecting the population when deciding if an endocrine disrupting chemical is having an effect on that population.

When studying the endocrine disrupting ability of a chemical there needs to be a signal that the chemical is having an effect on an animal. One of the most commonly used signals is the production of vitellogenin in male fish (Clark, 2002). Vitellogenin is an egg yolk protein which is synthesised in the liver under the control of estrogens. The enzymes that produce it are present in both male and female fish but are normally only activated by estrogens in maturing females (Clark, 2002).

One of the most cited studies on the environmental effects of endocrine disrupting chemicals is Jobling et al. (1998). This study investigated whether endocrine disrupting chemicals exist in the environment at concentrations high enough to exert adverse reproductive effects on fish. It was already known that exposure to estrogens and alkylphenols could cause synthesis and secretion of vitellogenin in male fish and could occur up to several kilometres distant from the point of the effluent entry where the disruptors are entering the environment. Jobling et al. (1998) investigated if this was occurring on a large scale in UK rivers. To do this, wild populations of roach (*Rutilus rutilus*) were sampled randomly both upstream and downstream of sewage treatment works. The control sites were a range of lakes and canals as no rivers could be found without any effluent from sewage treatment works. There was also a population reared in the laboratory in water from a spring. Overall, 60 to 100 adult roach were collected from each location. Their blood was sampled and the length, total weight and gonadal weight were determined for each fish. Their results found that all of the fish appeared to be either male or female physically but histological examination of the gonads revealed that a large proportion of the males were actually intersex. This means they had both male and female characteristics. These intersex fish were found at all sites, although the incidence was higher in effluent impacted regions of rivers than the control regions. Downstream of the sewage treatment works the intersexuality ranged from 16% in the River Wreake/Eye to 100% in the River Nene and the River Aire. Upstream of the sewage treatment works the intersexuality ranged from 12% in the River Lea to 44% in the River Nene. The percentage of intersexuality in the control populations was 4% to 18%. This study provided the first real evidence to show that there was a major pollution issue associated with endocrine disrupting chemicals in the UK.

Fish are most susceptible to endocrine disruption in the time prior to morphological sex differentiation, just following hatching or at the juvenile stage (Jobling et al., 1998). In this study, it was impossible to tell the genetic sex of the fish that were examined, therefore Jobling et al. (1998) were unable to determine whether intersexuality

was due to feminisation or masculinisation. They established that the number of fish with normal testes was inversely proportional to the number of intersex fish which implies that the intersex fish were originally male. This was supported by the knowledge that sewage contains estrogenic substances so Jobling et al. (1998) concluded that intersexuality was due to the feminisation of male fish. Jobling et al. (1998) also showed that the plasma vitellogenin concentrations provided strong evidence that some populations of fish were being exposed to estrogenic chemicals. In general, the concentration of vitellogenin found in intersex fish were intermediate between the concentrations found in males and those found in females. In their study they found that higher concentrations of effluent from the sewage treatment works gave higher incidence of intersexuality, although when assessing the results the authors stated that it was important to remember that a "typical" effluent does not exist. For instance, there were not just natural and synthetic estrogens present but also alkylphenolic compounds and other chemicals. Effluent levels can also change with the seasons, with lower levels of rainfall in the summer and so less dilution of the effluents at the time when juveniles are undergoing sex differentiation (Jobling et al., 1998).

The conclusion to this study was that discharges are inevitable for human existence and if global warming continues allied to water use increases and water reuse schemes are implemented, then the impacts of sewage discharges on fish will be expected to increase (Jobling et al., 1998).

2.4 Natural hormones and pharmaceuticals - estrogens

Estrogens are biologically active steroid hormones that are synthesised from cholesterol and have in common a cyclopentan-*o*-perhydrophenanthrene ring (Figure 2.8) (Ying et al., 2002a).

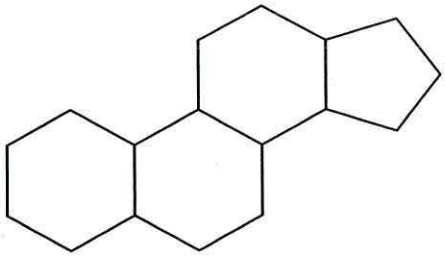

Figure 2.8: cyclopentan-*o*-perhydrophenanthrene ring

Steroid hormones are excreted by all humans (Ying et al., 2002a). Women can excrete levels of 17 β-estradiol from 2 – 12 μg person^{-1} day^{-1} and estrone from 3 – 20 μg person^{-1} day^{-1}. This amount can vary greatly depending on other factors such as pregnancy and menopause (Ying et al., 2002a). Men can excrete levels of estradiol of approx 5 μg person^{-1} day^{-1}. Based on the amounts in oral contraceptive pills, it is estimated that 35 μg person^{-1} day^{-1} of ethynyl estradiol is excreted

when they are used (Ying et al., 2002a). In the UK, concentrations of estrogenic hormones from sewage treatment plants have been found to be 1.4 to 76 ng l^{-1} for estrone and 2.7 to 48 ng l^{-1} for 17 β-estradiol (Ying et al., 2002a). When the daily levels of excretion and previous measurements are taken into account it was estimated that ng l^{-1} levels of estrogens were expected in English rivers. There is therefore potential to exceed the level of 1 ng l^{-1} where vitellogenesis was reported in male fish (Ying et al., 2002a). This was supported by Hansen et al. (1998) who found that vitellogenesis in fish was occurring at concentrations of 1 ng l^{-1}.

Animals also excrete steroid hormones. Lange et al. (2002) estimated the daily estrogen excretion rate by the predominant mammalian farm animal species (Table 2.1).

Table 2.1: The total daily excretion of estrogens by various farm animal species (Lange et al., 2002).

Farm animal		**Total daily excretion of estrogens (μg animal^{-1})**
Cattle	Cycling cows	299
	Bulls	540
Pigs	Cycling sows	120
	Boars	2300
Sheep	Cycling ewes	23
	Rams	25

Plotka et al. (1969) studied the excretion of estrogens in urine from ewes and found that the highest average rate of excretion occurred during oestrus which was 394 μg sheep^{-1} day^{-1} for estrone and 479 μg sheep^{-1} day^{-1} for estradiol. The lowest concentrations were the second day after oestrus when the levels had fallen to 20 μg day^{-1} for estrone and 17 μg day^{-1} for estradiol. As well as the natural hormones present, cattle overseas are often fed steroids which can affect the steroid content of their urine. The major difference between human waste and animal waste is that human waste passes through wastewater treatment plants whereas animal waste is often left to rot in manure heaps before it is applied to land (Lange et al., 2002).

The fate of estrogenic chemicals in the environment has been considered (Ying et al., 2002a) and was affected by the distribution and partitioning of estrogenic steroids in different matrices and so was determined by their physicochemical properties and site-specific environmental conditions.

From measurements in three English rivers it was found that bed sediments accounted for between 13% and 92% of the estrogenic loads in the river system. K_{oc} and K_{ow} values from various studies predicted high binding with sediment/soil particles however, surprisingly estrogenic compounds are often reported in surface and groundwater at high concentrations and so more work is needed to understand their behaviour (Ying et al., 2002a). The half lives of estrogenic steroids are estimated to be 2 – 6 days in water and sediment (Ying et al., 2002a). 17 β-estradiol is abiotically transformed into estrone in sterile and non-sterile soils. Estrone and ethynyl estradiol are microbially degraded but more work also needs to be done in this area (Ying et al., 2002a).

There have been a few studies on groundwater. Studies that have been carried out have shown that using animal manure as a fertiliser can lead to the movement of estrogenic chemicals into surface and groundwater. In the review by Ying et al. (2002a), it was stated that 17 β-estradiol was mobile in water and detected in run off from manured land.

2.5 Sewage sludge and animal manures

Since 1989/90 the level of sewage sludge disposal to land in the UK has remained constant at between 1 to 1.1 million tonnes per year. In 1998/9, 936000 tonnes of sewage sludge was produced in England and Wales with 1058000 tonnes in the UK as a whole (Defra, 2003). Around half a million tonnes is disposed of on farmland and until recently the next largest disposal route has been the sea in areas such as the Thames Estuary and Liverpool Bay. However, the disposal of sewage sludge at sea stopped at the end of 1998 and incineration is now the second largest disposal route as shown in Figure 2.9. Figure 2.9 also shows that farmland is the most important disposal route and with increasing regulations on the landfilling of waste it will continue to be important in the future (Defra, 2003).

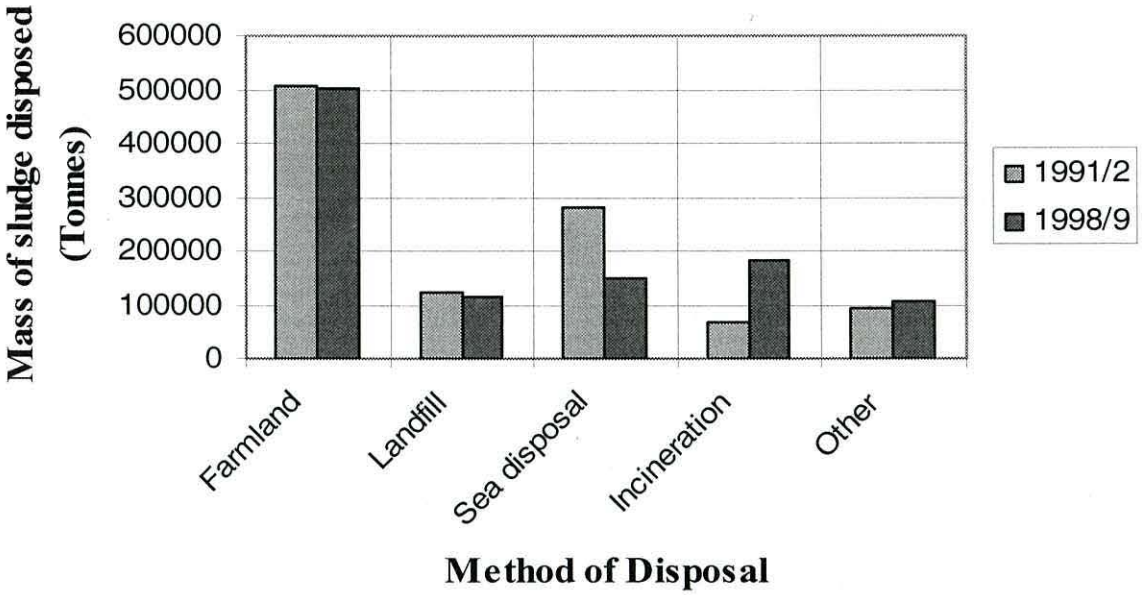

Figure 2.9: The changes in the methods of disposal of sewage sludge in the UK between 1991/2 and 1998/9 (Defra, 2003).

The legislation governing the spreading of sewage sludge on land is the Sludge (use in agriculture) Regulations 1989 (as amended) (Defra, 2003). Estrogens enter the sewage system in a form that is largely not estrogenic. This is because estrogens are excreted primarily by humans as a variety of inactive glucuronide or sulfonide conjugates (Johnson and Sumpter, 2001). These have the same structure as unconjugated estrogens except that either a sulphate or glucuronide group is substituted at C3 and or C17 position (Figures 2.10 and 2.11) (Hanselman et al., 2003). However, in the sewage system there are many bacteria such as *Escherichia coli* that contain the β – glucuronidase enzyme (D'Ascenzo et al., 2003). This means that the glucuronidase region of the molecule would be rapidly cleaved and metabolised (Johnson and Sumpter, 2001). This is supported by Andersen et al. (2003) who found that the estradiol-3-glucuronide conjugate of estradiol was readily converted to the active hormone 17 β-estradiol. This suggests that no glucuronide conjugates would survive the sewage system.

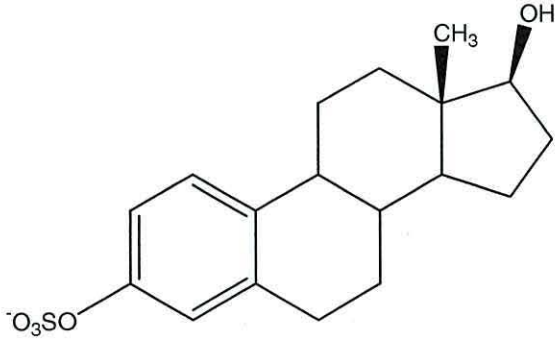

Figure 2.10: 17 β-estradiol -3 - sulphate

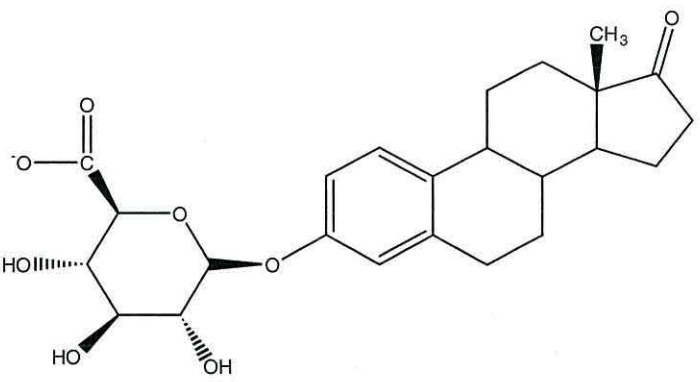

Figure 2.11: Estrone -3-glucuronide

Only approximate predictions of the sorption potential of estrogens to sludge can be made. 17 β-estradiol is considered to be weakly hydrophobic with a log K_{ow} of 3.1 (Andersen et al., 2003). A modelled value for estrone has been given as log K_{ow} 3.4. The synthetic steroid ethynyl estradiol is more hydrophobic with a log K_{ow} of approximately 3.9 and so its removal into sludge is likely to be important during the sewage treatment process (Andersen et al., 2003).

As well as sewage sludge that is applied to fields, animal waste is also often applied as a fertiliser and a means of disposal. In 1997, the annual amount of livestock manure that required collection, handling, storage and land application in the UK was 90 million tonnes, of this 73 million tonnes was cattle manure (Smith et al., 2001). Hanselman et al. (2003) reviewed manure-borne estrogens as potential environmental contaminants, detailing the work that had been carried out up to 2003 and work that should be carried out in the future. The authors identified that different farm animal species can excrete estrogens by different routes; cattle mostly excrete estrogens in their faeces whereas swine and poultry excrete estrogens mostly in urine. However, since urine and faeces are not generally handled separately the route of excretion is not an important environmental consideration. Hanselmann et al. (2003) also identified work that was needed that had not been carried out yet. This included looking at the sorption of hormones to soil when manure was present as no studies had been carried out with additions of manure to soil.

Both cattle and poultry manure have been reported to contain 17 β-estradiol and estrone in both conjugated and unconjugated forms (Lee et al., 2003). The concentration of 17 β-estradiol in poultry manure depends on the gender and age of the poultry and has been found to range from 14 μg kg^{-1} in immature male broilers to 533 μg kg^{-1} in laying hens (Nichols et al., 1997). Cattle urine can contain 13 ng l^{-1} of 17 β-estradiol (Peterson et al., 2000).

2.6 Estrogen behaviour in soil

Studies have been carried out on the behaviour of estrogens in soils. This is important as endocrine disrupting chemicals in soil can either affect soil organisms or they can be mobile and run off into water supplies and so affect aquatic ecosystems.

A problem with the study of estrogens is that the concentrations involved are very low and the hormones are also active at low concentrations. One study by Colucci and Topp (2001) addressed this problem by identifying a way to measure low concentrations (ng kg^{-1}) by using radio-labelled 17 β-estradiol and ethynyl estradiol.

Three different soils were tested by the addition of ^{3}H- or ^{14}C-labelled hormone. Hormone residue analysis was carried out by periodically opening the jars containing the soil and removing 5 g of moist soil using a sterile spatula. Radioactivity was measured with a liquid scintillation counter and the parent compounds and the transformation products were analysed by reverse phase HPLC with radioactivity detection. The detection limit was found to be 11 ng kg^{-1}, which is lower than previous studies. 17 β-estradiol was reduced to the detection limit within 24 hours of incubation at 30°C which was accompanied by an accumulation of estrone which then dissipated to undetectable limits in the sandy loam in 1 day, loam in 2 days and silt loam in 3 days. It was concluded by Colucci and Topp (2001) that environmentally meaningful concentrations of 17 β-estradiol were rapidly dissipated in agricultural soils.

In a study by Lee et al. (2003), the simultaneous sorption and dissipation of three reproductive hormones (17 β-estradiol, ethynyl estradiol and testosterone) were studied using batch equilibrium techniques. It was found that the half-life of 17 β-estradiol in soil/water slurries was 4.5 to 9.7 days in fresh water sediment from a river and 0.8 to 1.1 days in a surface soil. The log K_{oc} values found showed that organic carbon is the primary sorption domain for 17 β-estradiol. In this study the authors criticised the work carried out by Casey et al. (2003) who also carried out experiments with 17 β-estradiol.

Casey et al. (2003) used radio-labelled (^{14}C) 17 β-estradiol, at a concentration similar to those found in manures applied to land, in a series of batch sorption and miscible displacement reactions. The concentrations applied to

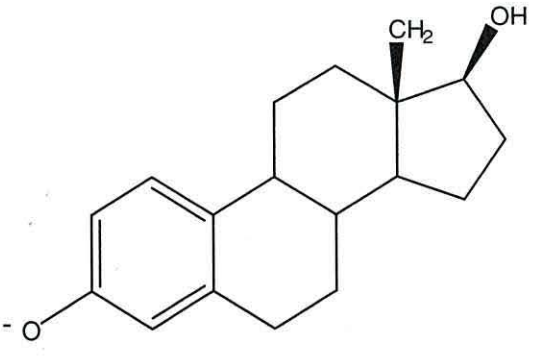

Figure 2.12: The anion formed by dissociation of the phenolate group

soil were 150 μg l^{-1}, 15 μg l^{-1} and 1.5 μg l^{-1}. The measurements taken after the experiment were complicated by transformation of the hormone and possible colloidal suspensions. During the first 48 hours the 17 β-estradiol sorption rates ranged from 0.002 μg g^{-1} soil $hour^{-1}$ for sand to 0.112 μg g^{-1} soil $hour^{-1}$ for bentonite clay. Casey et al. (2003) found that there was a strong correlation between sorption and specific surface area (m^2 g^{-1}) of the soil. However, these correlations may also have shown a sorption mechanism governed by interactions between surface ion exchange sites and charged or polar solutes. Cation exchange complexes are associated with clay minerals and organic matter and result in the sorption of polar compounds, such as phenolates. Casey et al. (2003) stated that this sorption mechanism may partially explain the difference found between two clays, bentonite (high affinity) and kaolinite (low affinity). Kaolinite has a low specific surface area and a low cation exchange capacity (3-5 cmol kg^{-1}) while bentonite has a high specific surface and a higher cation exchange capacity ranging from 100 – 150 cmol kg^{-1}.

Lee et al. (2003) argued that this is not the case as 17 β-estradiol has a phenolic group with a pKa of 10.71 that will deprotonate under very basic conditions, pH < 8.7. (Figure 2.12). This will form an organic anion but will never be positively charged which is required for cation exchange.

Also Lee et al. (2003) argue that the hormone sorption in Casey et al. (2003) was estimated by calculating the difference between the mass of chemical applied and the mass in the solution phase after a specific amount of time. This is a problem when the organic species applied can undergo microbial degradation or surface–induced abiotic transformations. Clay and oxide minerals often catalyse abiotic reactions, which if interpreted as sorption would over estimate the sorption affinity and could cause wrong correlation with soil properties. Casey et al. (2003) had incubated the experiments for 48 hours and so this was possible. Casey et al. (2003) also found a correlation between sorption and silt content, however, the authors were not certain because silt was also correlated to the organic matter content and the statistical model used was unable to significantly distinguish between the two effects. Casey et al. (2003) decided that the organic matter content was more likely to be significant than the particle size and that the correlation between sorption and silt content was coincidental.

Thin layer chromatography was also used by Casey et al. (2003) and showed that the majority of the sorbed ^{14}C was contained in 17 β–estradiol and estrone. Estriol was detected in trace amounts. Generally, they showed that 17 β–estradiol entered the soil column, then readily partitioned to the solid phase and underwent rapid transformation to

form at least three metabolites of different polarities. The lower polarity estrone was adsorbed to the soil and the higher polarity metabolites were more mobile and transported in the aqueous phase.

Lee et al. (2003) concluded that hydrophobic partitioning into organic carbon in soils and sediments plays a dominant role in the sorption of the hormones and the associated transformation products. This can lead to reduced amount of leaching of the hormones in soils and so minimising ground and surface contamination. However Lee et al. (2003) concluded that further research was needed to understand the role of microbial and abiotic processes as well as the effect of soil type and the concentration of hormone present. Casey et al. (2003) also identified further work which is needed to understand the long term persistence of estrone in soil and its impacts on water and soil quality.

Other studies have been conducted to find out how estrogens partition to sediments (Collucci et al., 2001; Emmerick et al., 2003; Lai et al., 2000). Lai et al.(2000) found that there was initial rapid sorption of estrogens (4.0 - 9.4 $\mu g\ g\ soil^{-1}\ h^{-1}$) between 0 and 0.5 h which was followed by a period of up to 1 hour of slower sorption (1.5 – 2.9 $\mu g\ g^{-1}\ h^{-1}$). Next there was a steady decrease in sorption (0.07 – 0.37 $\mu g\ g\ soil^{-1}\ h^{-1}$). The decrease in sorption rate between 0.5 and 1 hour may reflect both the progressive saturation of the sorbent binding sites and reduction in available estrogens for binding. This pattern of binding was supported by Emmerik et al. (2003) in which sorption to a range of soil minerals was investigated and found that there was an initial very rapid phase of sorption followed by slower uptake. The amount of sediment added was varied and it was found that the loss of estrogens from the aqueous phase increased with the amount of sediment present which may be due to a greater number of binding sites. However, the mass of estrogens sorbed per gram of sediment decreased with an increased amount of sediment due to the supply of estrogens being exhausted (Emmerick et al., 2003).

The importance of total organic carbon (TOC) and particle size distribution in estrogen sorption has been studied Lai et al. (2000). Generally, the more TOC there is, the greater the binding. There was only a weak correlation between the particle size distribution and the binding. This supports Casey et al. (2003) when it was not known if there was correlation between silt content and sorption or organic matter and sorption and it were speculated that the carbon content was more significant.

Lai et al. (2000) also investigated the effect of salinity of water on sorption of hormones to sediment and found that with increased salinity the sorption increased. This was due to aggregation and flocculation in the higher ionic strength NaCl solutions which

is what typically happens in estuaries and results in higher rates of sedimentation. The conclusions were that estrogens discharged to the environment may rapidly become sorbed on contact to solids. There will be competition for binding sites between estrogens and other hydrophobic chemicals such as DDT, PCBs and alkylphenols and so the results for sorption from laboratory experiments could be different to those occurring in natural environments.

Others factors which could also influence the binding of estrogens to soils is the temperature and moisture of the soil. Colluci et al. (2003) found that in a loam soil, dissipation of 17 β-estradiol did not vary greatly at temperatures of 10, 19, 30 and 37°C but they did at 4°C when the rate was significantly lower compared with the rates observed at the higher temperatures. In the sandy loam soil the moisture content was varied from air-dried, 7%, 15% and field capacity with removal of estradiol generally increasing with increasing moisture content. However, with different soils the results may be different.

Colluci et al. (2003) concluded that 17 β-estradiol and estrone were readily biodegradable under a range of moisture and temperature conditions and they predicted that they would be rapidly dissipated in aerated agricultural soils following application of manures in a temperate growing season. These results show that manure application methods that maximise contact with soil and minimise the chance of surface or bypass flow would best protect ground and surface waters from contamination (Emmerick et al., 2003).

The pH of the soil could also affect adsorption; Emmerick et al. (2003) investigated the sorption on a range of soil minerals (monmorillonite, kaolinite, illite and goethite), using different pHs ranging from pH 3-10. Sorption to clay particles was found to be independent of the pH but for goethite there was a maximum sorption between pH 7 to 7.5 and significantly less 17 β-estradiol was taken up at a higher and lower pH. This suggests that 17 β-estradiol binds primarily to the uncharged surface hydroxyl groups on the geothite which are most abundant at the point of zero charge, *ca.* pH 8.5. The decrease in sorption at lower pH may result from the increasing polarity of the goethite surface, as more of the surface hydroxyls become protonated. The drop in sorption at a higher pH may be due to the progressive ionisation of the phenolic hydroxide on the estrogen above the point of zero charge of goethite, which would produce an electrostatic repulsion between 17 β-estradiol and the surface (Emmerick et al., 2003).

2.7 Runoff from agricultural land

If a hormone has high solubility then it will be most likely to partition into water and so it will be possible for it to be transported long distances from its original point of application.

The runoff of estrogens from poultry litter has been investigated by Nicols et al. (1997) by collecting pond and stream water samples below fields receiving poultry litter. In the streams the concentration of estrogens were found to increase from <0.5 ng l^{-1} to approx 5.4 ng l^{-1} following the application of poultry litter. The concentration of estrogen in ponds decreased from 23 ng l^{-1} to 5 ng l^{-1} during the study period. However, as this experiment did not include the collection of edge of field samples, controls or background samples the actual contribution of the poultry litter could not be assessed. Nicols et al. (1997) also investigated the run off of 17 β-estradiol. Poultry litter was applied to plots in differing amounts (1.76, 3.52, 5.28 and 7.05 Mg ha^{-1} dry weight). The results found that 17 β-estradiol can persist for at least 7 days under field conditions. Storms were simulated by irrigating the plots with a sprinkler immediately after application (first storm event) and 7 days later (second storm event) In the first storm event the concentration of estradiol in the run off increased with the increasing amount of litter applied as shown in Figure 2.13. The graph shows how in the second storm event the losses of 17 β-estradiol were less clearly influenced by the application rate. There are still questions that need to be answered; this study only involved the measurement of one of many hormones present in animal manures and sewage sludges. The losses of testosterone, estrone and other hormones are still uncertain (Nichols et al., 2003).

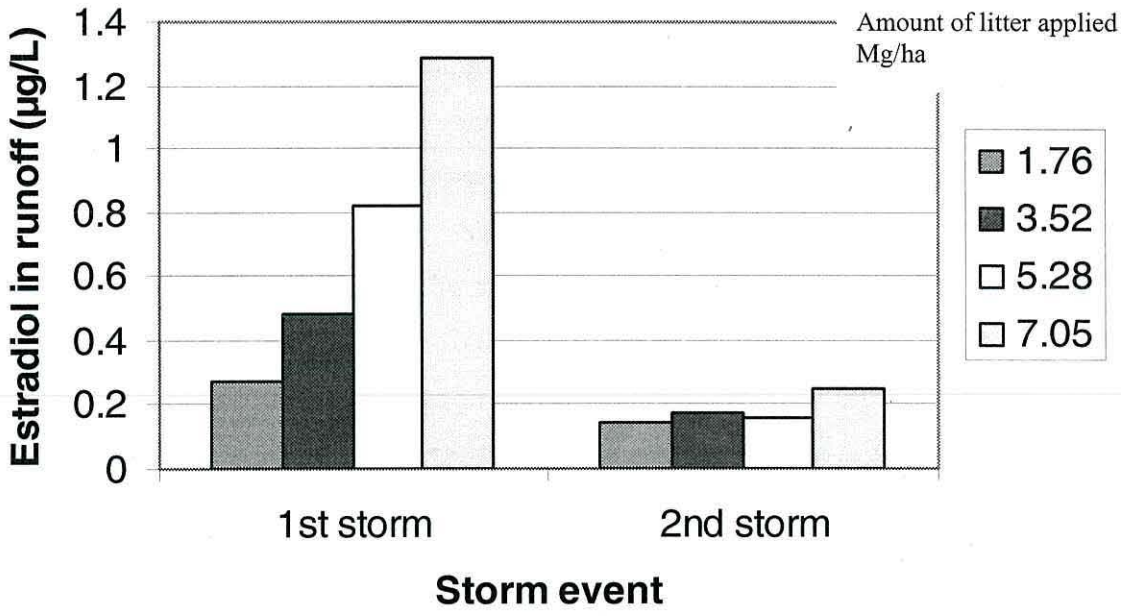

Figure 2.13: The concentration of 17 β-estradiol in the run off samples after first and second storm events (Nichols et al. 1997).

2.8 The future

It is becoming more accepted that endocrine disrupting chemicals are having a significant effect on wild populations of species of fish, amphibians, birds and mammals but the extent to which this is occurring is not fully understood. The first paper to document the widespread sexual disruption in wild populations of any vertebrate was by Jobling et al. (1998). This showed that reproductive and developmental effects do result from exposure to ambient levels of chemicals present in typical British rivers but has only been recognised relatively recently.

There is degradation of all the chemicals once they reach the environment and also sorption to solids, which will reduce the transport of the chemicals to other places. More research needs to be carried out on the mechanism of biodegradation of the hormones especially estrone which is a degradation product of 17 β-estradiol.

In the future as the human population increases further, the amount of effluent and sludge from sewage treatment works and animal wastes will increase. This will increase the amount that needs to be disposed of and so may increase the amount of hormones and chemicals reaching the environment. In addition, climate change could affect the behaviour of these chemicals once they reach the land and water, leading to unexpected effects. There

may also be problems arising from different hormones such as those that affect the thyroid and possible new pharmaceutical chemicals, for example unexpected effects from the male pill.

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CHAPTER 3

Biodegradation of estrone and 17 β-estradiol in grassland soils amended with animal wastes

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Published in Soil Biology & Biochemistry 38 (2006) 2803-2815

ELSEVIER

Soil Biology & Biochemistry 38 (2006) 2803–2815

Soil Biology & Biochemistry

www.elsevier.com/locate/soilbio

Biodegradation of estrone and 17 β-estradiol in grassland soils amended with animal wastes

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Received 16 September 2005; received in revised form 11 April 2006; accepted 19 April 2006
Available online 19 May 2006

Abstract

The release of endocrine disrupting chemicals into the environment is of increasing concern due to the formation of an intersex state in freshwater organisms and potential risks to human health. The aim of this study was to investigate the persistence of the naturally occurring hormones, estrone and 17 β-estradiol in three agricultural grassland soils in the presence and absence of cattle and sheep wastes (urine and manure). Biodegradation was investigated using ^{14}C-labelled hormones which were applied to soil in three different solvents (water, artificial urine and natural sheep urine). When applied directly to soil the two hormones degraded at a similar rate, however, the speed of mineralization was soil type and solvent dependant. The half-life ($t_{1/2}$) of the hormones in soils ranged from 5 to 25 d. The hormones were also applied to the soils in sheep and cattle manure of different ages (7 d to 2 yr). Generally, the rate of degradation in the animal manure amended soils was more rapid than in the unamended soils ($t_{1/2}$ = 1–9 d), with mineralization being largely independent of manure age and type. We conclude that in comparison to many xenobiotics, estrogens are not persistent in agricultural soils. However, our calculations suggest that if they are lost to freshwater via runoff or leaching then they may have an appreciable effect on freshwater organisms. Assuming normal landspreading rates our results suggest that the risk of estrogen contamination of freshwater associated with manure spreading is very low.

Keywords: Animal waste; Bovine; Cow; Estrone; 17 β-estradiol; Faeces; Landspreading; Mineralisation; Oestrogen; Ovine

1. Introduction

The release of endocrine disrupting chemicals (EDCs) into the environment is of increasing concern due to their detrimental impact on freshwater organisms, ecosystem sustainability and human health (van der Kraak, 1998). While many EDCs released into the environment are of anthropogenic origin (e.g. pesticides, detergents, etc.), many naturally occurring estrogens and androgens are also released in large quantities from agricultural animal excrement (e.g. cattle, sheep), wastewater treatment (e.g. sewage sludge, effluent discharges) or industrial processing of natural products (e.g. phytoestrogens; Ternes et al., 1999). The main estrogens released into the environment are the steroidal hormones estrone and 17 β-estradiol which are excreted by all humans and animals. Other estrogens which are released to a lesser extent include the naturally occurring estriol and the synthetic estrogens ethynylestradiol and mestranol which are components of human contraceptives (Ying et al., 2002). It has been shown that exposure to estrogen levels as low as 1 ng/l (<10 pM) is sufficient to cause the feminization of male trout (Hansen et al., 1998) and the development of intersex roach in rivers (Jobling et al., 1998).

In agricultural environments, estrogens from humans can potentially reach freshwater environments via the spreading of biosolids and sewage to land. Estrogens from animals may enter the environment due to direct addition (e.g. urine and faeces) and from the spreading of animal wastes to land (e.g. manure, slurry). Different farm animals excrete estrogens by different routes; cattle mostly in their faeces (58%), whereas, swine and poultry excrete estrogens mostly in urine (96% and 69%, respectively; Hanselman et al., 2003). The total amount of estrogens excreted each day is dependant on animal age, gender and reproductive

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0038-0717/$ - see front matter © 2006 Elsevier Ltd. All rights reserved.
doi:10.1016/j.soilbio.2006.04.033

state and typically ranges from 300 to 550 μg/animal d for cattle and 25–400 μg/animal day for sheep (Plotka and Erb, 1969; Lange et al., 2002). Typically, estrogen excretion rates are maximal during estrous when hormone excretion rates can reach 1000 μg/animal d. Consequently, based upon the responses of fish to low water concentrations (1 ng/l), either major dilution, abiotic immobilization or biodegradation of the hormones must occur if they are to be environmentally benign.

The persistence and movement of estrogens in the environment has been investigated in several studies. Hormones can undergo several fates after entering soil including microbial immobilization and mineralization, abiotic transformation, sorption to the solid phase, uptake by plants, leaching and runoff (Lai et al., 2000; Collucci et al., 2001; Emmerick et al., 2003; Jacobsen et al., 2005; Casey et al., 2005). The relative contribution of these individual flux pathways remains unknown. Collucci and Topp (2001) and Jacobsen et al. (2005) found that 17 β-estradiol was degraded within 24 h of addition to soil at 30 °C which was accompanied by an accumulation of estrone which subsequently degraded within 72 h. 17 β-estradiol can also be abiotically degraded to estrone in soil (Ying et al., 2002). Studies have found that 17 β-estradiol has a relatively short half-life ($t_{1/2}$) in freshwater sediments ($t_{1/2} = 5$–10 d) and soil ($t_{1/2} = 1$ d; Lee et al., 2003).

In a comprehensive review on estrogen behaviour in soils, Hanselman et al. (2003) identified that research on hormone persistence in animal wastes was required. Research to date has involved the addition of hormones to soil in distilled water, however, additions in a manure or urine matrix would more realistically reflect the natural environment. We hypothesize that the chemical and biological characteristics of the waste matrix will significantly affect hormone behaviour in soil. Therefore, we aimed to study the effect of waste matrix on hormone behaviour in three contrasting agricultural soils.

2. Materials and methods

2.1. Soil

Soil was obtained from three contrasting temperate oceanic agricultural grasslands located in Abergwyngregyn, Gwynedd, North Wales (53°14′N, 4°01′W; Table 1; Jones et al., 2004). All the soils regularly receive urine and faecal inputs from grazing sheep which occurs throughout the year. Soil A (sandy clay loam textured Eutric cambisol) was collected from the surface Ah horizon (5–20 cm) of a lowland (15 m altitude) freely draining, heavily sheep-grazed grassland which receives regular fertilization (120 kg N, 60 kg K and 10 kg P/yr) and occasional sheep manure addition. Soil B (sand textured Eutric cambisol) was collected from the surface Ah horizon of a lowland (5 m altitude) freely draining, lightly sheep-grazed soil which receives no fertilization. Soil C (sandy loam textured Haplic podzol) was collected from the surface Ah horizon (5–20 cm) of an upland (200 m altitude) freely draining, heavily leached, lightly sheep-grazed grassland that receives no fertilization. The mean annual soil surface temperature at 10 cm varies from 8 to 10 °C and the annual rainfall at the lowland site is 1250 mm and at the upland site 1700 mm. All soils were stored field-moist and unsieved at 4 °C until use.

Table 1
Chemical and physical characteristics of the three soils used in the study

	Soil A (Eutric cambisol)	Soil B (Eutric cambisol)	Soil C (Haplic podzol)
$EC_{1:1}$ (μS/cm)	80±4	76±19	46±7
pH ($_{1:1}$, H_2O)	6.06±0.07	5.31±0.10	4.63±0.08
$CaCO_3$ (g/kg)	0.11±0.02	<0.01	<0.01
Water holding capacity (g/kg)	520±20	251±10	690±40
Moisture content (g/kg)	160±10	62±4	260±2
Organic C (g/kg)	2.1±0.1	2.7±0.1	1.2±0.1
Total N (g/kg)	0.16±0.01	0.20±0.01	0.08±0.01
C-to-N ratio	13.3±0.6	13.4±0.3	15.6±1.3
Soil solution NO_3^-, (mg N/l)	13.7±1.3	2.7±0.1	0.5±0.106
Soil solution NH_4^+ (mg N/l)	1.4±0.1	28.8±14.2	1.1±0.1
Exchangeable cations			
Na (mg/kg)	29±3	17±1	37±1
K (mg/kg)	116±18	5±1	77±12
Ca (mg/kg)	1595±217	48±1	89±8
Mg (mg/kg)	89±19	35±5	15±2
Al (mg/kg)	22±2	ND	323±55
Extractable P (mg/kg)	9.9±0.3	1.8±0.2	0.22±0.09
Root biomass (g/m²)	245±6	337±101	107±38
Soil respiration (g CO_2/m² h¹)	0.60±0.02	ND	0.25±0.02

All values represent means ± SEM ($n = 3$).
ND indicates not determined.

2.2. Sheep urine, sheep manure and cattle manure

Sheep urine was collected from two individual ewes held in a stainless-steel crush which had previously been grazing on *Lolium perenne* grassland at soil A. Immediately after collection the urine was centrifuged at 8000*g* at 10 °C for 15 min to remove particulate matter. The samples were then frozen at −20 °C in polypropylene containers until required. Manure samples were collected from two commercial farms in North Wales namely, Pandy Tudur, Conwy and Abergwyngregyn, Gwynedd. Manure A was collected from the surface of a <7-d-old cattle-derived manure heap stored outside on concrete. Manure B was collected from the inside of a 3–6-month-old cattle-derived manure heap stored outside on grass. Manure C was collected from the inside of a >1-yr-old cattle-derived manure heap stored outside on grass. Manure D was <7 d old and was collected from the floor of a lambing shed. Manure E was 14 d old and was collected from the inside of a sheep-derived manure heap which was stored undercover. All the manures consisted of a mixture of faeces, urine and

Table 2
Chemical and physical characteristics of the five manures and two urine samples used in the study

	Age	pH	EC (mS/cm)	(g/kg)		C-to-N ratio	(mg/kg)				Ash (g/kg)	DOC (g/l)	Moisture content (g/kg)
				Total C	Total N		K	Na	Ca	P			
Manure													
Cattle A	<7 d	7.45	8.6	340±40	14±1	24.2	71±7	16±2	14±1	949±160	48±30	2.3±0.1	830
Cattle B	0.25–0.5 y	7.57	4.0	438±2	20±1	21.9	54±3	15±1	14±1	351±61	19±1	0.7±0.1	860
Cattle C	>1 y	7.89	5.8	375±12	34±1	11.0	95±10	38±10	24±4	1062±340	52±7	1.4±0.5	790
Sheep D	<7 d	7.40	3.4	416±7	22±1	18.9	48±9	39±14	29±1	992±201	45±2	0.3±0.2	690
Sheep E	14 d	8.09	10.7	296±16	24±1	12.3	79±28	20±9	47±6	1106±259	179±26	2.0±0.4	710
Urine				(gl^{-1})			(mgl^{-1})						
Artificial	<1 d	7.8	12.7	2.2±0.0	4.2	0.52	9208±38	603±8	205±2	1.2±0.4	ND	ND	ND
Natural	<1 d	8.6	26.2	1.5±0.2	2.0	0.76	2089±8	1352±8	271±4	2.4±0.1	ND	ND	ND

All values represent means±SEM ($n = 3$).
DOC indicates dissolved organic carbon and EC indicates electrical conductivity.

bedding straw and were stored at 4 °C until use. The characteristics of the manure and urine samples are shown in Table 2.

2.3. Artificial sheep urine

The artificial sheep urine was made in distilled water and contained the following: $KHCO_3$ (6 g/l), KCl (3.5 g/l), Na_2SO_4 (0.4 g/l), urea (6.4 g/l), creatine (0.85 g/l), hippuric acid (1.85 g/l), allantoin (0.6 g/l), glycine (0.01 g/l), creatinine (0.015 g/l), uric acid (0.005 g/l), hypoxanthine (0.001 g/l) and ammonium chloride (0.015 g/l). The solution was frozen at −20 °C until required. The composition of the artificial urine was based primarily on Bathurst (1952), Bristow et al. (1992) and Anger et al. (2003).

2.4. Chemical analysis

Soil and manure pH and electrical conductivity were determined in 1:1 (v/v) solid:H_2O extracts (Smith and Doran, 1996) and moisture by drying at 80 °C for 24 h. Total C and total N were determined with a CHN-2000 analyzer (Leco Corp., St. Joseph, MI). Exchangeable cations were estimated by performing 1:10 (w/v) soil:0.5 M $BaCl_2$ extractions on a reciprocating shaker (60 min, 20 °C) followed by centrifugation at 10,000g (30 min, 4 °C) and storage of supernatant solutions at −20 °C. Exchangeable cations were determined by ICP-OES (Jobin Yvon, JY138 Ultrace). $CaCO_3$ content was determined by the van Slyke manometric method (Nelson, 1982). Extractable P was measured by extraction with 0.5 M acetic acid (1 h, 200 rev/min) followed by centrifugation at 10,000g (30 min, 20 °C) and P analysis by the method of Murphy and Riley (1962). Soil solution was removed by the centrifugal-drainage method of Giesler and Lundström (1993) with NO_3^- and NH_4^+ in solution determined with a Skalar San$^+$ segmented flow autoanalyser (Skalar UK Ltd., York, UK). Root biomass was determined by wet sieving and drying the roots at 80 °C overnight. Soil respiration was determined with a SR1 automated soil respirometer (PP Systems Ltd., Hitchin, UK). Dissolved organic carbon (DOC) and total dissolved nitrogen (TDN) concentrations were measured using a Shimadzu TC-TNV analyzer (Shimadzu Corp., Kyoto, Japan). Hormone analysis of the urine samples was undertaken by reverse phase gradient elution HPLC (Laserchrom Analytical Ltd., Rochester, Kent, UK) using a Evolution® C18 column (Laserchrom Analytical Ltd.) at 40 °C. An LA2100 gradient pump (Laserchrom Analytical Ltd.) created an elution profile from 10% acetonitrile to 90% acetonitrile (pH 6) with a 100 min run time. Compounds were detected by a combination of fluorescence detection (Ex. 275 nm, Em. 300 nm) and UV (215 nm). A UV diode array was also used to resolve peaks and aid in peak identification which was based upon retention time and performed with Clarity® (DataApex, Prague, Czech Republic). Analysis indicated that the concentration of estrone and 17 β-estradiol in the urine were below detection limits (< 1 mg/l).

2.5. Soil mineralization studies

A ^{14}C-radiolabelled solution of either estrone or 17 β-estradiol (81 µg/l; 0.3 µM; 500 µl; specific activity 0.17 kBq/ml) was added to 5 g of field-moist soil contained in 50 ml polypropylene tubes. Two estrogens, ^{14}C-estrone (4-^{14}C; American Radiolabelled Chemicals Inc., St. Louis, MO; 1.99 GBq/mmol) and ^{14}C-17 β-estradiol (4-^{14}C; American Radiolabelled Chemicals Inc.; 2.04 GBq/mmol) were used in the mineralization assays. The estrogens were added to soil in either distilled water, artificial urine or field-collected sheep urine. Following addition, the soil was shaken gently to ensure mixing and a 1 M NaOH trap placed inside the tube to capture respired $^{14}CO_2$. After sealing, the tubes were placed in a temperature-controlled incubator at 10 °C to reflect the mean annual temperature of the soils. The NaOH traps were changed at regular intervals over a 100 d incubation period. At the end of the 100 d incubation period the soils were serially extracted

with 25 ml of 0.01 M $CaCl_2$ and 20 ml of methanol to determine the total amount of ^{14}C label remaining in the soil aqueous and organic phases, respectively. Additional soil samples were set up to allow for extractions at times other than 100 d. ^{14}C was determined by liquid scintillation counting (Wallac 1404 scintillation counter, Wallac EG&G, Milton Keynes, UK) using alkali compatible scintillation fluid (Wallac Optiphase 3; Wallac EG&G, Milton Keynes, UK).

Previous studies have shown that the mineralization of naturally occurring substrates in soils follows a similar two phase pattern to those observed here (Jones et al., 2004). The first rapid phase of $^{14}CO_2$ production can be attributed to the immediate use of the substrate in catabolic processes within the microbial biomass (i.e. respiration). The remaining substrate taken up into the microbial community is incorporated and immobilized by anabolic processes (i.e. formation of new biomass). Abiotic mineralization was assumed to be negligible (Jacobsen et al., 2005). The slower second phase of $^{14}CO_2$ production can be attributed to the subsequent turnover of the soil microbial community leading to the production of $^{14}CO_2$. Assuming that the primary mineralization of the substrate and the secondary mineralization of the microbial community both follow a first order kinetic model (Paul and Clark, 1996; Casey et al., 2005) then the half-life of the hormone in soil can be calculated using the double first order exponential decay equation

$$S = [a_1 \exp(-b_1 t)] + [a_2 \exp(-b_2 t)], \quad (1)$$

where S is the ^{14}C-label remaining in the soil, b_1 is the rate constant describing the primary mineralization phase, b_2 is the rate constant describing the secondary mineralization of the microbial biomass, a_1 and a_2 describe the size of pools b_1 and b_2 and t is time. The half-life ($t_{1/2}$) of the pool a_1 is therefore defined as

$$t_{1/2} = \ln(2)/b_1. \quad (2)$$

2.6. Manure mineralization studies

^{14}C-labelled estrone or 17 β-estradiol contained in distilled water (81 μg/l; 0.3 μM; 100 μl; specific activity 0.17 kBq/ml) were added to 1 g of field-moist sheep or cattle manure contained in 50 ml polypropylene tubes. Field-moist soil (4 g) was then added and roughly mixed with the manure and then incubated at 10 °C in sealed tubes and $^{14}CO_2$ captured as described above over a 100 d period. At the end of the incubation period the soils were serially extracted with 25 ml of 0.01 M $CaCl_2$ and 20 ml of methanol as described above.

2.7. Statistical analysis

Statistical analysis (ANOVA with Univariate GLM, t-tests) of the hormone mineralization results were carried out using SPSS 12.0 (SPSS Inc., Chicago, IL). Fitting of a double first order kinetic model to the experimental mineralization data was undertaken with Sigmaplot 8.0 (SPSS Inc.).

3. Results

3.1. Hormone mineralization in soil

The rate of hormone mineralization was strongly influenced by both soil type and the matrix in which the hormone was added to the soil (Fig. 1). Generally, the rate of hormone mineralization followed the series: soil B (Eutric cambisol) > soil A (Eutric cambisol) > soil C (Haplic podzol). In addition, the overall patterns of estrone and 17 β-estradiol mineralization were similar in the individual soils although significant differences did occur between treatments.

In soil A (Eutric cambisol) the rate of hormone mineralization was initially very rapid (0–10 d) and then became progressively slower over the 100 d incubation period (Fig. 1). This pattern of $^{14}CO_2$ evolution was similar irrespective of the solvent the hormone was applied in (distilled water, artificial or field-collected urine). While the mineralization patterns were similar, there were significant differences in the total amount of $^{14}CO_2$ recovered from the treatments ($P<0.001$). The highest rate of estrone and 17 β-estradiol degradation was seen after addition to soil in the field-collected sheep urine treatment while the lowest rate occurred in the presence of distilled water for estrone and artificial sheep urine for 17 β-estradiol. In soil A, there were no significant differences between the amount of estrone and 17 β-estradiol mineralized ($P>0.05$).

The mineralization profiles of both estrone and 17 β-estradiol in soil B (Eutric cambisol) were significantly different from the results for soils A and C for all three matrix types ($P<0.001$; Fig. 1). In soil B there was initially a relatively low rate of hormone degradation in all of the matrices (0–5 d). However, after an initial lag phase, at approximately 10 d there was a sudden increase in the rate of hormone mineralization particularly in the artificial sheep urine and the natural sheep urine treatments. After 100 d, large treatment differences in the amount of estrone and 17 β-estradiol biodegraded were apparent with only 15–16% of the hormone mineralized when added in distilled water, 62–68% when applied in artificial sheep urine and 85–90% when applied in natural sheep urine.

In comparison to soils A and B, the rates of both estrone and 17 β-estradiol mineralization in soil C (Haplic podzol) were much lower ($P<0.001$) irrespective of matrix type (Fig. 1). For soil C, the hormones had the greatest rate of degradation when applied in distilled water followed by the artificial sheep urine and the field-collected sheep urine. A 10 d lag period in 17 β-estradiol mineralization was observed when the hormone was added to soil in field-collected sheep urine, however, this was not apparent when the hormone was added in distilled water ($P<0.05$).

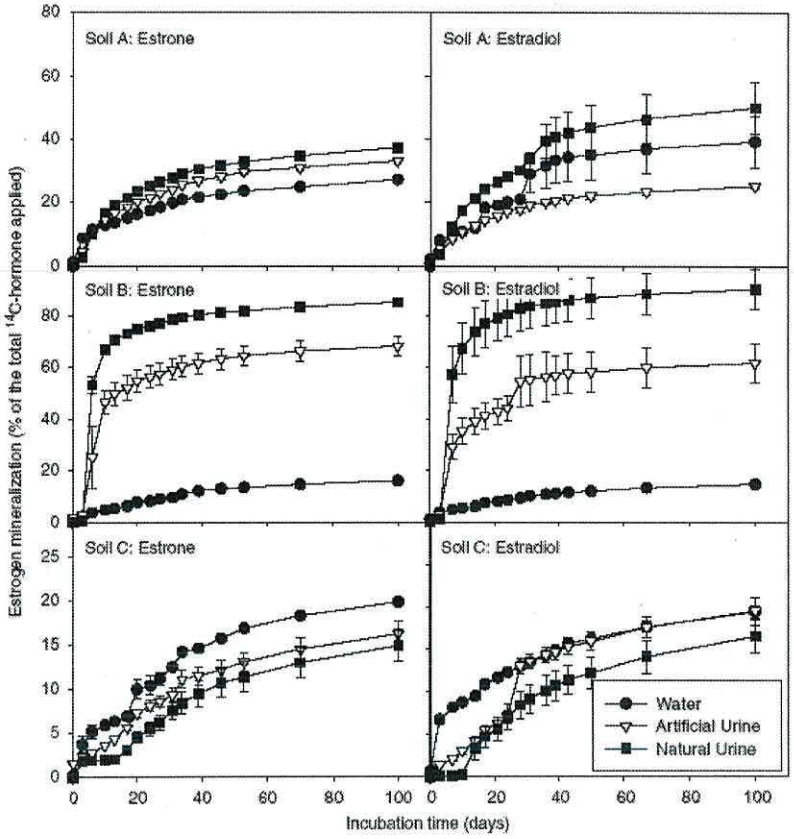

Fig. 1. Cumulative degradation of ^{14}C-labelled estrone and 17 β-estradiol after the addition to three soils in either distilled water, artificial urine or natural field-collected sheep urine. Values represent means $\pm$ SEM ($n = 3$).

3.2. *Hormone availability in urine amended soil*

During the 100 d incubation period, the additional soil samples were serially extracted with $CaCl_2$ and methanol to assess the availability of the ^{14}C-labelled hormones. In soil A only small amounts of ^{14}C label could be recovered by $CaCl_2$ when they were added to the soil in a distilled water matrix even after only 24 h incubation (< 5% recovered of total hormone added). In contrast, when the hormones were added in urine, large amounts of the estrogens could be initially recovered (20–30% of total added after 24 h) after which time the amount recovered gradually decreased over time (Fig. 2). The patterns of estrone and 17 β-estradiol recovery in the $CaCl_2$ extracts in soil A were very similar. A similar pattern of hormone recovery to that observed with $CaCl_2$ was also seen when soil A was subsequently extracted with methanol (Fig. 2). Generally, the amount of ^{14}C-label recovered in the methanol extract was approximately twice that recovered with the $CaCl_2$ extract. The general pattern of ^{14}C-label recovery in the $CaCl_2$ and methanol extracts in soil B were similar to those observed for soil A. In contrast, in soil C, large amounts of ^{14}C-label could be recovered by $CaCl_2$ even after 14 d although after 100 d the ^{14}C-label had largely disappeared.

Table 3 shows the total amount of radioactivity recovered by each extraction and by difference, the total amount of ^{14}C remaining in the soil after 100 d. Generally, in all soils and treatments the recovery of ^{14}C as a proportion of the total ^{14}C-hormone added was very low. On average across all treatments, the recovery of ^{14}C in the $CaCl_2$ extract was $3.3 \pm 0.2\%$ of the total ^{14}C added while for the methanol extract it represented $5.0 \pm 0.5\%$ of the total. Overall, there was no significant difference between the amount of ^{14}C recovered in the $CaCl_2$ and methanol extracts after 100 d ($P > 0.05$). In addition, there was no significant difference in the amount of estrone and 17 β-estradiol recovered in the extracts ($P > 0.05$). No differences in hormone recovery in the $CaCl_2$ and methanol extracts were observed between the three soil types ($P > 0.05$). After accounting for the amount of hormone recovered in the extracts or as $^{14}CO_2$, on average, $54 \pm 6\%$ of the ^{14}C label still remained unaccounted for in the soil

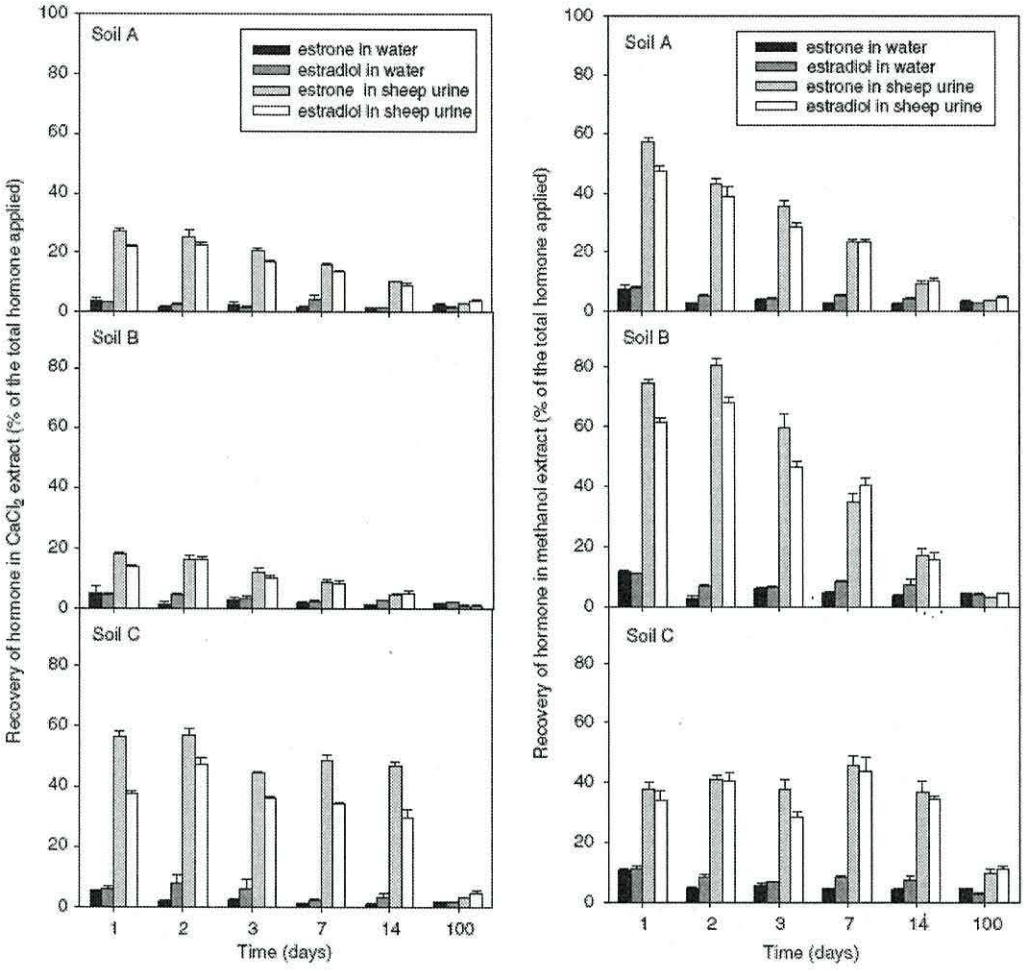

Fig. 2. The amount of ^{14}C-label recovered over time after the incubation of ^{14}C-estrone and ^{14}C-17 β-estradiol in three soils for 100 d and after extraction with either $CaCl_2$ or methanol. Hormone was added to the soil in either distilled water or natural field-collected sheep urine. Values represent means $\pm$ SEM ($n = 3$).

after 100 d. The amount of ^{14}C unaccounted for in Soil C was significantly greater than in soils A and B ($P<0.05$). Although there was no overall difference in the amount of estrone and 17 β-estradiol unrecovered from the soil after 100 d, significant differences existed between treatments ($P<0.05$) and followed the series: distilled water > artificial urine > field collected urine.

3.3. Effect of urine addition on soil chemistry

The addition of urine had a significant effect on the chemistry of the soil in comparison to the addition of distilled water (Fig. 3). While the addition of urine caused a significant increase in soil nutrient concentrations (data not shown) it also caused significant changes in soil pH and electrical conductivity. Generally, these effects persisted for up to 14 d.

3.4. Hormone mineralization in manure amended soil

Generally, the rate of hormone mineralization was more rapid in cattle and sheep manure amended soil than in urine amended soil (Figs. 4 and 5). For example, after 100 d the total mineralization of estrone in sheep urine applied to soil A was 37% whereas in cattle manure A it was 83%, in cattle manure B it was 90% and in cattle manure C it was 52%. The rate of $^{14}CO_2$ production was significantly affected by the age of the manure.

Fig. 4 shows that the highest rate of hormone mineralization for all of the soil types was in cattle manure B that was 6 months old. In contrast, the lowest rate of

Table 3
Amount of ^{14}C-label recovered after the incubation of ^{14}C-estrone and ^{14}C-17 β-estradiol in three soils for 100 d and after extraction with either $CaCl_2$ or methanol

Soil	Hormone	Treatment	Extractable hormone (% of total recovered)			Amount not recovered (%)
			$CaCl_2$	Methanol	Sum	
Soil A	Estrone	Water	3.3±0.4	3.4±0.3	34.4	65.6
		Artificial urine	3.3±0.5	5.1±0.5	42.1	57.9
		Natural urine	3.7±0.3	3.6±0.1	45.1	54.9
	17 β-estradiol	Water	2.4±0.6	2.7±0.2	44.9	55.1
		Artificial urine	2.6±0.3	3.8±0.3	32.2	67.8
		Natural urine	4.9±0.6	4.6±0.5	60.1	39.9
Soil B	Estrone	Water	2.9±0.1	4.5±0.3	24.0	76.0
		Artificial urine	3.2±0.5	5.8±0.1	77.7	22.3
		Natural urine	2.1±0.4	3.2±0.1	91.0	9.0
	17 β-estradiol	Water	3.2±0.3	4.1±0.4	22.6	77.4
		Artificial urine	2.4±0.4	3.7±0.3	68.3	31.7
		Natural urine	2.3±0.2	4.4±0.4	97.4	2.6
Soil C	Estrone	Water	2.6±0.2	4.5±0.2	27.5	72.5
		Artificial urine	4.8±1.3	6.6±0.6	28.2	71.8
		Natural urine	4.1±0.4	9.7±1.7	29.2	70.8
	17 β-estradiol	Water	2.6±0.1	2.9±0.2	25.5	74.5
		Artificial urine	3.2±0.2	5.8±0.4	29.0	71.0
		Natural urine	5.9±0.6	11.2±0.8	34.3	65.7

Hormone was added to the soil in either distilled water, artificial sheep urine or natural field-collected sheep urine.
The 'sum' column includes the total recovered as $^{14}CO_2$, methanol and $CaCl_2$.
Values represent means ± SEM ($n = 3$).

mineralization was observed in manure C (> 2 yr old) and intermediate in the very fresh cattle manure A (7 d old; $P < 0.001$). There were no significant differences between hormone mineralization in the three soil types ($P > 0.05$).

There was a significant difference in the rate of mineralization between the two sheep manures ($P < 0.001$; Fig. 5). The highest amount of $^{14}CO_2$ production for both hormones and all soil combinations was in manure E that had been composted for approximately 2 yr. The mineralization of the hormones in the sheep manure amended soils was generally higher than in the non-manure unamended soils.

In the $CaCl_2$ and methanol extractions performed after 100 d there was a significantly lower amount of hormone unaccounted for in the manure amended soil when compared to the experiments in the absence of manures ($P < 0.001$; Table 4). On average, across all treatments 28 ± 3% of the total ^{14}C added was unaccounted for after 100 d in the manure amended soils. In the sheep manure experiment generally more of the hormone was recovered from the $CaCl_2$ and methanol extraction of the soil in comparison to the cattle manure treatments ($P < 0.01$).

3.5. Hormone persistence in soil

Fitting of the double first order exponential decay model (Eq. (1)) to the experimental results yielded r^2 values ranging from 0.948 to 0.999 indicating a very close fit of the experimental data to the model (r^2 mean ± SEM values = 0.975 ± 0.006 for the urine amended soils and 0.991 ± 0.003 for the manure amended soils). Using this approach, we calculate that the average half-life of the hormones was 13 ± 3 d in soil A, 10 ± 3 d in soil B and 28 ± 6 d in soil C (Table 5). In the presence of animal manures the average half-life of the hormones in all the soils was calculated to be 5 ± 1 d (Table 5). When the calculated half-life values were compared to the few experimental examples where a direct measure of $t_{1/2}$ was observed during the initial rapid mineralization phase (i.e. 50% recovered as $^{14}CO_2$), a close agreement of half-life values was observed providing experimental validation of the kinetic modelling approach (Figs. 1, 4 and 5). In addition, the calculated half-life values are also consistent with the recovery of ^{14}C-label in the $CaCl_2$ extractions (Fig. 2).

4. Discussion

4.1. Hormone persistence in soil

It is clear from this study that in comparison to many xenobiotics the estrogens, estrone and 17 β-estradiol are not particularly persistent in soil especially when present in a natural matrix. Our study shows that the hormones estrone and 17 β-estradiol behave very similarly to each other in soil but that the rate of mineralization is directly regulated by soil type and the matrix in which the hormones are added. In this study we measured the ultimate mineralization of ^{14}C-labelled hormones to $^{14}CO_2$ and therefore the intermediate hormone degradation

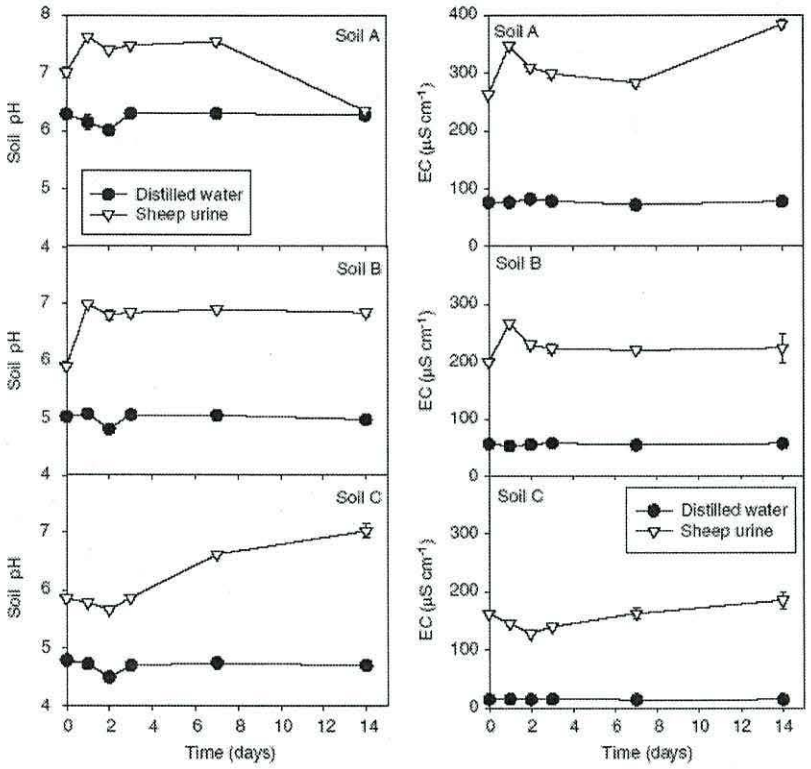

Fig. 3. Effect of the addition of natural sheep urine and distilled water on the pH and electrical conductivity of three soils. Values represent means ± SEM ($n = 3$).

products were not measured. Based upon previous work and the extractions performed at the end of the 100 d incubation period we speculate that the removal of side groups from the hormones is faster than that reported here and therefore our results represent the worse case scenario for persistence. The removal of side groups would in many cases result in a loss of its endocrine functionality. The pattern of the mineralization curves is consistent with many other substrate degradation studies in which a proportion of the added substrates are immediately mineralized while the remainder is immobilized, most likely within the microbial biomass (Jones, 1999). If significant proportions of the hormone remained in their natural state in soil after 100 d, they would have been recovered in the $CaCl_2$ and methanol extracts (Fig. 2; Garcia-Pelaez et al., 2004). The fact that they were not recovered in these extracts lends credence to their immobilization within the microbial biomass particularly as sorption of the hormones to the solid phase of our soils was low (data not presented).

4.2. Hormone biodegradation pathways

In some of the experiments involving the addition of hormones to soil, a lag phase in mineralization was observed. This lag phase typically lasted for 2–10 d and was particularly apparent in the treatments involving sheep urine. We hypothesize that this lag phase occurs as a result of the time taken for the soil microbial community to adapt to the urine. This adaptation may be due to metabolic changes caused by the alteration in chemical environment (Sakadevan et al., 1993) or the induction of enzymes and membrane transporters required for hormone biodegradation. Although estrogen membrane transporters are widespread in animals (e.g. hOat2 and hOat3; Asif et al., 2005; Kobayashi et al., 2005), in most cases these transporters are only capable of transporting sulfonated inactive forms of the hormones (e.g. estrone-sulphate; E_1S). In animals, after transport to the target cell the estrogens are then converted to active estrone by the activity of estrogen sulfotransferase (Hosoya et al., 2000). As E_1S is also a major estrogen component of domesticated animal urine (Yang et al., 2003) it can be expected that sulfatase present in the soil solution will be the first step in the E_1S biodegradation pathway. In the case of the non-sulfonated estrone and estradiol used in our experiments, it is unlikely that the soil microbial community possesses specific estrogen transporters for taking hormones into the cell (Schlenker et al., 1999), although further work is required to clarify this. Due to the relatively lipophilic nature of the hormones (log K_{OW}

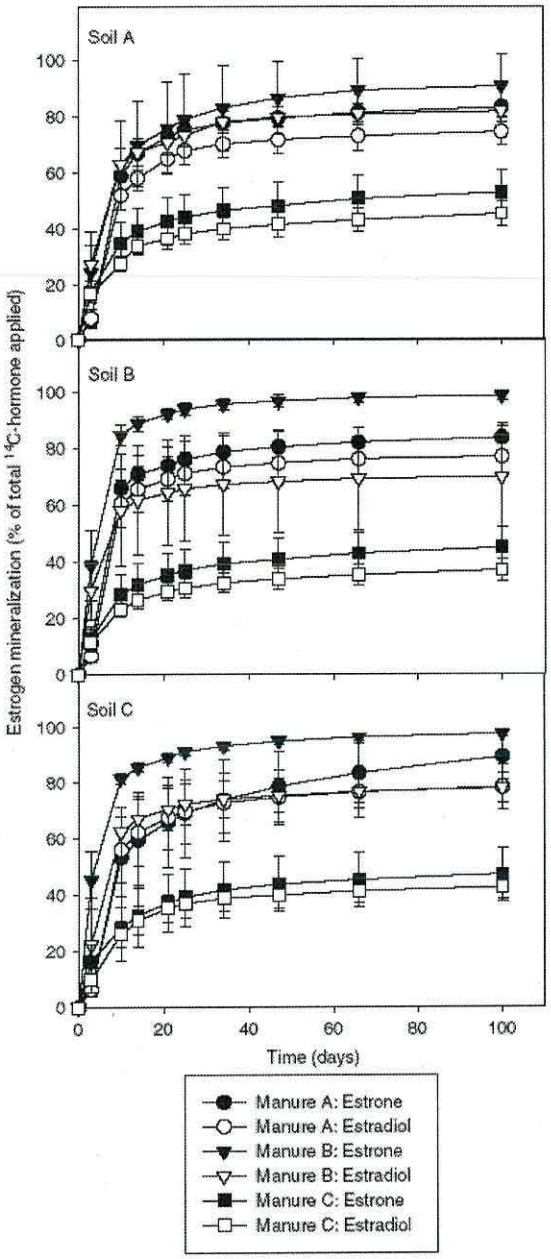

Fig. 4. Cumulative degradation of ^{14}C-labelled estrone and 17 β-estradiol after the addition to three soils in three types of cattle manure. Values represent means $\pm$ SEM ($n = 3$).

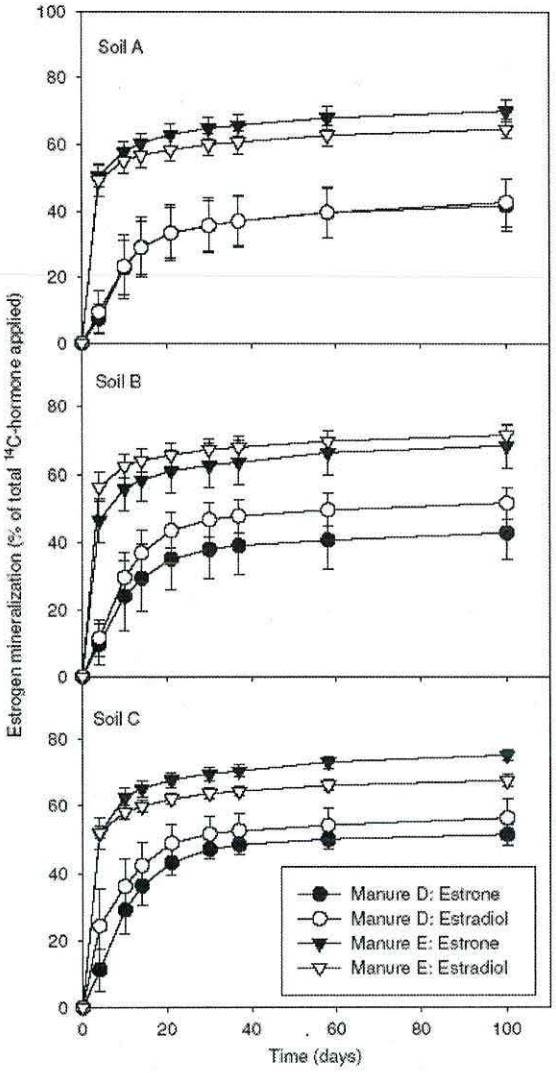

Fig. 5. Cumulative degradation of ^{14}C-labelled estrone and 17 β-estradiol after the addition to three soils in two types of sheep manure. Values represent means $\pm$ SEM ($n = 3$).

3.4–3.9) they have the potential to passively enter both plant and microbial cells (Bromilow et al., 1991; Lai et al., 2002; Casey et al., 2005) where they can potentially be degraded by a variety of enzymes including cytochrome P450 s and catechol-*o*-methyltransferase like enzymes, etc (Lakhani et al., 2003). However, estrogen biodegradation is also likely to occur extracellularly. The evidence available from sewage treatment works and freshwater sediments suggests that degradation of estrogens to intermediates that can be readily used directly in microbial respiration (e.g. TCA cycle) may occur via multiple pathways involving at least five individual enzymatic reactions (Lee and Liu, 2002; Shi et al., 2004). While some of the enzymes involved in these reactions may be common in agricultural soils (e.g. phenol oxidase) further work is required to fully elucidate the breakdown pathway under a range of scenarios.

Table 4
Amount of ^{14}C-label recovered from three soils after the addition of five manures (A–E) containing ^{14}C-labelled estrone and 17 β-estradiol and incubation for 100 d and after extraction with either $CaCl_2$ or methanol

		Extractable hormone (% of total recovered)			Amount not recovered (%)
		$CaCl_2$	Methanol	Sum	
Estrone					
Soil A	Manure A	2.3±0.4	2.4±0.5	87.2	12.8
	Manure B	0.8±0.2	1.7±0.3	92.5	7.5
	Manure C	3.3±1.1	2.7±0.1	58.1	41.9
	Manure D	9.7±2.8	2.8±0.5	53.7	46.3
	Manure E	3.8±0.3	2.1±0.1	75.6	24.4
Soil B	Manure A	3.0±0.7	2.3±0.1	88.7	11.3
	Manure B	1.4±0.2	2.1±0.2	100	0.0
	Manure C	3.4±0.9	3.4±0.3	51.4	48.6
	Manure D	11.5±3.0	8.2±2.1	62.1	37.9
	Manure E	1.5±0.9	2.6±0.3	72.1	27.9
Soil C	Manure A	2.2±0.7	8.3±3.2	99.5	0.5
	Manure B	0.9±0.5	1.9±0.4	100	0.0
	Manure C	4.0±0.8	3.4±0.2	54.2	45.8
	Manure D	10.9±2.5	3.2±0.5	65.3	34.7
	Manure E	1.5±0.6	1.8±0.2	78.1	21.9
Estradiol					
Soil A	Manure A	3.5±0.6	2.1±0.4	79.3	20.6
	Manure B	3.9±1.1	2.1±0.3	87.1	12.9
	Manure C	4.0±0.3	2.0±0.2	50.6	49.4
	Manure D	9.1±1.5	2.1±0.5	53.4	46.6
	Manure E	3.5±0.2	2.4±0.4	70.1	29.9
Soil B	Manure A	1.2±0.4	2.7±0.1	80.6	19.4
	Manure B	0.5±0.2	1.8±0.1	71.7	28.3
	Manure C	2.1±0.6	2.4±0.2	41.1	58.9
	Manure D	11.0±2.8	4.8±0.4	66.9	33.1
	Manure E	4.4±0.5	2.9±0.6	78.4	21.6
Soil C	Manure A	0.6±0.3	3.6±1.1	82.2	17.8
	Manure B	1.3±0.6	2.4±0.2	81.3	18.7
	Manure C	2.7±0.4	5.2±1.3	50.1	49.9
	Manure D	11.0±1.7	4.1±0.3	71.0	29.0
	Manure E	1.1±0.3	1.8±0.2	70.1	29.9

The 'sum' column includes the total recovered as $^{14}CO_2$, methanol and $CaCl_2$.
Values represent means±SEM ($n = 3$).

Table 5
Calculated half-life ($t_{1/2}$ in days) of estrone and 17 β-estradiol in three soils after addition in three different solvents (distilled water, artificial urine and natural field-collected sheep urine) or five different types of animal manure

	Soil A (Eutric cambisol)		Soil B (Eutric cambisol)		Soil C (Haplic podzol)	
	Estrone	17 β-estradiol	Estrone	17 β-estradiol	Estrone	17 β-estradiol
Distilled water	4.7±0.8	23.1±8.8	24.6±5.6	12.2±2.2	24.8±7.6	13.9±0.7
Artificial urine	9.1±0.8	11.4±2.9	6.4±1.0	8.9±1.4	30.0±10.4	28.1±14.2
Natural urine	9.5±0.9	19.0±3.2	4.8±0.9	5.3±0.9	45.5±27.8	33.9±16.9
Manure A	6.1±1.2	6.3±1.1	5.2±1.1	5.5±1.2	6.1±1.5	6.0±1.3
Manure B	4.9±0.4	4.2±0.4	3.5±0.3	3.3±0.1	2.8±0.1	4.2±0.3
Manure C	4.3±0.2	4.4±0.6	5.0±0.3	4.9±0.3	5.2±0.7	6.2±0.2
Manure D	7.8±1.4	7.8±1.4	7.8±1.4	8.0±1.3	8.6±1.5	7.0±1.0
Manure E	1.5±0.2	1.3±0.1	1.5±0.2	1.3±0.1	1.7±0.1	1.3±0.1
Overall mean	6.0±1.0	9.7±2.7	7.4±2.6	6.2±1.2	15.6±5.7	12.6±4.2

Values represent means±SEM ($n = 3$) except for the overall mean ($n = 8$).

A more likely explanation for the apparent lag phase in estrogen mineralization is the presence of other organic compounds in the urine which may either directly or indirectly suppress hormone biodegradation. The presence of these compounds may affect the internal anabolic and catabolic partitioning of the hormone-C once inside the microbial cell. Evidence for differences in partitioning of the C contained in the hormones in the presence and absence of urine can be seen in Fig. 1 and Table 5 where although the treatments had vastly different mineralization profiles they possessed relatively similar half-lives. The high ionic strength, NH_4^+ content and pH of the urine may also inhibit components of the microbial community involved in hormone degradation (Petersen et al., 2004). The lag phase may therefore represent the growth of estrogen utilizing microorganisms adapted to these conditions.

The presence of compounds in the urine can be expected to block solid phase sorption sites possibly enhancing the bioavailability of the hormones. Currently, the relative importance of estrogen sorption on bioavailability in soil remains unknown. Studies in sewage treatment plants, however, have indicated that solid phase sorption does not greatly influence estrogen persistence in sludges (Andersen et al., 2005).

4.3. Mineralization studies with manure

In the manure amended soil the mineralization of the estrogens was generally two- to seven-fold higher than in the urine amended soil. This was probably due to the presence of indigenous microorganisms in the manures that are both adapted to the chemical conditions and possess the intrinsic capability for hormone degradation (Jacobsen et al., 2005). In general, the half-life of 17 β-estradiol was almost identical to that of estrone in all the manure amended soils. As estrone typically represents the first stage of 17 β-estradiol degradation (Ying et al., 2002), we conclude that this initial transformation step of 17 β-estradiol to estrone is not a rate limiting step in the biodegradation pathway. This would also imply that the transformation of 17 β-estradiol to estrone is rapid and that its half-life is actually much shorter than reported here. This is consistent with previous reports from sewage treatment works where 17 β-estradiol is rapidly converted to estrone which in comparison is relatively recalcitrant (Weber et al., 2005).

4.4. Environmental risk of estrogens in agricultural environments

Our results indicate that estrone and 17 β-estradiol are not particularly persistent, lasting only a few days in most soils. Similar results have also been reported for the hormone, testosterone in biosolids and manure amended soil (Jacobsen et al., 2005). The potential for estrogen loss to freshwater, however, is still large considering that cattle may produce 20–70 l of excreta/cow d (Bannink et al., 1999; de Boer et al., 2002) and that runoff or subsurface flow from agricultural land to freshwater frequently occurs during heavy rainfall events. Further, it is well documented that other components of animal faeces are regularly lost to freshwater (e.g. faecal coliforms; Oliver et al., 2005). One key question which remains unanswered is the extent to which the hormones become diluted during passage to watercourses. Here we will assume that the threshold estrogen concentration (TEC) at which an effect on freshwater organisms is observed is 1 ng/l. Assuming that an average animal releases 0.5 mg estrogen/d and that the stocking density is 10 animal/ha then to reach the TEC each day the animal wastes need diluting with 500 m^3 water/ha. If we assume that the soil depth is 1 m and the soil water content is 0.3 kg/kg then the total amount of soil water in a field can be calculated to be 3000 $m^3\,ha^{-1}$. Assuming an estrogen half-life of 5 d, it is clear that this is relatively close to the attenuation capacity of the soil. Under storm conditions (e.g. 50 mm rainfall/d = 500 m^3/ha) when high rates of runoff are likely to occur, then the amount of dilution can be expected to be close to the TEC level. These concentrations, however, are still likely to be an order of magnitude lower than the estrogens continuously discharged by wastewater treatment plants to freshwater (0.2–35 ng/l; D'Ascenzo et al., 2003; Rodriguez-Mozaz et al., 2004; Johnson et al., 2005; Labadie and Budzinski, 2005). Although there have been few direct studies on the concentration of estrogens in agricultural freshwater catchments devoid of sewage treatment plants, one study has reported estrogen concentrations close to the TEC limit (Soto et al., 2004). However, in that study significant concentrations of estrogens were also found in a non-agricultural control area. From our calculations and previous studies it is clear that the levels of estrogens released from agricultural environments are close to the TEC limit. Consequently, more work is required to measure the concentration of estrogens in soil, freshwater and groundwater under a range of environmental scenarios (e.g. stocking density, animal type and point in estrous cycle, different hydrological events, soil types, etc.). Our studies suggest that estrogen mineralization in manure is rapid and consequently storage of animal wastes should effectively reduce estrogen concentrations to very low limits. The risk associated with spreading manures to land is therefore probably very small.

Acknowledgements

The authors would like to acknowledge the European Union, European Social Fund for financial assistance.

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CHAPTER 4

Urine enhances the leaching and persistence of estrogens in soils

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Submitted to Soil Biology & Biochemistry

4.1. Abstract

The release of endocrine disrupting chemicals (EDCs) into the environment is of concern due to their potential deleterious effects on freshwater ecosystems and human health. Estrogen losses from agricultural land to freshwater have been implicated as a diffuse source of EDC pollution, however, uncertainty exists about the magnitude of this flux in comparison to other point sources (e.g. sewage treatment works). Recent reviews have all highlighted the need for more mechanistic studies on hormone behaviour in soil environments. The aim of this study was to investigate the influence of aqueous matrix on the leaching, sorption and persistence of two naturally occurring hormones, estrone and 17β-estradiol in three agricultural grassland soils. The hormones were applied to the surface of the soil in two solvents, distilled water and natural sheep urine. Rainfall was subsequently applied to the top of the soil columns and the leachate collected. In comparison to distilled water, the presence of sheep urine both enhanced and prolonged the amount of estrogen leaching from soil. We hypothesized that this enhanced rate of estrogen migration in soil was due to changes in either estrogen sorption or microbial immobilization. While the presence of urine did not greatly affect the rate and amount of estrogen sorption to soil it did significantly reduce their rate of mineralization. Overall, our study shows that vertical estrogen movement is rapid, soil type dependent and regulated by the aqueous matrix in which the hormones are contained. In terms of risk assessment and environmental fate modelling, we conclude that previous studies performed using hormones contained in artificial matrices (e.g. distilled water) may underestimate their rate of dissipation in the environment.

Keywords: animal waste; estrogen; estrone; 17β-estradiol; leaching; oestrogen; ovine; sorption

4.2. Introduction

The release of endocrine disrupting chemicals (EDCs) into the environment is of increasing concern due to their detrimental impact on freshwater organisms, ecosystem sustainability and human health (van der Kraak, 1998; Mills and Chichester, 2005; Campbell et al., 2006). Many EDCs released into the environment are naturally occurring estrogens, released in large quantities from agricultural animal excrement (e.g. cattle, sheep), wastewater treatment (e.g. sewage sludge, effluent discharges) or industrial processing of natural products (e.g. phytoestrogens; Lee et al., 2007). The main estrogens released into the environment are the steroidal hormones; estrone and 17β-estradiol, which are excreted by all humans and animals (Ying et al., 2002). It has been shown that exposure to estrogen at levels as low as 1 ng l^{-1} (<10 pM) are sufficient to cause the feminisation of male trout (Hansen et al., 1998) and the development of intersex roach in rivers (Jobling et al., 1998). There is, however, limited understanding of how these EDC's are reaching rivers (Lee et al., 2007). Some are released directly into freshwater from sewage treatment works. For example, Körner et al. (2000) reported effluent discharge concentrations of 6 ng l^{-1} of 17β-estradiol equivalents from a sewage treatment works in Germany. However, there could be further indirect inputs to surface and ground waters from the application of treated and untreated animal wastes to agricultural land.

Nichols et al. (1997) demonstrated that the land application of poultry manure can potentially lead to hormone losses in surface runoff from agricultural fields. Peterson et al. (2000) also investigated the subsurface movement of 17β-estradiol from poultry and cattle manure applied to land into aquifers and its subsequent movement into surface waters. Their study found significant contamination of 17β-estradiol in spring waters (6 to 67 ng l^{-1}) which were hydrologically connected to the initial site of waste application. They also studied the faecal coliform and *E. coli* concentrations and found a positive correlation with estrogen concentrations providing further direct evidence that the animal waste was the source of hormone contamination. Column leaching experiments undertaken in the laboratory have also demonstrated the potential for vertical migration and microbial transformation of hormones in the soil (Casey et al., 2005). However, Casey et al. (2005) concluded that their experimental conditions were unlikely to be representative of field conditions due to their continuous method of hormone delivery to the soil. Consequently, they concluded that under natural conditions the sorption and degradation rates of the hormone were most likely to result in little mobility and little persistence. This contrasts with work by Lucas and Jones (2006) who suggested that 17β-estradiol and estrone in

animal pastures could lead to significant contamination of freshwaters during rainfall events. We hypothesize that this discrepancy in viewpoint could be associated with the aqueous matrix in which the hormones are applied to the soil (Lucas and Jones, 2006).

When hormones enter the soil they can undergo a number of fates of which the major ones include biotic transformation and solid phase sorption (Lee et al., 2007; Zhou et al., 2007). Shareef et al. (2006) demonstrated differential sorption affinities and binding kinetics for estrone to a range of common minerals including goethite, kaolinite and montmorillonite. These differences may be due to different bonding mechanisms in the minerals and may also cause differences in their desorption potential (van Emmerik et al., 2003). It is likely that the alkaline pH and very high concentrations of salts and organic compounds in animal urine may also affect the sorption and leaching potential of hormones in soil due either to a blockage of sorption sites or alteration of surface charges on both organic and inorganic colloids. In addition, the high concentration of labile C and N in the urine may also suppress microbial transformation of the hormones thereby indirectly facilitating their movement in soil.

Our poor understanding of the fate of hormones in animal wastes applied to agricultural land has recently been highlighted (Hanselman et al., 2003; Lee et al., 2007). Consequently, the primary aim of this study was to investigate the influence of aqueous matrix (sheep urine or artificial rainwater) on the leaching and sorption of estrone and 17β-estradiol in three contrasting grassland soils.

4.3. Materials and methods

4.3.1. Soil

Soil was obtained from three contrasting temperate oceanic agricultural grasslands located in Abergwyngregyn, Gwynedd, North Wales (53°14' N, 4°01' W; Table 1). All the soils regularly receive urine and faecal inputs from grazing sheep that occurs throughout the year. Soil A (sandy clay loam textured Eutric cambisol) was collected from the surface Ah horizon (5-20 cm) of a lowland (15 m altitude) freely-draining, heavily sheep-grazed grassland which receives regular fertilization (120 kg N, 60 kg K and 10 kg P y^{-1}) and occasional manure addition. Soil B (sand textured Eutric cambisol) was collected from the surface Ah horizon of a lowland (5 m altitude) freely draining, lightly sheep-grazed soil that receives no fertilization. Soil C (sandy loam textured Haplic podzol) was collected from the surface Ah horizon (5-20 cm) of an upland (200 m altitude) freely draining, heavily leached, lightly sheep-grazed grassland that receives no fertilization. The mean

annual soil surface temperature at 10 cm varies from 8 to 10°C and the annual rainfall at the lowland site is 1250 mm and at the upland site 1700 mm. All soils were stored field-moist and unsieved at 4°C until use.

Table 4.1: Chemical and physical characteristics of the three soils used in the study. All values represent means ± SEM (n = 3). ND indicates not determined.

	Soil A	Soil B	Soil C
	Eutric cambisol	Eutric cambisol	Haplic podzol
$EC_{1:1}$, µS cm^{-1}	80 ± 4	76 ± 19	46 ± 7
pH ($_{1:1}$, H_2O)	6.06 ± 0.07	5.31 ± 0.10	4.63 ± 0.08
$CaCO_3$, g kg^{-1}	0.11 ± 0.02	<0.01	<0.01
Water holding capacity, g kg^{-1}	520 ± 20	251 ± 10	690 ± 40
Moisture content, g kg^{-1}	160 ± 10	62 ± 4	260 ± 2
Organic C, g kg^{-1}	2.1 ± 0.1	2.7 ± 0.1	1.2 ± 0.1
Total N, g kg^{-1}	0.16 ± 0.01	0.20 ± 0.01	0.08 ± 0.01
C-to-N ratio	13.3 ± 0.6	13.4 ± 0.3	15.6 ± 1.3
Soil solution NO_3^-, mg N l^{-1}	13.7 ± 1.3	2.7 ± 0.1	0.5 ± 0.1
Soil solution NH_4^+, mg N l^{-1}	1.4 ± 0.1	28.8 ± 14.2	1.1 ± 0.1
Exchangeable cations			
Na, mg kg^{-1}	29 ± 3	17 ± 1	37 ± 1
K, mg kg^{-1}	116 ± 18	5 ± 1	77 ± 12
Ca, mg kg^{-1}	1595 ± 217	48 ± 1	89 ± 8
Mg, mg kg^{-1}	89 ± 19	35 ± 5	15 ± 2
Extractable P, mg kg^{-1}	9.9 ± 0.3	1.8 ± 0.2	0.2 ± 0.1
Soil respiration, g CO_2 m^{-2} h^{-1}	0.60 ± 0.02	ND	0.25 ± 0.02

4.3.2. Sheep urine

Successive replicate batches of sheep urine were collected from two individual ewes held in a stainless steel crush which had previously been grazing on *Lolium perenne* grassland on soil A. Immediately after collection, aliquots of the urine was centrifuged at 8000*g* at 10°C for 15 min to remove particulate matter. The samples were then frozen at –20°C in polypropylene containers until required. The pH of the urine was 8.6 and the electrical conductivity was 26 mS cm^{-1}. Its soluble C content was 1.5 ± 0.2 g C l^{-1} and the

total soluble N content was 2.0 ± 0.1 g N l^{-1}. The urine contained 2089 ± 8 mg K l^{-1}, 1352 ± 8 mg Na l^{-1}, 271 ± 4 mg Ca l^{-1} and 2.4 ± 0.1 mg P l^{-1}.

4.3.3. Chemical analysis

Soil pH and electrical conductivity were determined in 1:1 (v/v) soil:H_2O extracts (Smith and Doran, 1996) and moisture by drying at 80°C for 24 h. Total C and total N were determined with a CHN-2000 analyser (Leco Corp., St. Joseph, MI). Exchangeable cations were estimated by performing 1:10 (w/v) soil:0.5 M $BaCl_2$ extractions on a reciprocating shaker (200 rev min^{-1}; 60 min, 20°C) followed by centrifugation at 10000*g* (30 min, 4°C) and storage of supernatant solutions at -20°C. Exchangeable cations were determined by ICP-OES (Jobin Yvon, JY138 Ultrace). $CaCO_3$ content was determined by the van Slyke manometric method (Nelson, 1982). Extractable P was measured by extraction with 0.5 M acetic acid (1 h, 200 rev min^{-1}) followed by centrifugation at 10000*g* (30 min, 20°C) and P analysis by the method of Murphy and Riley (1962). Soil solution was recovered by the centrifugal method of Giesler and Lundström (1993) with NO_3^- and NH_4^+ in solution determined with a Skalar San^{++} segmented flow autoanalyser (Skalar UK Ltd., York, UK). Soil respiration was determined with a SR1 automated soil respirometer equipped with an infra-red gas analyzer (PP systems Ltd., Hitchin, UK). Total organic carbon and total dissolved nitrogen concentration were measured using a Shimadzu TC-TNV analyser (Shimadzu Corp., Kyoto, Japan). Hormone analysis of the urine samples was undertaken by reverse phase gradient elution HPLC (Laserchrom Analytical Ltd., Rochester, Kent, UK) using a Evolution® C18 column (Laserchrom Analytical Ltd.) at 40°C. An LA2100 gradient pump (Laserchrom Analytical Ltd.) created an elution profile from 10% acetonitrile to 90% acetonitrile (pH 6) with a 100 min run time. Compounds were detected by a combination of fluorescence detection (Ex. 275 nm, Em. 300 nm) and UV (215 nm). A UV diode array was also used to resolve peaks and aid in peak identification, which was based on retention time and performed with Clarity® (DataApex, Prague, Czech Republic). Analysis indicated that the concentration of estrone and 17β-estradiol in the urine were below detection limits (< 1 mg l^{-1}) and indicated that neither of the ewes were pregnant which is associated with high intrinsic hormone loads.

4.3.4. Artificial rainwater

Artificial rainwater was made to simulate natural rainwater and was based upon the mean composition of 1152 individual field samples taken throughout Wales (Stevens et al.,

1997). The rainwater contained the following: NaCl (5.6 mg l^{-1}), K_2SO_4 (1.75 mg l^{-1}), $CaCl_2.2H_2O$ (0.74 mg l^{-1}), $MgCl_2.6H_2O$ (1.21 mg l^{-1}), NH_4NO_3 (1.2 μM) and KH_2PO_4 (0.14 mg l^{-1}) dissolved in distilled water and stored at 4°C until use.

4.3.5. Soil leaching studies

Leaching columns were constructed from 7 cm long, polypropylene tubes to which mesh (1.5 mm) was secured over the base to prevent soil loss. As our primary objective was to critically assess the influence of aqueous matrix type, we used repacked soil columns to ensure a more homogeneous pore matrix and to minimize preferential flow pathways (Sangsupan et al., 2006). All the soils possess a natural crumb (Soils A and B) or granular structure (Soil C) with 1 to 2 mm diameter aggregates that packed easily to achieve a bulk density of 1 g cm^{-3}. Two estrogens, ^{14}C-estrone (4-^{14}C; American Radiolabeled Chemicals Inc., St. Louis, MO; 1.99 GBq $mmol^{-1}$) and ^{14}C-17β-estradiol (4-^{14}C; American Radiolabeled Chemicals Inc.; 2.04 GBq $mmol^{-1}$) were used in the study. A ^{14}C-radiolabeled estrogen solution (500 μl, 50 μg l^{-1} in distilled water or sheep urine) was added to the centre of the surface of the field-moist soil contained in the leaching columns. The initial estrogen concentration was based upon Lange et al. (2002). Artificial rainwater was then applied to the surface of the leaching columns at a rate of 1.4 mm min^{-1} using a peristaltic pump (205U, Watson-Marlow, Cornwall, England). This rate was chosen to simulate a worst case scenario storm force rainfall event (during 2005-07 there were 211 ± 6 rain days $year^{-1}$ of which 13 ± 2% were >10 mm in intensity). To catch the leachate, polypropylene vials were placed underneath the leaching columns and replaced every 15 min over a 5 h period. The amount of ^{14}C in the leachate was determined using a Wallac 1404 liquid scintillation counter (Wallac EG&G, Milton Keynes, UK) and Optiphase 3 scintillation fluid (Wallac EG&G). After the leaching had ended, the columns were divided into 3 equal depth sections, the soil dried at 40°C and its ^{14}C content determined with a OX-400 Biological Sample Oxidiser (RJ Harvey Instrument Corp., Hillsdale, NJ) and Oxosol scintillation fluid (National Diagnostics, Hessle, UK).

To estimate the amount of hormone leached from the soil, a first order exponential decay model was fitted to the time-dependent leaching experimental data where

$$H = S_0 + (A_1 \times \exp^{-k_1 t}) \quad \text{(Eqn. 1)}$$

and where H is the amount of hormone remaining in the soil, S_0 is the amount of hormone sequestered biotically or abiotically in the soil, A_1 is the total amount of hormone leached

from the soil, k_1 is the first order rate constant describing the rate of hormone leaching and t is time. The half-time ($A_{½}$) required for 50% of the hormone to be leached from soil was determined following

$$A_{½} = \ln(2)/k_1 \quad \text{(Eqn. 2)}$$

4.3.6. Sorption studies

^{14}C-labeled estrone or 17β-estradiol contained in distilled water or sheep urine (50 μg l^{-1}; 5 ml) was added to 1 g of soil placed in a 20 ml polypropylene vial. The vials were then shaken for period of up to 5 h on a flat bed shaker at 200 rev min^{-1}. At known times after hormone addition, the tubes were centrifuged at 16000*g* for 5 min and the supernatant solution recovered for ^{14}C determination as described previously. The sorption experiments were performed on soil that had been heat-sterilized (80°C, 30 min) prior to use to minimize microbial mineralization of the hormones (Kuzyakov and Jones, 2006). The equilibrium solution concentration (C_i) and amount sorbed to the solid phase (A_i) were used to calculate the solid-to-solution partition coefficient (K_d) where

$$K_d = A_i / C_i \quad \text{(Eqn. 3)}$$

In addition, a first order kinetic model with asymptote was fitted to the time-dependent sorption data where

$$H = S_1 + (A_s \times \exp^{-k_2 t}) \quad \text{(Eqn. 4)}$$

and where H is the percentage hormone remaining in the soil, S_1 is the percentage of hormone remaining in solution, A_s is the percentage of hormone sorbed to the solid phase and k_2 is the first order rate constant describing the rate of sorption to the solid phase. The half-time ($S_{½}$) required for 50% of the hormone to be sorbed was determined using

$$S_{½} = \ln(2)/k_2 \quad \text{(Eqn. 5)}$$

4.3.7. Statistical analysis

The first order exponential decay model was fitted to the experimental leaching data using a least squares optimization routine in Sigmaplot v8.0 (SPSS Inc., Chicago, IL). Statistical analysis (ANOVA with Tukey pair-wise comparison, *t*-tests) was carried out using SPSS 12.0 (SPSS Inc.).

4.4. Results

4.4.1. Hormone leaching in soil

The leaching of the hormones from soil was strongly influenced by both soil type ($P < 0.001$) and the matrix in which the hormone was added to the soil ($P < 0.01$; Fig. 1). Generally, the overall temporal patterns of estrone and 17β-estradiol leaching were very similar to each other in the individual soils. In all three soils the rate of hormone leaching progressively declined over time with a significant proportion remaining unrecovered in the leachate at the end of the 5 h collection period (i.e. that immobilized in the soil or lost as $^{14}CO_2$). Although the magnitude of the difference was very small, significantly more 17β-estradiol than estrone was recovered in the leachate across all three soils ($P < 0.05$). To estimate the amount of hormone that could be leached from soil, a single first order kinetic model with asymptote was fitted to the experimental data. Overall, the model gave extremely good fits to the experimental results with r^2 values of 0.9991 ± 0.0002 for Soils A and B. In contrast, the fits to the model for Soil C were satisfactory but less good producing r^2 values of 0.976 ± 0.003. A summary of the parameter estimates produced from the kinetic model are presented in Table 2. The kinetic model revealed that the average half-time ($A_{½}$) for hormone leaching from soil was similar for both estrone ($A_{½}$ = 2.3 ± 1.0 h) and 17β-estradiol ($A_{½}$ = 2.2 ± 0.9 h; $P > 0.05$). Particularly in soils A and B, the presence of sheep urine significantly increased both the total amount of hormone available for leaching and increased the half-time for leaching ($P < 0.001$; Table 2). The average half-time for hormone leaching in water across all soils and hormones was 1.4 ± 0.3 h while in a sheep urine matrix the half-time was 2-fold greater at 3.2 ± 1.2 h. Similarly, across all soils and hormones, only 42 ± 6% of the hormone could be attributed to the leachable pool when applied in a water matrix whilst the model predicted that 80 ± 4% of the hormones would be leached when applied in a sheep urine matrix.

The amount of ^{14}C-label remaining in the soil after the 5 h leaching period is shown in Figure 2. This pool includes ^{14}C still present in a hormonal form (e.g. sorbed to the solid phase) and that contained in ^{14}C-labeled microbial transformation products. Overall, the total amount of ^{14}C-label remaining in the soil was inversely related to that recovered in the leachate. The vertical distribution of the ^{14}C-label in the soil columns was also soil dependent ($P < 0.001$) with a progressive decline in ^{14}C content with depth seen in Soils A and B while a more even vertical distribution was observed in Soil C. Overall, there were no significant differences in the amount of ^{14}C derived from either estrone of 17β-estradiol remaining in the three soils ($P > 0.05$). In all three soils, the presence of sheep urine

significantly reduced the amount of ^{14}C-label persisting in the soil ($P < 0.001$) and promoted its downward vertical migration in Soils A and B.

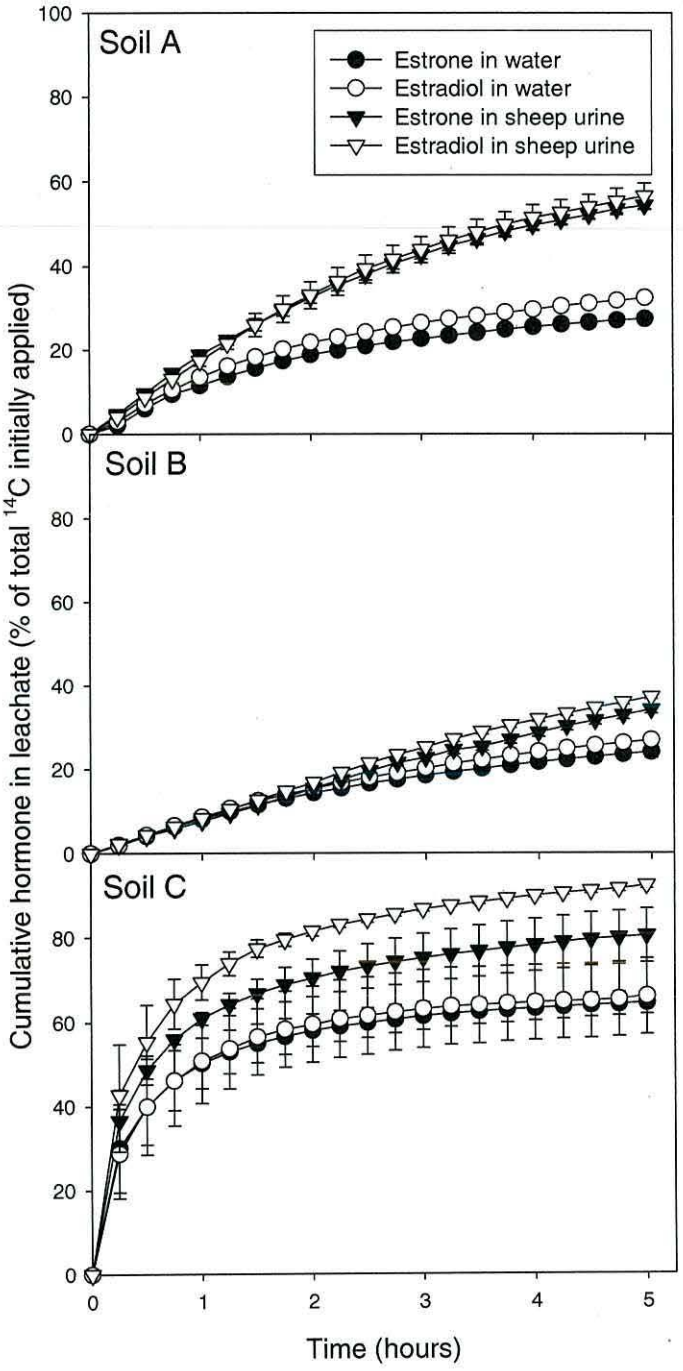

Figure 4.1: The cumulative amount of ^{14}C-labeled estrone or 17β-estradiol recovered in the leachate from soil columns after the addition of artificial rainfall to the surface. Hormones were added to each of the three soils in either water or sheep urine and leachate was collected every 15 minutes. Values represent means ± SEM ($n = 3$).

Table 4.2: Predicted total amounts of estrone or 17β-estradiol that can be leached from soil (pool size) and the half-time ($A_{½}$) for 50% of the hormone to be lost via leaching after the addition of the hormones to soil in either a distilled water or sheep urine matrix. Values represent means ± SEM ($n = 3$).

	Soil A		Soil B		Soil C		All soils	
	Pool size (% of total)	Half-time (h)	Pool size (% of total)	Half-time (h)	Pool size (% of total)	Half-time (h)	Pool size (% of total)	Half-time (h)
Estrone in water	30.0 ± 0.3	1.37 ± 0.04	29.9 ± 0.2	2.10 ± 0.03	58.4 ± 2.2	0.40 ± 0.03	39.4 ± 9.4	1.29 ± 0.49
Estradiol in water	35.2 ± 0.3	1.43 ± 0.04	35.3 ± 0.3	2.38 ± 0.05	60.3 ± 1.9	0.42 ± 0.03	43.6 ± 8.3	1.41 ± 0.51
Estrone in urine	68.2 ± 0.4	2.09 ± 0.03	91.0 ± 8.0	7.24 ± 0.74	71.3 ± 3.1	0.43 ± 0.03	76.8 ± 7.1	3.25 ± 2.05
Estradiol in urine	74.1 ± 1.2	2.23 ± 0.08	92.3 ± 8.0	6.46 ± 0.66	81.9 ± 3.4	0.42 ± 0.03	82.8 ± 5.2	3.04 ± 1.78

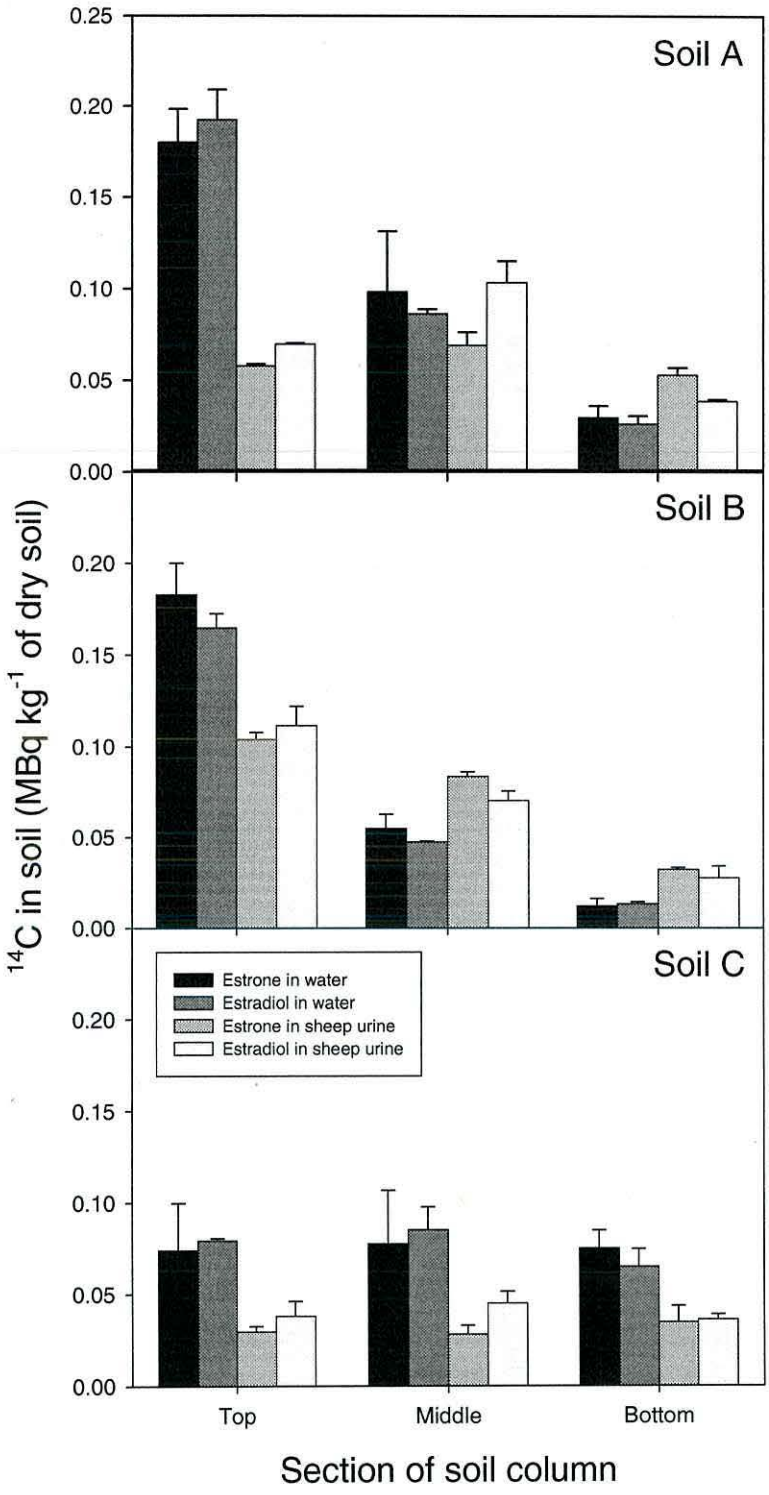

Figure 4.2: Vertical distribution of ^{14}C-label remaining in the soil after the addition of ^{14}C-labeled estrone and 17β-estradiol to the soil surface and leaching with artificial rainfall for 5 h. Hormones were added in either a water or sheep urine matrix. Values represent means ± SEM (n = 3).

4.4.2. Hormone sorption in soil

The time-dependent sorption of the hormones to the three soils is shown in Figure 3. Overall, the temporal pattern of sorption was similar in all soils with a solid-solution equilibrium reached extremely quickly after ^{14}C-hormone addition. A first order kinetic model with asymptote fitted well to the experimental data with r^2 values for the model fit being 0.95 ± 0.02 for Soil A, 0.98 ± 0.01 for Soil B and 0.94 ± 0.01 for Soil C. From the kinetic model, the total amount of hormone sorbed to the solid phase was estimated to be 69 ± 1% for soil A, 76 ± 2% for Soil B and 46 ± 3% for Soil C with few significant differences observed between hormone or matrix type ($P > 0.05$). The solid-to-solution partition coefficients (K_d) were significantly different between the soils ($P < 0.001$) and estimated to be 11 ± 1 for Soil A, 16 ± 2 for Soil B and 4 ± 1 for Soil C. The sorption half-time ($S_{½}$), at which point the amount sorbed is half maximal, was estimated from the kinetic model and shown to be similar for all soils ($P < 0.05$). Across all treatments, the average $S_{½}$ values were 2.4 ± 0.6 min for Soil A, 1.6 ± 0.5 min for Soil B and 2.1 ± 0.8 min for Soil C. There was no significant difference in the $S_{½}$ values in the presence (1.7 ± 0.6 min) or absence of sheep urine (2.3 ± 0.3 min; $P < 0.05$).

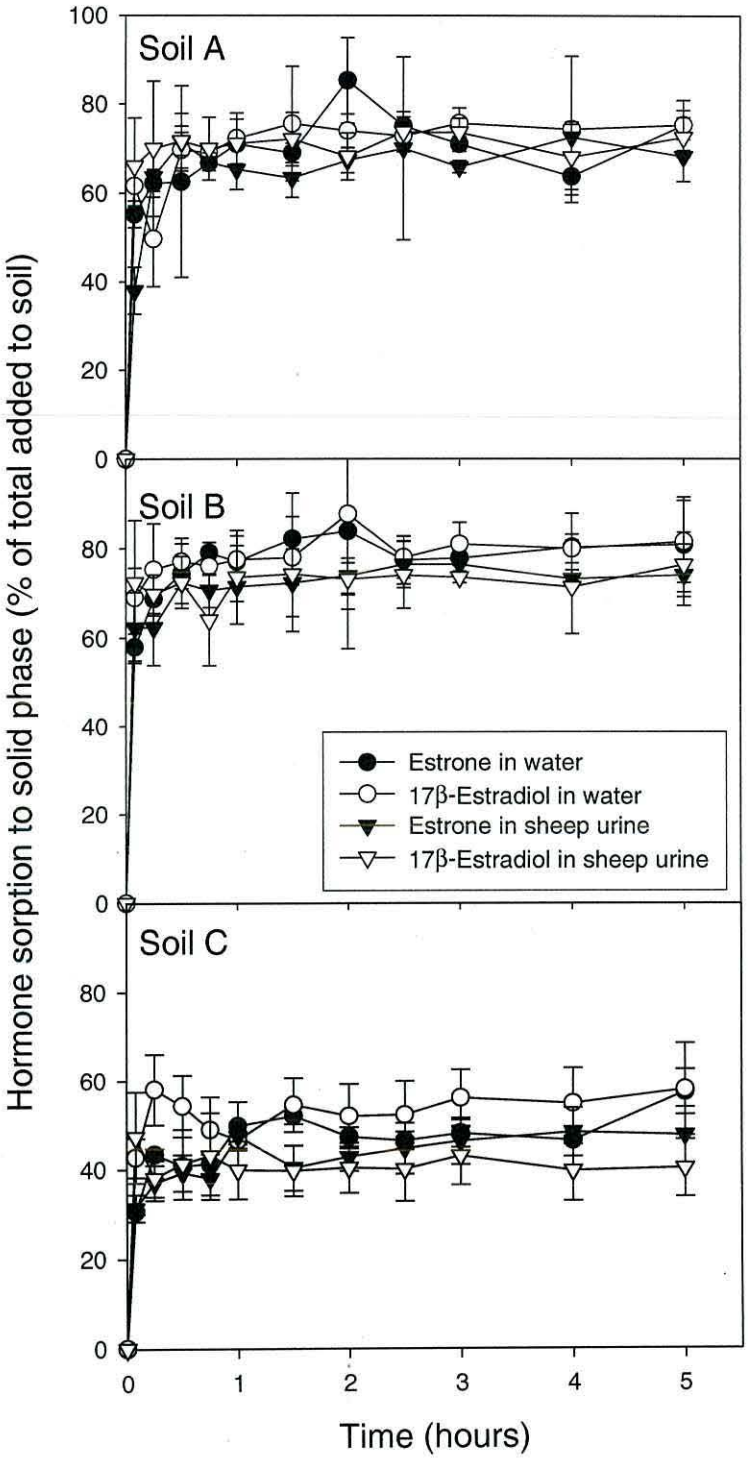

Figure 4.3: Time-dependent sorption of either estrone or 17β-estradiol to three soils when added in either a distilled water or sheep urine matrix. Values represent means ± SEM (n = 3).

4.5. Discussion

4.5.1. Hormone dissipation in the environment

Scientific investigations in Europe, USA and Japan have demonstrated the occurrence of the intersex condition and elevated concentrations of plasma vitellogenin in fish in freshwater and estuarine environments (Environment Agency, 2004). A direct link between this intersex/vitellogenin response in fish and the presence of steroid estrogens in freshwaters (e.g. estrone and 17β-estradiol) has now been firmly established and shown to be of widespread concern. Both point sources (e.g. sewage treatment works) and diffuse sources (e.g. agricultural fields) have been implicated as major causes of EDC pollution, however, the relative importance and interaction of these sources remains poorly understood. Further, recent reviews by Hanselman et al. (2003), Wang et al. (2004) and Khanal et al. (2006) have all indicated the need for more research to understand estrogen behaviour in agricultural soils and potential losses to freshwaters. In agreement with previous studies, the results presented here on grassland soils clearly show that the environmental fate of estrone and 17β-estradiol is soil type specific. Of importance, however, is our demonstration that the behaviour of estrogens in soil is critically dependent upon the aqueous matrix type in which it is delivered to the soil. Previous studies on estrogen sorption and dissipation in soil have studied their behaviour after their addition to soil in unnatural matrices such as distilled water or dilute salt solutions (e.g. 10 mM $CaCl_2$; Lee at al., 2003; Sangsupan et al., 2006; Fan et al., 2007). Here we show that their addition in realistic matrices such as animal urine results in an increased capacity to be lost via leaching from soil and an increased capacity to leach for longer periods. This increased loss in the presence of sheep urine could be attributable to: (1) the blockage of hormone sorption sites by solutes presents in the urine, (2) changes in the net charge of solid phase sorption sites due to urine induced shifts in soil pH and ionic strength, (3) osmotic-induced disruption of the soil microbial community reducing hormone mineralization, and (4) inhibition or alteration of microbial hormone transport and assimilation systems by components in the urine.

4.5.2. Hormone sorption in soil

Sheep urine contains very high concentrations of dissolved solutes (ca. 100-300 mM) which could potentially block a range of estrogen sorption sites in soil. In addition, the presence of urine can cause significant increases in soil pH both directly (e.g. through chemical buffering, displacement of H^+ from mineral surfaces) and indirectly (e.g. through

urea hydrolysis) which can affect the net charge of soil and consequently its sorption properties (Keerthinarayana and Bandyopadhyay, 1998; Lucas and Jones, 2006). The results presented here, however, suggest that the organic and inorganic compounds present in urine did not greatly affect either the amount or rate of hormone sorption to the soil's solid phase. Taken together, we conclude that the observed increase in estrogen leaching in the presence of urine is not mediated through a change in the soil's estrogen sorption properties. Our findings do, however, add to the debate on hormone sorption mechanisms in soil which are poorly understood. Our results support the hypothesis that soil mineral composition and not pH exerts a major effect on hormone sorption capacity (Shareef et al., 2006). Van Emmerik et al. (2003) suggested that the mechanism for 17β-estradiol sorption was via hydrogen bonding while Shareef et al. (2006) suggested that based upon their octanol-water partition coefficients (log *K*ow = 3.2-4.0), estrogens would be attracted to relatively hydrophobic surfaces. This is supported by the results presented here and by Lee et al. (2003) and Jones et al. (2006) who reported that estrogen sorption correlated well with soil/sediment organic carbon content. If the hormones are sorbed to hydrophobic binding domains in soil then it is unlikely that the hydrophilic solutes which dominate urine chemistry will interfere with estrogen sorption (e.g. urea log *K*ow = -3.2 to -1.5)(Bi et al., 2007). Our sorption results also concur with studies in the aquatic environment showing that the sorption behaviour of estrone and 17β-estradiol were similar, probably due to their similar physicochemical properties (Bowman et al., 2002; Bowman et al., 2003).

Although hormone desorption reactions were not explicitly studied here, our results suggest that a significant proportion of the hormone could not be recovered from the soil during the 5 h leaching period. Estimates from the first order kinetic modelling in the absence of urine suggested that 67 ± 3% of the hormones would not be leached from soil A, 66 ± 2% from Soil B and 41 ± 1% from Soil C. This corresponds reasonably well to the theoretical maximum amount of sorption predicted from the batch sorption studies also performed in the absence of urine (70 ± 1% for soil A, 78 ± 1% for Soil B and 51 ± 3% for Soil C). However, in the presence of urine much more estrogen was leached than would have been predicted from the batch sorption studies. Studies on the extractability of estrogens with methanol and water performed on the same soils indicated significant differences in estrogen desorption ability in the presence and absence of urine (Lucas and Jones, 2006). This implies that although the presence of urine may not affect estrogen adsorption it may affect their desorption kinetics. Studies by Hildebrand et al. (2006) have

suggested that estrogens are weakly bound to soil and easily desorbed, however, Shore et al. (1993) suggested that estrogens were almost irreversibly sorbed. Clearly, further work is required to ascertain the major factors regulating estrogen desorption from soil.

4.5.3. Hormone mineralization in soil

We hypothesized that the presence of urine may have direct and indirect effects on soil microbial functioning which could significantly affect the persistence and dissipation of estrogens in soil. Based upon previous studies, we presume that in the context of our experiment that urine addition will cause immediate changes in soil microbial activity but will not significantly affect microbial community structure (Bol et al., 2004; Rooney et al., 2006). Current evidence suggests that soil microorganisms do not possess specific transport systems for estrogens and that transfer into the cell occurs passively after which they can be potentially degraded by a range of enzymes (Lucas and Jones, 2006). Consequently, we expected the rate of degradation in soil to be low. In a previous study, we examined the long-term persistence of ^{14}C-labeled estrogens in the same soils as those used here (up to 100 d; Lucas and Jones, 2006). Extracting the data for the first few hours, we determined the amount of hormone mineralization to $^{14}CO_2$ in each soil (3 h after hormone addition to soil). The results presented in Figure 5 clearly show that both estrogen and estradiol mineralization are significantly reduced in all three soils ($P < 0.05$). Although the amount of hormone mineralized to $^{14}CO_2$ was low, this method only provides a measure of complete degradation of the hormones to CO_2 (i.e. it doesn't provide a measure of estrogen immobilization in the biomass). Taken together with the other results presented in Lucas and Jones (2006), it does imply that the addition of urine causes significant changes in microbial activity and their capacity to degrade estrogens.

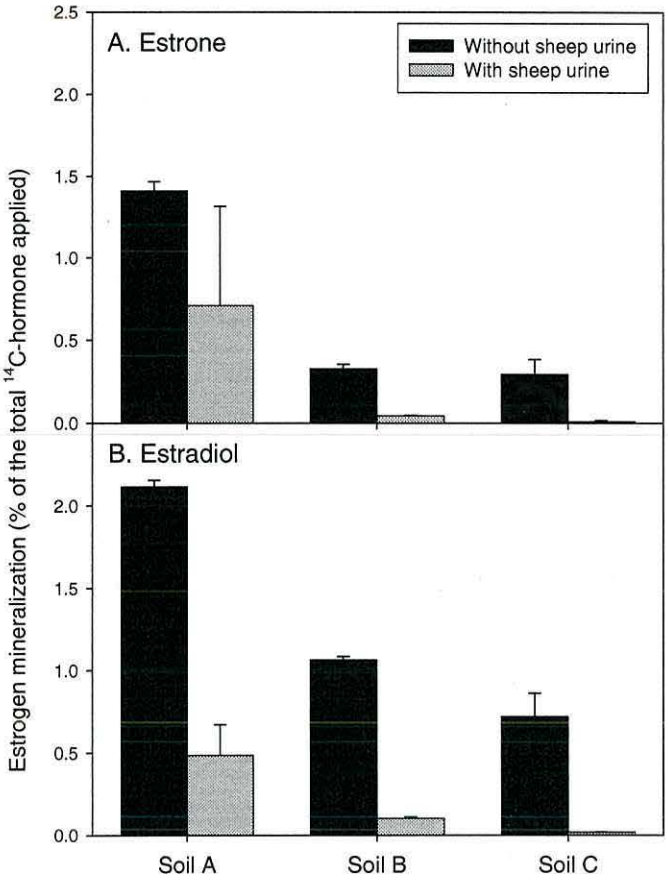

Figure 4.4: Short-term mineralization of ^{14}C-labeled estrone or 17β-estradiol in the three soils when added in either a distilled water or sheep urine matrix. Values represent means ± SEM (n = 3). The incubation time was 3 h. The legend is the same for all panels. Adapted from Lucas and Jones (2006).

4.5.4. Conclusions

The loss of small quantities of estrogens from agricultural land via overland and sub-surface flow may represent a significant route of entry for endocrine disrupting chemicals into freshwaters. The results presented here suggest that most previous studies may have underestimated the potential for estrogens to migrate in soil due to the lack of consideration of the intrinsic matrix in which the hormones are contained. Our results suggest that while urine does not affect the sorption of estrogens in soil it might either enhance their rate of desorption or more likely suppress their rate of microbial degradation allowing faster and more prolonged rates of vertical migration. Further studies of estrogen behaviour in soil should therefore use realistic matrices. To improve our mechanistic understanding of estrogen fate in soil, further work is required to separate out the direct and indirect effects of urine components on estrogen sorption/desorption reactions and

degradation pathways in soil. In addition, the influence of different cattle urine types on estrogen behaviour also needs investigation for inclusion into risk assessment models.

4.6 Acknowledgements

The authors would like to acknowledge the European Union, European Social Fund for financial assistance.

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CHAPTER 5

Literature Review - Nitrate and Turbidity in Groundwater

5.1 Groundwater

Groundwater is a vital natural resource the importance of which is frequently overlooked (Downing, 1998). When looking at the world's water balance, the importance of groundwater is clear. If the 94% of the world's water that is present in oceans and seas is ignored, groundwater accounts for about two thirds of the freshwater resources of the world. If the ice caps and glaciers are not included, then groundwater accounts for almost the total volume of utilisable water resources (Freeze and Cherry, 1979). Groundwater provides approximately a third of public water supplies in England and Wales. The total abstraction each year including use by industry and agriculture in the UK is 2400 million m^3 $year^{-1}$. The south-east of England has the highest percentage of groundwater use at over 70 % of total public supply (Downing, 1998).

Groundwater is largely located in aquifers; these are defined as a permeable rocks that store groundwater and allow it to flow readily through the pore spaces in the rock into a well or borehole (Downing, 1998). In the UK, the principle aquifers are the Chalk, the Permo-Triassic sandstones, the Jurassic limestones and the Lower Greensand (Figure 5.1). Of these, chalk is the most important providing 85% of the water that is pumped (Downing, 1998). The way that the water enters these aquifers is illustrated schematically in Figure 5.2. The rock type of the aquifer represents a major factor regulating the chemistry of the groundwater held within it. Further, the water chemistry at depth changes more slowly than at the surface and as a result of a series of often interrelated geochemical reactions. These reactions include mineral dissolution and precipitation, redox reactions, cation exchange, sorption and mixing (Edmunds et al., 2003).

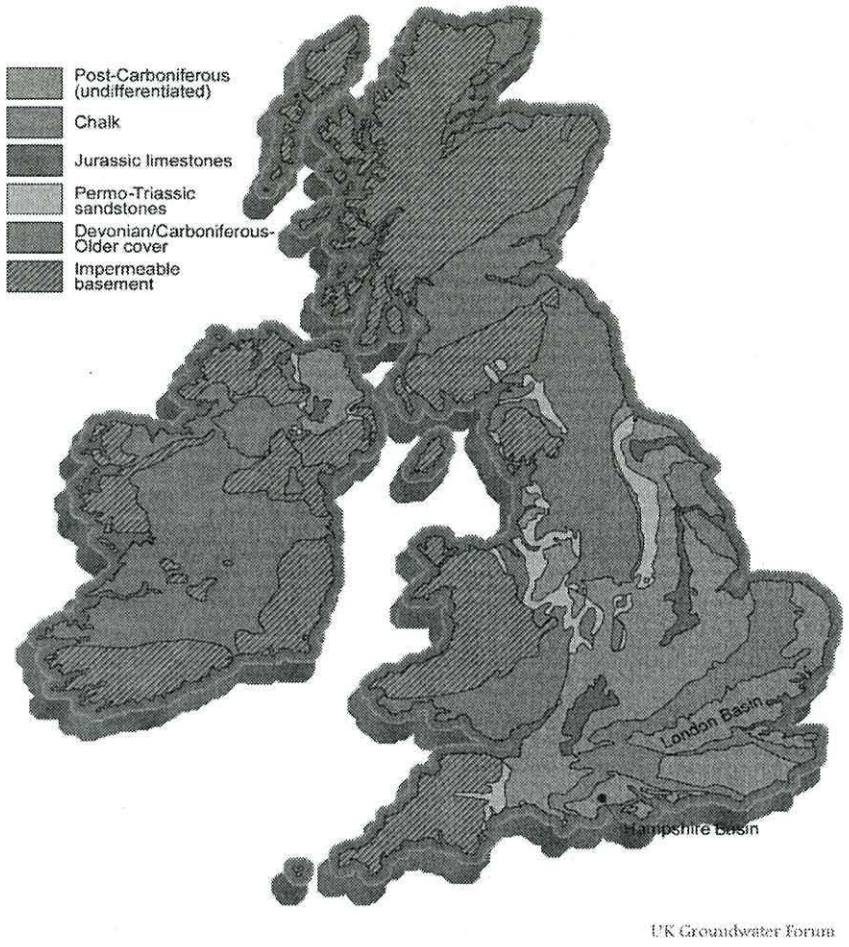

Figure 5.1: The aquifers of the British Isles (source: UK Groundwater Forum).

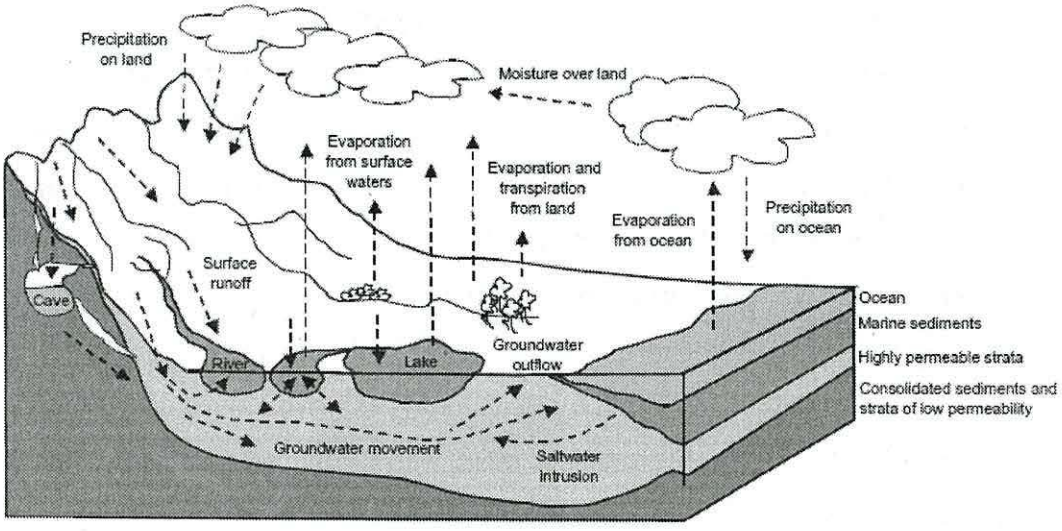

Figure 5.2: The hydrological cycle which regulates aquifer recharge (Danielopol et al., 2003).

Worldwide there are many different pressures and impacts on groundwater resources. Examples include, arsenic contamination of groundwater in some parts of Asia (Mukerjee et al., 2006; Harvey et al., 2005) and endocrine disrupting chemicals entering groundwater systems (Swartz et al., 2006; Ying et al., 2003). In the UK, the most commonly identified pollutant of groundwater is nitrate, which can potentially have deleterious effects on both the environment and human health (Freeze and Cherry, 1979).

5.2 Nitrate

5.2.1 Human health

The nature of groundwater recharge means that if it becomes contaminated it may take decades to recover (Knapp, 2005). As groundwater has such an important role in the water supplies of the nation its contamination may represent a significant threat to human health and the environment. Nitrate is the pollutant most commonly identified in groundwater (Freeze and Cherry, 1979). It can affect the environment and human health but its actual effects remain controversial. From a human health perspective there are two main concerns about nitrate in drinking water: methaemoglobinaemia and stomach cancer.

Methaemoglobinaemia (blue baby syndrome) is thought to arise when ingested nitrate impairs the oxygen carrying capacity of the blood in newborn infants (Heathwaite et al., 1993). However, the risk is very low with the last reported case in Britain being in 1972. There is also a link between stomach cancer and nitrogen containing compounds although this is not well understood and remains controversial. It is known that nitrosamines are carcinogenic but the link between nitrate ingestion and nitrosamine formation is less clear. Since nitrite is present in human saliva in concentrations in the range of 6–10 $\mu g\ g^{-1}$ and a variety of secondary and tertiary amines may be ingested in the form of food additives, pesticides residues and normal food constituents, there is a strong probability that some nitrosation must occur in the human stomach (Magee, 1982).

In contrast, Forman et al. (1985) measured the levels of nitrate and nitrite in the saliva of two populations who differed in their risk of developing gastric cancer. Surprisingly, they found that the levels of both ions were significantly higher in the low risk group. This is supported by Beresford (1985) who investigated the mortality data from 1969-1973 in 253 urban areas in relation to drinking water nitrate levels. They found no evidence of a positive association between nitrate in the drinking water and the risk of stomach cancer. In support of this, Wild (1977) also reported that nitrate ingestion does not

pose a serious risk to human health. A more recent review by Addiscott and Benjamin (2004) agreed that nitrate does not cause either methaemoglobinaemia or stomach cancer. They go as far to say that nitrate preserves health rather than threatens it. When nitrate is reduced by microbes on the tongue to nitrite, it reacts when acidified to form nitric oxide which is part of a vital anti-bacterial defence mechanism. This provides protection in the stomach against *Salmonella*, *Escherichia coli* and other organisms that cause gastroenteritis (Addiscott and Benjamin, 2004). The antibacterial properties of nitric oxide in saliva may also help explain the instinctive reaction of humans to lick wounds (Benjamin et al., 1997)

It has also been shown that nitric oxide is generated on normal human skin and it was suggested that this is being produced by the reduction of nitrate to nitrite, which is then acidified by acids in the skin. The production of nitric acid on the skin may provide a defence against skin infections especially those caused by fungi. Weller et al. (1998) found that nitrite acidified with an organic acid was an effective treatment for *Tinea pedis* (athletes foot). These examples all show that there is disagreement on the effects of nitrate on human health and therefore it is important that the public is not put in danger while the risks are assessed further.

5.2.2 Environmental effects

Whilst the effect of nitrate on human health remains controversial, there is clear evidence that nitrate does represent a significant environmental hazard. The contamination of ecosystems by nitrate is becoming increasingly widespread due to the increasing use of inorganic fertilisers, ploughing of old grassland and disposal of organic material (farmyard manure, slurry, sewage sludge) on or beneath the land surface (Burt and Trudgill, 1993). The main problem caused by nitrate is eutrophication. This is where a water body receives a large input of plant-available nutrients which can promote excessive plant growth in the water body. When the microbial community starts to degrade the inevitable plant necromass that is formed, the water body can become depleted of oxygen (Manahan, 2000). In freshwater ecosystems the limiting nutrient is usually phosphorous, however, in marine systems the most likely nutrient to be limiting is often nitrogen (and often co-limited by Fe) and so elevated concentrations of NH_4^+, NO_2^- and NO_3^- derived from human activities can stimulate or enhance the development of man-made eutrophication in coastal ecosystems (Camargo and Alonso, 2006). There is evidence of N limitation or co-limitation with P in upland lakes. Maberly et al. (2002) studied 30 upland lakes located in

Britain and found that over the whole season the percentage frequency of P, N and co-limitation was 24, 13 and 63 % respectively for rate of phytoplankton growth and 20, 22 and 58 % respectively for phytoplankton yield. This work was extended by investigating the types of land use where the N in the lake catchment originated from. They found that N leakage was linked to the proportion of rough grass and marsh and deciduous and mixed woodland present in the catchment (Maberly et al., 2003).

Withers and Lord (2002) reviewed the current state of groundwater in the UK. They found that typically, arable and managed grasslands in the UK receive on average 125 kg N ha^{-1} in inorganic fertilisers each year. In addition, inputs of manure must also be considered (e.g. deposited during grazing or applied as an organic fertiliser). Nitrate concentrations consistently exceeded the limit of 50 mg NO_3^- l^{-1} in 7 of 14 abstraction wells examined in a study in Birmingham (Shepherd et al., 2006) and in a study of chalk aquifer in Dorset mean nitrate concentrations have increased from 4.6 mg NO_3 l^{-1} to 28.2 mg NO_3^- l^{-1} (Limbrick, 2003). This problem is not restricted to the UK. Chae et al. (2004) found nitrate concentrations up to 218 mg N l^{-1} in a study of groundwater in an agricultural area of South Korea and Schilling et al. (2006) found nitrate concentrations up to 88 mg N l^{-1} in Iowa, USA.

One pathway by which pollutants may enter aquifers is though leakage from slurry contained in lagoons. Gooddy et al. (1998) drilled two boreholes to a depth of 35 m in a chalk aquifer beneath an unlined, earth-banked cow slurry lagoon. Figure 5.3 shows the nitrate concentrations down the profile of these boreholes. The depth profiles revealed high concentrations of nitrate migrating down to 30 m (in less than 20 years) and therefore storage of slurry in this way poses a potential threat to groundwater quality. These concentrations were well in excess of those normally found in the chalk in a control borehole. As well as nitrate, there are many other water quality issues that can arise from the storage of slurry in lagoons such as *Escherichia coli*, *Cryptosporidium*, high DOC and heavy metal concentrations (Gooddy et al., 2001).

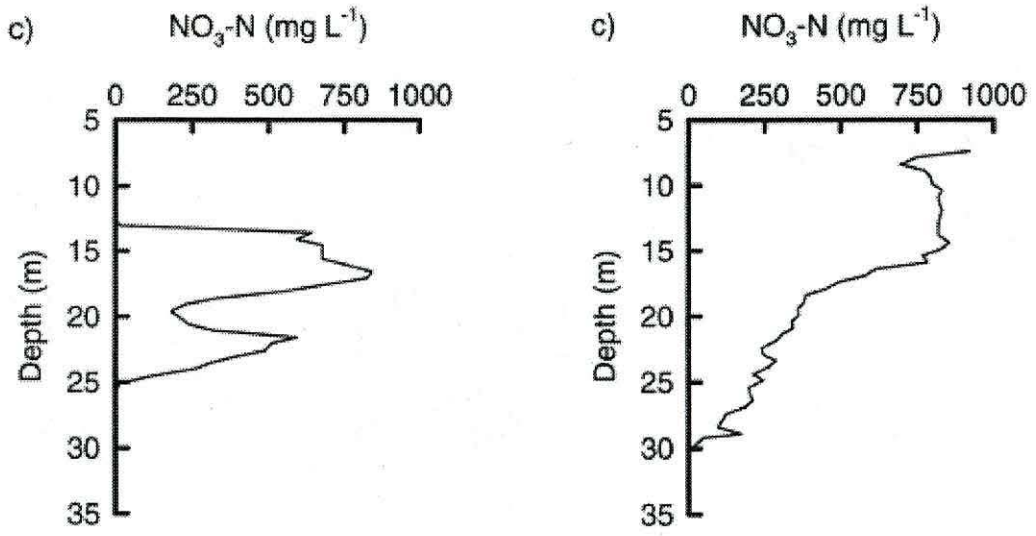

Figure 5.3: Concentration of nitrate in porewater in two boreholes drilled into an aquifer where there had previously been a cow slurry lagoon. On the left is borehole 1 and on the right is borehole 2 (Gooddy et al., 1998).

As well as the agricultural sources of nitrate there are also inputs from other sources. In urban areas the nitrate concentration can be similar or higher to those in the surrounding agricultural areas (Wakida and Lerner, 2005). Possible sources include sewage and mains leakage, septic tanks, industrial spillages, contaminated land, landfills, animal wastes, river or channel infiltration, fertilisers used in gardens, house building, storm water and direct recharge. A study in Birmingham found nitrate groundwater levels to range from 9 to 90 mg l^{-1} (Ford and Tellam, 1994). In Nottingham, the results of many studies were combined to estimate the total nitrogen loading to the aquifer. It was found in recent times to be 21 kg N ha^{-1} y^{-1} with the major components of this being contaminated land including landfills (38 %) and mains leakage (37 %) (Wakida and Lerner, 2005).

Nitrate levels in many lowland surface waters now exceed the EC drinking water limit of 50 mg NO_3 l^{-1} for at least part of the winter and many groundwaters in central and eastern England have nitrate concentrations that exceed or approach this limit. Yorkshire Water measured the nitrate levels in four of their boreholes over the last three decades and all show a clear increasing trend in nitrate concentration since 1997, especially in the chalk sources (Figure 5.4; Knapp, 2005).

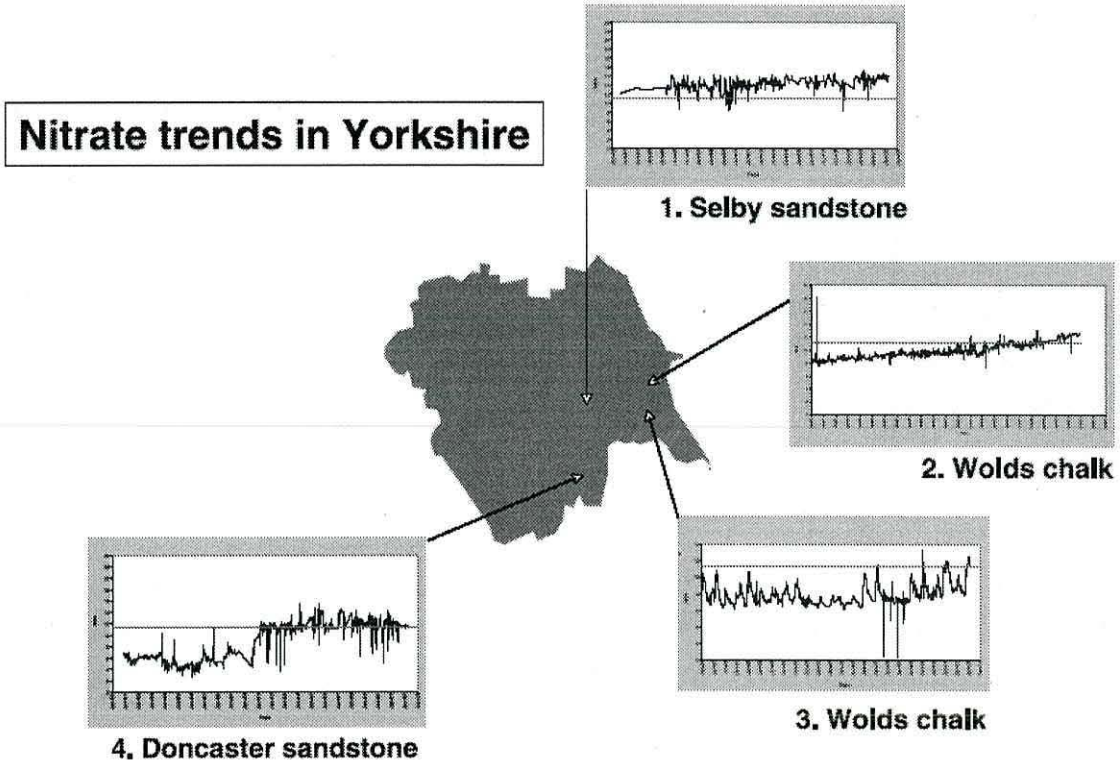

Figure 5.4: The changes in nitrate concentration in four boreholes located in Yorkshire from January 1980 to January 2004, the horizontal line in the graphs represents the EC nitrate limit (50 mg $NO_3^- l^{-1}$) (Knapp, 2005).

The nitrate dynamics in individual boreholes and aquifers is variable and depends on many factors including rainfall, soil type, topography, well depth and application of fertiliser (Rasiah et al., 2005). A study in Australia found that the nitrate concentration increased rapidly from early January, fluctuated from February to April and then decreased from May to June. The nitrate concentration was higher;

- During high rainfall seasons (increased leaching),
- In coarser soil than fine (increasing leaching),

and the nitrate concentration was lower;

- With increasing depth of water in the wells (dilution effect),
- With increasing well depth (retarded vertical transport).

Often nitrate is found in groundwater that is well oxygenated and in that situation it is relatively stable and mobile. Frequently, however, oxygen can be consumed and become limiting to aerobic respiration. This means that nitrate becomes the preferred electron acceptor and is reduced by nitrate-respiring micro-organisms (Smith et al., 1991). Since nitrate does not form precipitates or is adsorbed in significant quantities under aquifer

conditions the only realistic means for in-situ removal is by reduction (Appelo and Postma, 1993). The reduction of nitrate to N_2 (denitrification) was found to be the main nitrate reducing mechanism in a nitrate contaminated sand and gravel aquifer (Smith and Duff, 1988). Nitrate is reduced to N_2 by overall reactions of the type (Appelo and Postma, 1993):

$$2NO_3^- + 12H^+ + 10e^- \rightarrow N_2 + 6H_2O$$

The overall reaction has a transfer of five electrons per N atom and therefore proceeds via a complicated pathway with intermediates such as NO_2^-, NO, and N_2O. For substantial nitrate reduction to occur in aquifers the reduction potential must be present within the sediments such as organic matter. The reaction is bacterially catalysed and can be written as an overall reaction (Appelo and Postma, 1993):

$$5CH_2O + 4NO_3^- \rightarrow 2N_2 + 4HCO_3^- + CO_2 + 3H_2O$$

The Water Framework Directive (WFD) requires the achievement or maintenance of good ecological status and therefore involves the preservation of good quality water as one factor, this can be achieved through the adoption of river basin management plans (Withers and Lord, 2002). As well as the Water Framework Directive there are directives relevant to nutrient pollution, which include the Nitrates Directive targeted at drinking water and the Integrated Pollution Prevention and Control Directive targeted at large livestock enterprises (Withers et al., 2000). The objectives are to:

a) prevent further deterioration, protect and enhance the status of aquatic ecosystems and the water need of terrestrial and wetland ecosystems;
b) promote sustainable water use based on the long-term protection of available water resources;
c) enhance protection and improvement of the aquatic environment;
d) ensure the progressive reduction of pollution of groundwater;
e) contribute to mitigating the effects of floods and droughts (Wilby et al., 2006).

When looking at nitrate in UK groundwaters, a vulnerability concept and methodology was developed which was designed to identify vulnerable aquifers (Holman et al., 2005). The

permeability of the aquifer, the presence of surface drift deposits and the presence and nature of the overlying soil were all assessed. Data from boreholes all over the country was used to develop the method, with the concentrations of nitrate in these boreholes shown in Figure 5.5.

This method of groundwater vulnerability assessment is based upon classification

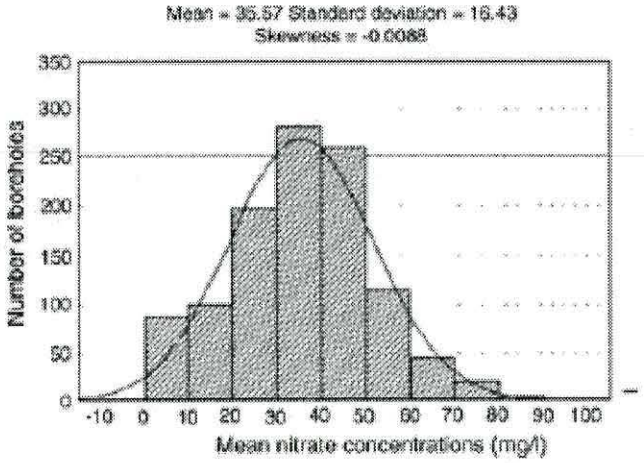

Figure 5.5: The distribution of the mean nitrate concentration (mg N l^{-1}) in 1108 boreholes in the Environment Agency's EC Nitrate Database (Holman et al., 2005)

of intrinsic properties of combined soil and aquifer layers. This leads to problems as a single pollutant such as nitrate can vary temporally as well as spatially. Therefore, the presence of nitrate in groundwater will also depend on the amount and timing of the surface loadings, soil nitrate levels and climatic factors such as rainfall. The authors (Holman et al., 2005) suggest that the next generation of groundwater protection tools should focus more on risk-based approaches with dynamic factors such as climate and pollutant loading integrated with the intrinsic characteristics of the aquifer.

5.3 Methods of Nitrate Analysis

There are several different methods of detecting nitrate in the many different matrices that it can occur. Moorcroft et al. (2001) and Sah (1994) reviewed these methods and identified four main techniques.

1. Capillary electrophoresis
2. Electrochemical detection
3. Chromatography

4. Spectroscopic detection.

Capillary electrophoresis is a useful method because it allows the fast simultaneous detection of a wide variety of anions. Other advantages include small sample sizes and low buffer consumption. It involves the separation of compounds in a narrow tube, driven by an electric field and then the detection of the cations and anions present using UV or fluorescence detection (Moorcroft et al., 2001).

A wide variety of electrode substrates have been investigated including copper, nickel, copper-nickel alloys, cadmium, platinum, gold and silver. Most commercially available nitrate-selective electrodes are based upon a membrane having a quarternary ammonium salt sensor trapped in an inert polymer matrix such as polyvinyl chloride. Trapped sensor molecules can diffuse out of the matrix which can reduce the lifetime of the electrode, making them unstable and in need of frequent calibration (Le Goff et al., 2002).

Chromatography techniques carried out are usually HPLC or ion chromatography based. End column detection systems include UV, fluorimetric, electron capture, electrochemical and mass spectroscopic analysis of the eluent (Moorcroft et al., 2001).

Spectroscopic techniques are the most widely used for nitrate and include UV/Vis, chemiluminescence, fluorimetric, IR, Raman and molecular cavity emission. These are discussed in detail in sections 5.3.1, 5.3.2 and 5.3.3 (Moorcroft et al., 2001).

5.3.1 Spectroscopic detection - Infrared analysis

The infrared region (IR) of the electromagnetic spectrum includes wavelengths from 0.8 μm to 1000 μm. Within this range there are three different regions. Near IR meets the visible region at 0.8 μm to about 2.5 μm. The mid infrared region is from 2.5 μm to 15 μm. Within this region there is a group frequency region and a fingerprint region. 15 μm to 1000 μm is the far-infrared region (Willard et al., 1988).

Infrared spectroscopy utilises the twisting, bending, rotation and vibrational motions of atoms in a molecule. The unique combination of these vibrations produces a highly complex absorption spectrum that is characteristic of the functional groups that make up the molecule and the molecule itself (Willard et al., 1988).

Infrared spectroscopy is most commonly used as a tool in the identification of organic compounds (Silverstein and Webster, 1998), however, the infrared spectra of inorganic materials has been well documented (Goulden and Manning, 1967; Miller and Wilkins, 1952). Goulden and Manning (1967) looked at the infrared spectra of inorganic

materials in aqueous solutions. Using aqueous solutions removes the problems associated with using powders and as the absorption bands for free ions appear over very limited frequency ranges compared with the broad ranges for solid state spectra, structural inorganic analysis is simplified.

For structural analysis, ion concentrations of the order of 0.2 M must be used. The composition of the cell material is an important consideration when using aqueous solutions. Calcium fluoride is probably the best material and also Irtran-2. The absorption cells that are used usually have a pathlength of about 50 μm. However, when aqueous solutions are measured there is a problem with the high absorbance of water, which masks the spectra of the ions. The transmission of IR through water with a pathlength of 50 μm is only about 10% (Manning, 1972) and so this is usually the maximum path length used when analysing water samples. The infrared spectra produced are commonly shown graphically as absorbance against wavelength (μm) or absorbance against wavenumber (cm^{-1}). Wavenumbers are reciprocally related to wavelength; $cm^{-1} = 10^4/\mu m$ (Silverstein and Webster, 1998).

A typical nitrate spectrum is shown in Figure 5.6. This spectrum was produced using solid sodium nitrate in a Nujol® mull. Nujol® is as an inert carrier agent which the solid sodium nitrate has been ground in, this allows the infrared radiation to pass through the solid sample (Harwood et al, 1999). The asterisked peaks are the ones caused by

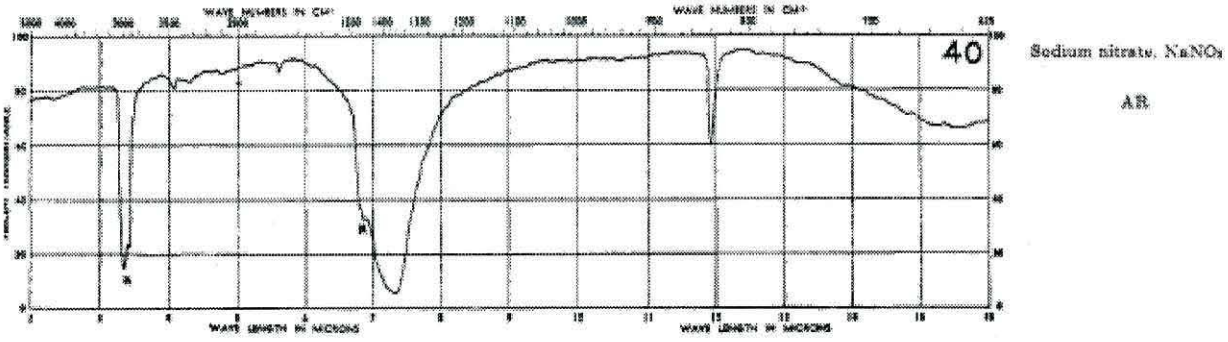

Figure 5.6: The IR spectra of sodium nitrate measured using a Nujol® mull (Miller and Wilkins, 1952).

Nujol®. For comparison, Figure 5.7 shows the spectrum of solid sodium nitrate produced using a KBr disk. Figure 5.8 shows the spectrum of nitrate using 0.5 and 5 M solutions of sodium nitrate in water. All three spectra show the peak at approx. 1400 cm^{-1} (7 μm), which could be used for the identification of nitrate.

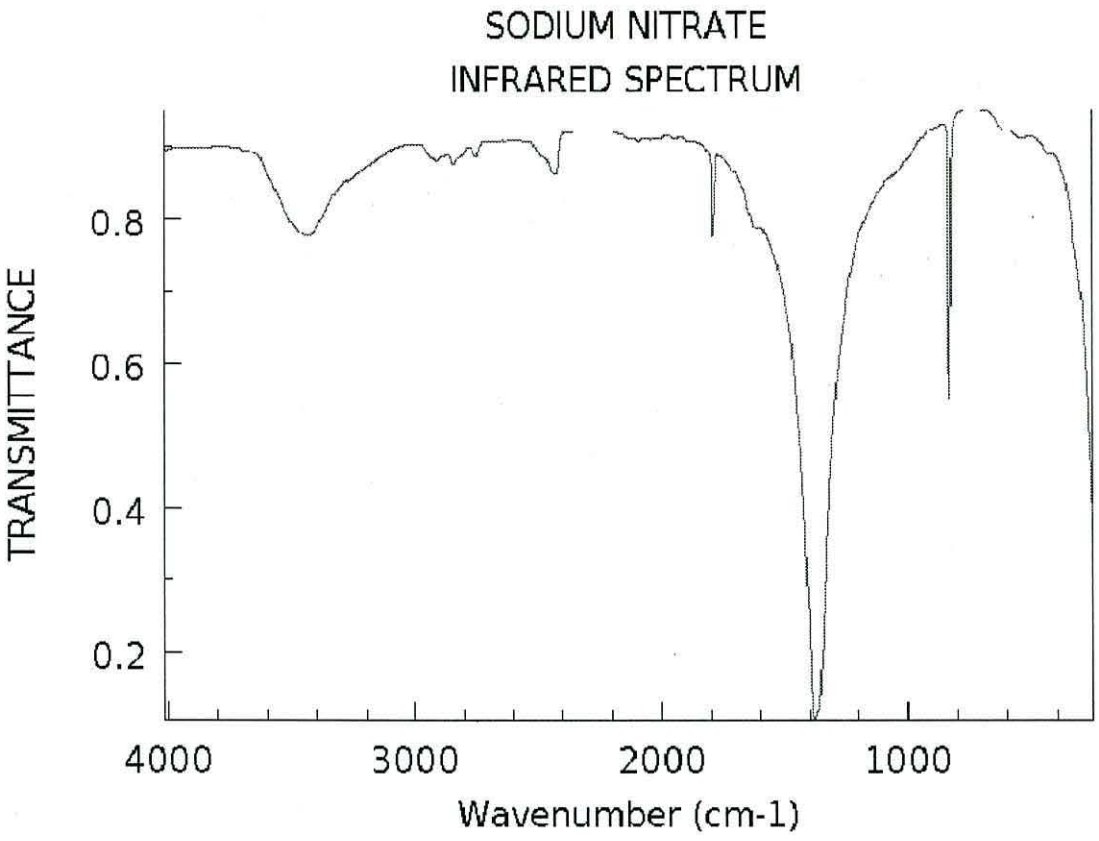

Figure 5.7: Infrared spectrum of solid sodium nitrate (NIST Chemistry WebBook (Source: http://webbook.nist.gov/chemistry)).

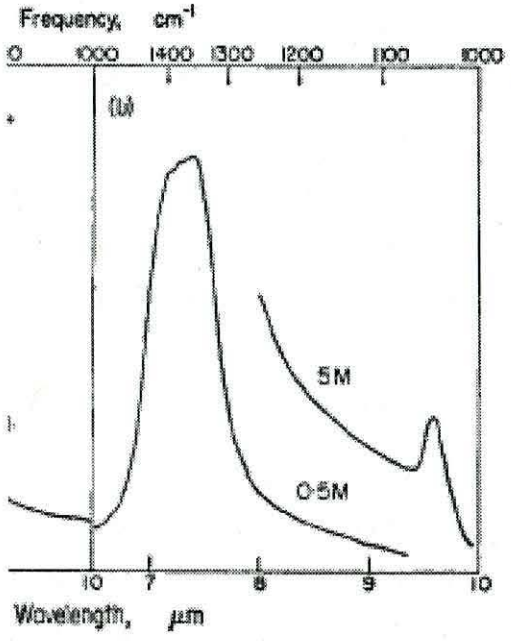

Figure 5.8: The absorbance of 5 M and 0.5 M solutions of nitrate (Goulden and Manning, 1967).

5.3.2 Spectroscopic detection - Near IR

Near IR is also used in various applications for detecting different compounds in the environment. Near IR is a non contact measurement method to provide rapid information about some soil physical and chemical properties including; organic matter content, nitrogen, potassium, phosphorous, pH, soil moisture content, particle size and mineral composition of the soil (Mouazen et al., 2005). It works on the basis that the characteristics of reflected light from the soil surface are different depending on the physical and chemical properties of it. When light is illuminated towards the soil surface, the radiant energy is distributed through three different processes; reflection, absorbance and transmission. As transmission in soils equals zero, the balance between reflection and absorbance is governed by the chemical and physical properties of the soil. These properties determine the colour and roughness of the soil surface, influencing the amount of light reflection and/or absorbance.

Adamchuk et al. (2004) examined the use of both mid and near IR to measure the concentration of different soil properties. They stated that it was possible to use near IR and mid IR for the measurement of organic matter, moisture, nitrate and CEC in soils. Eshani et al. (1999) found that NIR reflectance spectra in the range of 1100 to 2500 nm were useful in determining soil moisture, organic carbon and nitrogen.

The actual wavelengths used for nitrogen are 1702, 1870 and 2052 nm. The free nitrate ion has D_{3h} symmetry (Vogt and Finlayson-Pitts, 1994) and so this gives 4 vibration modes in the IR region. These are symmetric stretch, ν_1, at 9524 nm, out of plane bending, ν_2 at 12034 nm, anti-symmetric stretch, ν_3 at 7194 nm and in-plane bending ν_4 at 13889 nm. Of these four, ν_1 is IR inactive and so usually very weak while ν_3, the anti-symmetric stretch, is usually very strong. However, these peaks will all occur in the mid IR region. In the near IR region from 1800 to 2300 nm the following peaks are likely to occur; second overtone of $(\nu_1 + \nu_3)$, $(\nu_2 + \nu_3)$, $(\nu_1 + \nu_3 + \nu_4)$ and third overtones of $(\nu_1 + \nu_2)$, $(\nu_1 + \nu_4)$, $(\nu_2 + \nu_4)$ (Eshani et al., 1999).

To test the potential for near IR, Eshani et al. (1999), used two different soils for a laboratory test with three sources of nitrogen; ammonium sulphate, calcium nitrate and ammonium nitrate. 660 samples were analysed using NIR reflectance from 1100 nm to 2500 nm. The results from this were very positive with all the samples having a good correlation with the concentration of fertiliser applied and a standard error of 6 mg kg^{-1} or less. Further, this technique would still work if there were interferences in the sample providing that the calibration samples also included these interferences.

Fox et al. (1993) studied the use of near IR to determine the amount of available nitrogen in soil. Sieved dry soil from soil cores was used in this experiment and various methods of nitrogen analysis were compared including near IR and UV analysis. In this case it was found that both near IR and UV methods offered alternatives to electrode methods. The advantages of using near IR were that no sample preparation was required beyond drying and sieving the soil sample and there were a number of commercial laboratories that could carry out the analysis with existing equipment.

Near IR has also been used for the detection of moisture in soils. Bowers and Hanks (1965) looked at the effects on absorbance and reflectance of near IR of surface moisture content, organic matter and particle size. At all wavelengths measured, on all of the samples, reflectance decreased and absorbance increased as moisture content increased. A method was also developed by Stafford et al. (1989a, 1989b) to build a detector for moisture levels in the field using a portable meter. It was used for the measurement of moisture in cut samples of grass. A linear relationship between grass moisture content and reflectance occurred at 1450 nm. The instrument operated satisfactorily over a range of 15 – 85% moisture content based on wet weight with the greatest accuracy between 40 – 80% moisture content. The use of NIR to measure moisture content in soils has been looked at more recently as well (Hummel et al., 2001; Mouazen et al., 2005).

Mouazen et al. (2005) developed an in-situ soil moisture content sensor. This sensor was attached to a subsoiler chisel backside to detect the light reflectance from the trench bottom side opened by the chisel. Fibre optics were used and the whole spectrophotometer was fast, precise and robust making it suitable to be permanently aligned on mobile machines.

Hummel et al. (2001) developed a non in-situ sensor for soil moisture content and organic matter determination for surface and sub-surface soils. Soil cores were used in the laboratory as this was not a portable sensor, however, undisturbed cores were used and so this was a step towards developing an in-situ sensor. The use of Near IR to develop a soil organic matter sensor was also studied by Coûteaux et al. (2003). They were using Near IR reflectance spectroscopy to determine labile compounds and microbial biomass containing C and N. The authors found that near IR worked well and gave good correlation with predicted results from previous experiments. Another application of near IR was found by McLellan et al. (1991a, 1991b) who used it to successfully measure the nitrogen, lignin and cellulose concentrations in green and senescent foliage. It was found that near IR

analysis produced errors considerably lower than those associated with wet chemistry methods and concluded that it was a viable alternative to wet chemistry methods.

The versatility of near IR was also shown by Goulden (1957) who used the technique to study dairy products such as butter, milk and cream. Near IR was useful to assess the globule and particle size of milk and butter and therefore to see the changes that occurred during homogenisation.

5.3.3 Spectroscopic detection- UV spectrophotometry

The use of UV spectrophotometry to measure the concentration of nitrate ions in solution has been developed progressively over time. One of the earlier reports, (Buck et al., 1954), showed the absorption spectra of different inorganic ions in aqueous solution. Sodium nitrate was used to produce a spectrum of the nitrate ion in the range of 210 nm to 400 nm. Many other anions were also measured including; sulphate, thiosulphate, nitrite, chlorate, bromate, borate and phosphate. Of these, only nitrate, nitrite and thiosulphate showed characteristic absorption maxima in the wavelength range studied. Nitrate had a peak maximum at 198 nm and there was also a peak present at 302 nm with low intensity (Buck et al., 1954; Meyerstein et al., 1961).

5.4 UV spectrophotometry

5.4.1 Detection of nitrate using UV spectrophotometry

The absorption of ultraviolet or visible radiation by a molecular species (M) can be considered to be a two-step process, the first of which involves excitation by a photon $h\nu$ as shown by the equation (Skoog, 1985),

$$M + h\nu \rightarrow M^*$$

The product of the reaction is an electronically excited particle (M*). The excited particle only exists for a very short amount of time (10^{-8} to 10^{-9} sec) with its existence being terminated by any of several relaxation processes. The most common type of relaxation involves conversion of the excitation energy to heat,

$$M^* \rightarrow M + \text{heat}$$

Relaxation may also occur by decomposition to form a new species, a photochemical reaction, or may involve fluorescence or phosphorescence. In the case of nitrate, the energy

is lost as heat. The lifetime of M* is so short that its concentration at any instant is normally negligible and the heat released during relaxation is not usually detectable so these measurements of absorption cause minimal disturbance to the system that is being studied (Skoog, 1985). As the absorption usually arises from the excitation of bonding electrons the wavelengths of the absorption peaks can be correlated with types of bonds that exist in the molecule. In a molecule there are different electronic energy levels as shown in Figure 5.9.

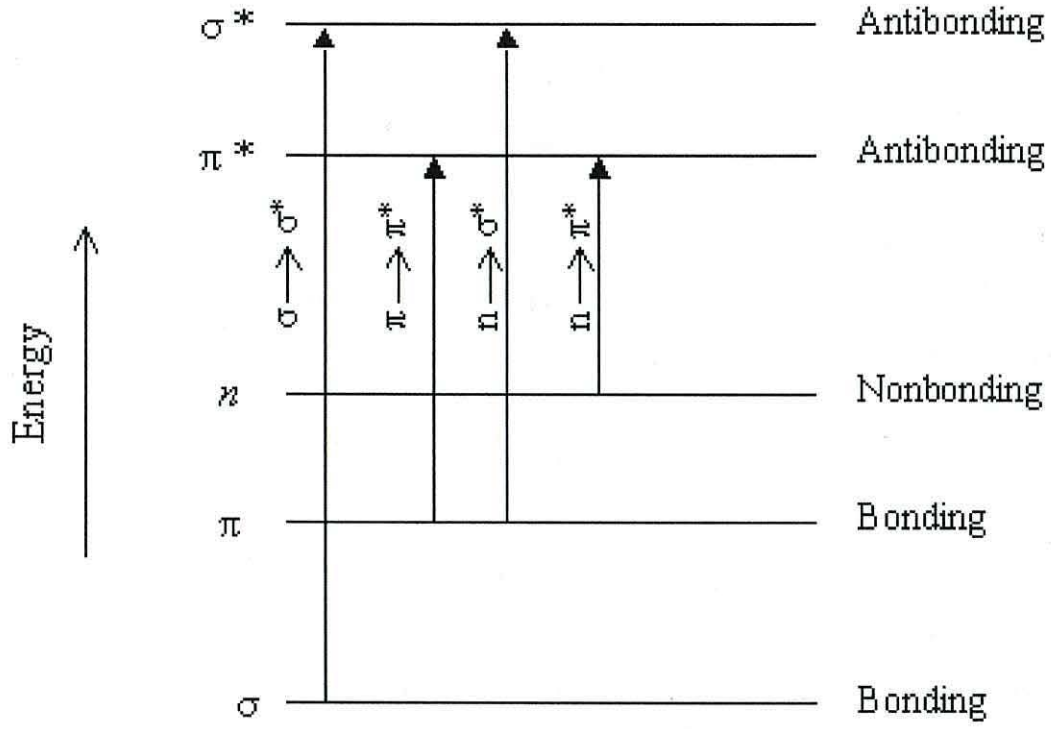

Figure 5.9: The electronic molecular energy levels and transitions that can occur between these levels during absorption of radiation.

The energy required for the $\sigma \rightarrow \sigma^*$ transition is relatively high and so the wavelengths that this transition occurs at are shorter than 185 nm. This makes the transitions very difficult to observe using normal UV spectrophotometry equipment as atmospheric gases also absorb at this wavelength (Skoog, 1985).

In nitrate the most commonly observed transition is the $\pi \rightarrow \pi^*$ transition. The transition occurs at around 200 nm (Tomišić et al., 2005) and generally has a relatively high molar absorbtivity. There is a $n \rightarrow \pi^*$ transition that occurs at approximately 300 nm, this transition has a relatively lower molar absorbtivity and so is only observed when high concentrations of nitrate are used (Fig. 5.10). The concentrations shown in Figure 5.10 were converted from mol dm^{-3} KNO_3 to mg l^{-1} of NO_3^- so the concentration in *plate a* was

5 mg l^{-1} and in *plate b* was 3370 mg l^{-1} and so shows the high concentration of nitrate required to observe the $n \rightarrow \pi^*$ transition.

There are several laws that govern the adsorption of light through a solution. If the initial intensity of light is described as I_0 and the intensity of light after travelling through the solution is I_1, then transmittance (T) can be described as:

$$T = I_1 / I_0$$

The relationship between absorbance (A) and transmittance (T) is a logarithmic one and can be described as:

$$A = \log_{10} I / T = -\log T$$

or

$$A = \log_{10} I_0 / I_1 = -\log I_1 / I_0$$

There is a logarithmic relationship between the transmission of light through an increasing pathlength. This is the basis for Lamberts Law where for a solution of a given concentration at a given wavelength:

$$A = a \times l = -\log T$$

(where a = absorptivity of the liquid (a constant) and l is the path length)

A similar relationship can be shown to occur for concentration versus absorption or transmittance and this forms the basis of Beer's Law:

$$A = a \times c = -\log T$$

(where c = concentration of the absorbing material)

The Beer-Lambert Law is a combination of the Beer's Law and Lambert's Law and can be described as:

$$A = a \times l \times c = -\log T$$

If the concentration is expressed in mol l^{-1} and the path length in centimetres, then the absorptivity is described as the molar extinction coefficient (ε). Therefore for a given sample where ε and pathlength are constant, absorption is linearly related to the concentration (Willard et al., 1998).

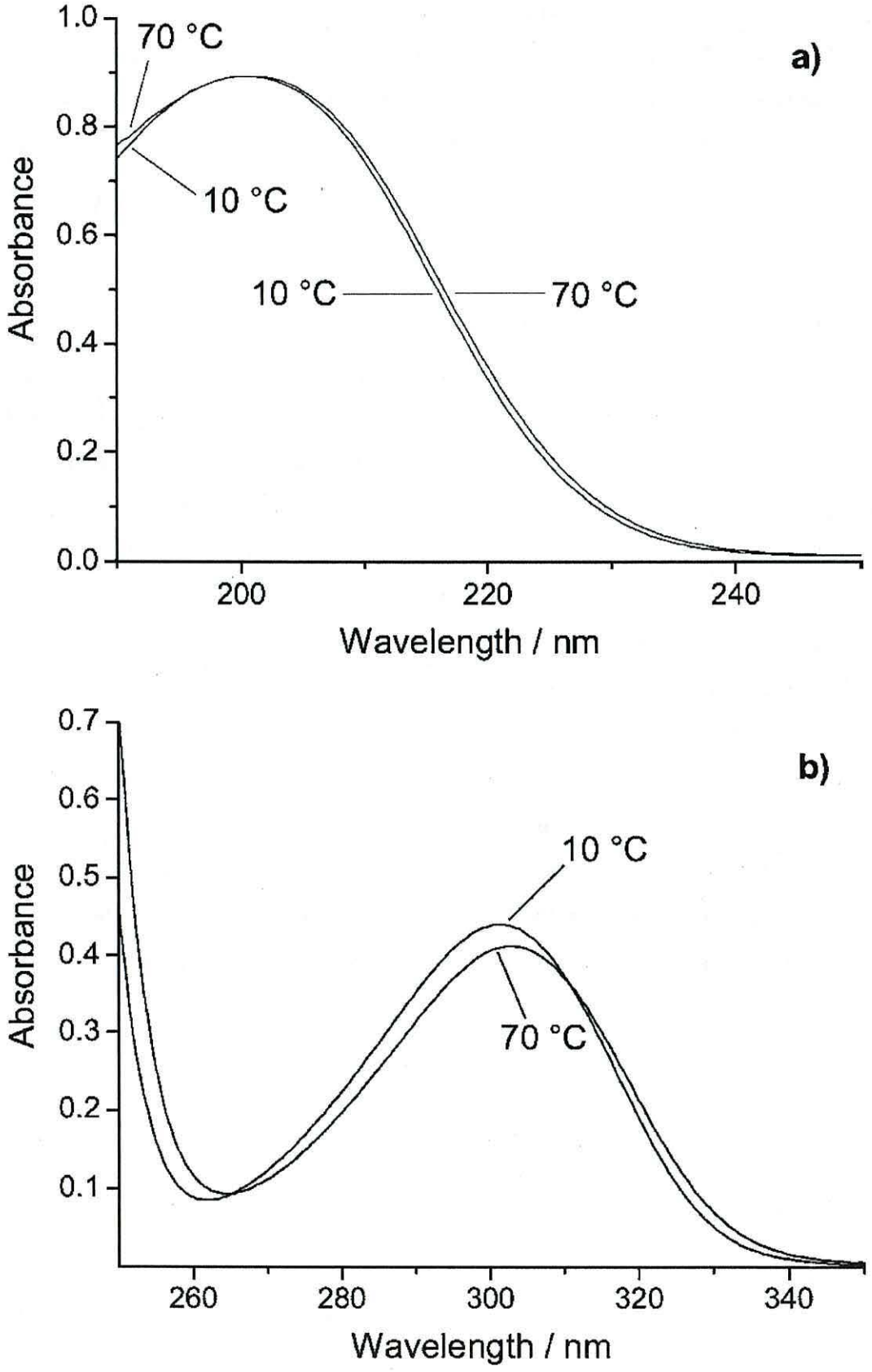

Figure 5.10: The UV absorbance of nitrate ($KNO_{3\,(aq)}$) at two different temperatures using cuvettes with a pathlength of 1 cm. (a) $\pi \rightarrow \pi^*$ transition, concentration = 8.9 x 10^{-5} mol dm^{-3} (b) $n \rightarrow \pi^*$, concentration = 0.06 mol dm^{-3} (Tomišić et al., 2005).

5.4.2 UV spectrophotometry of seawater

The use of UV spectrophotometry in environmental analysis has gradually improved over the years. In 1966 a report by Ogura and Hanya (1966) was published about the nature of ultraviolet absorption of seawater. Samples of seawater were collected from the western North Pacific and Sagami Nada, filtered and stored in the cold. UV absorption was measured between 210 and 300 nm with a 1 cm cuvette. This study found that in the wavelength range from 210 and 230 nm the absorption of seawater could be mostly attributed to organic matter, nitrate and bromide.

The use of UV spectrophotometry has been further refined over the years. Foster and Morris (1971) looked at the use of UV measurements for the quantification of pollutants in inshore waters. Seawater samples were collected from the Menai Straits, filtered and the spectra compared with the absorbances from nearby rivers. Smooth absorption spectra were obtained (Figure 5.11) over the wavelength range selected except for a nitrate peak in the seawater sample. The smooth shape was obtained from the diverse nature and low individual concentrations of the organic compounds, which comprise the total DOC of natural waters. The relatively large amount of DOC in rivers in comparison with seawater is reflected in these spectra. This method was used to look at the long-term changes in the nitrate concentration using a wavelength of 225 nm. It was found that the absorption fell rapidly from March to May during the spring burst of phytoplankton growth. This seasonal variation in nitrate concentration found from the absorbance measurements closely follows the actual nitrate measurements for the same site made by Ewins and Spencer (1967), which shows the validity of the method.

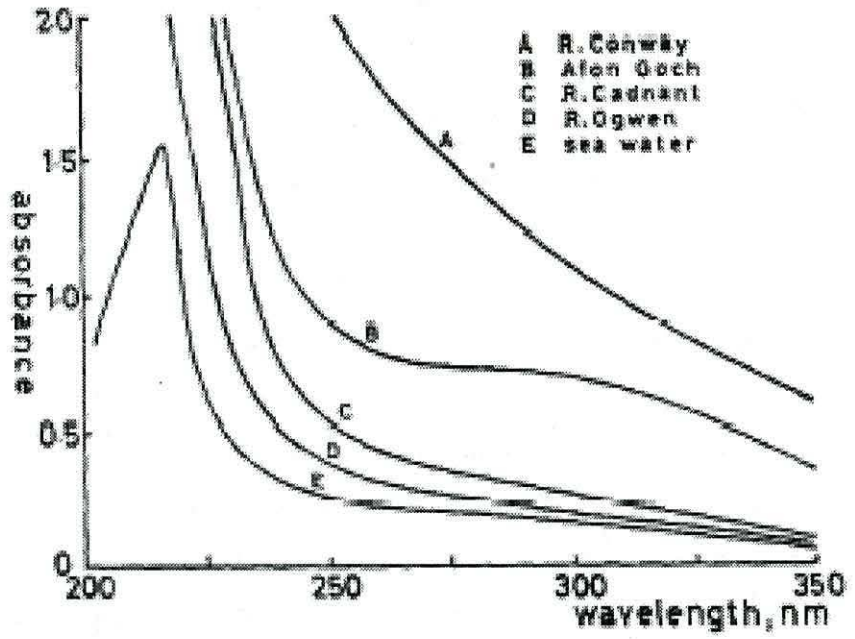

FIG. 1. Typical u.v. absorption spectra of river waters and of sea water.

Figure 5.11: The spectra of seawater, and different river waters in North Wales (Foster and Morris, 1971).

Finch et al. (1998) developed the method even further. In this study, the authors wanted to produce a self contained monitoring system which would allow for towed or lowered oceanic deployment in addition to long term unattended deployment using a battery power source. They also wanted the sensor to have a rapid response time (<1 s) to enable a resolution of 1 m when lowered through seawater vertically and a resolution of ~5 m on an undulating towed vehicle. They tested many different components for use in the sensor and decided on the following specification.

- Light sources – A low overall power was needed as the instrument was required to be capable of battery operation over extended periods. A xenon flashlamp was selected as it had an excellent UV output coupled with low power consumption.
- UV prefilters – Most detectors are several orders of magnitude more sensitive to visible and IR light than they are to UV. Two dichromatic filters were used to prevent stray scattered visible light from reaching the detectors.
- Lenses – Required for collimation of the light from the source and for imaging the spectrum upon the detectors. UV-grade fused silica lenses with a transmission of roughly 90 % at 200 nm were selected.

- Windows – fused silica windows with a thickness of 3 mm and a diameter of 25 mm were used in the prototype.
- Spectrometer – A 1800 mm^{-1} holographic grating spectrometer was used to disperse the UV light onto several output plane apertures to select the operational wavelengths. The advantages were that it has low light-loss, flexibility and cheapness in comparison to multiple filter systems.
- Detectors – A number of detector types including UV- enhanced silicon photodiodes, flame sensors and photomultipliers were initially tested. For the boxed prototype, discrete photodiode detectors were employed in conjunction with FET transimpedance amplifiers. A lock–in amplifier was used to monitor the detected energies in three spectral bands. For the fully submersible version, a linear photodiode array was used in conjunction with an imaging lens used to give spectral intervals of 20 nm at the photodiode spacing. Specially designed low-power electronic circuits then integrated the photodiode output current pulses, converting them to voltages proportional to the detected energies in the three spectral bands.
- Electronic systems – The boxed prototype was operated using standard laboratory units for power supply, control of flash rate and for lock-in detection of the transimpedance amplifier output signals. For the fully submersible version, compact low-power electronic circuits were designed to take the place of these units.
- Mechanical arrangement – The fully submersible prototype version had a rugged aluminium housing, rated to 50 MPa external pressure.

The layout of the prototype sensor is shown in Figure 5.12 (Finch et al., 1998). A three-wavelength operation was chosen to provide:

- A measurement channel at a wavelength where both nitrate and halide have significant absorption,
- A channel close to wavelength (1) where absorption due to halide is very small, and
- A reference channel that is primarily affected by obscuring particles and changes in the intensity of the light source, allowing normalization of the two channels for these variations.

The prototype method was compared with a NED/sulphanilamide chromophore method for nitrate analysis. The results showed that there was an acceptable correlation ($P < 0.05$) between the measured nitrate and the peak height derived from the UV absorbance signal. The differences found between the two techniques were thought to be due to absorbance by other seawater ions. From these data it was decided that there was enough evidence to indicate that there were worthwhile grounds for further development of a fully working sensor. The submersible sensor was calibrated using seawater or distilled water with aliquots of 50 and 100 µl of a 10 mM nitrate solution. The response to a range of salinities was also tested, as was the temperature stability at room temperature, 20 °C and 5 °C. Calibration of the nitrate sensor was derived from measuring the changes in absorbance produced by known concentrations of nitrate and using the method of least squares to derive a line of best fit. The line of best fit was found to be a second order equation with a between run variation of approximately 3 %. Therefore, during normal use, the sensor would require calibration between each new run. This appeared to be related to the "zero voltage line", the output voltage value of the sensor at the start of each run. The absorbance (A) is calculated using the formula

$$A = \ln V_0/V$$

where V_0 is the initial voltage and V is the voltage after each addition of nitrate. This means that the calculation is directly reliant on this initial voltage value and if this varies then so does the calibration line.

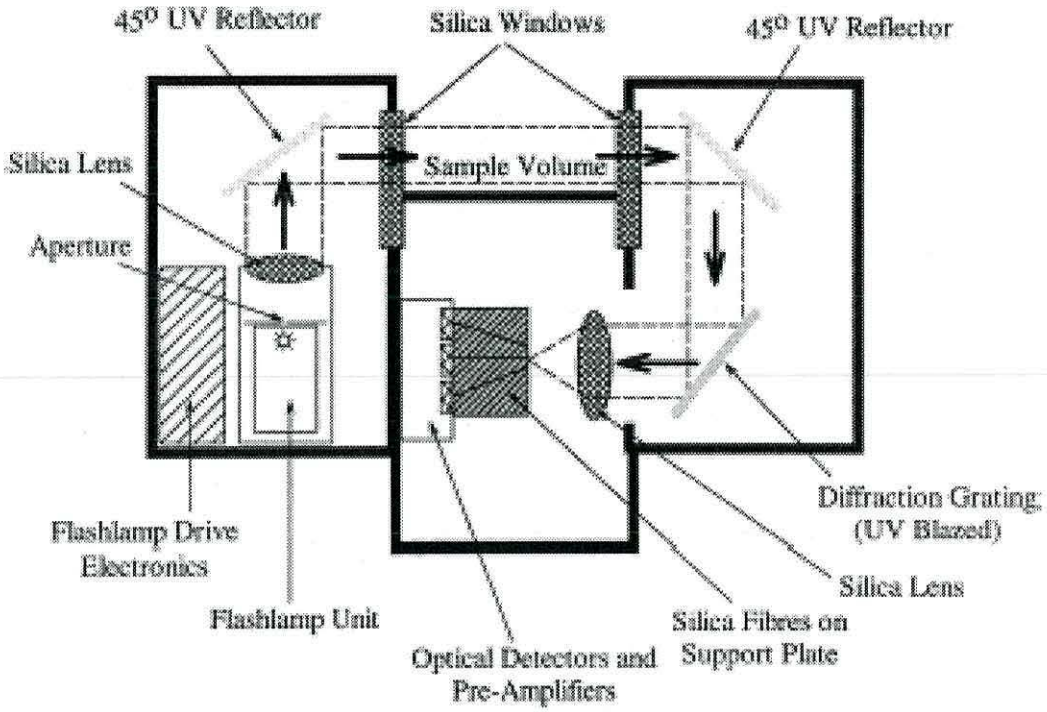

Fig. 2. The Mark I ultra violet nitrate sensor.

Figure 5.12: The design of the sensor produced by Finch et al. (1998).

During a cruise, the UV sensor was deployed and gave good correlation with the alternative methods of nitrate measurements. The graph produced from the comparison of the two values gave an r^2 value of 0.99 for the line of best fit. It also had a small y-axis intercept (-0.151) suggesting minimal interference from components that are not changing in parallel to the changes in the concentration of nitrate. Overall, it was concluded that UV has the potential for a rapid response time sensor for the measurement of dissolved nitrate in the marine environment.

Johnson and Coletti (2002) also developed an in-situ UV sensor for the measurement of nitrate as well as bromide and bisulfide. They produced an instrument capable of working throughout the ocean and freshwaters, on conductivity temperature depth profilers, undulating towed vehicles, remotely operated vehicles or on deep sea moorings. Their instrument was composed of four key components, the UV light source, an optically coupled sensing probe, the high resolution spectrometer and a low power instrument controller with a large amount of data storage.

5.4.3 UV spectrophotometry of freshwater

The determination of nitrate in freshwater using UV has also been investigated. One of the earliest studies was by Hoather and Rackham (1959). The units used at this time were angstroms (Å) instead of nm and so they measured the absorption of nitrate at 2100 Å (i.e. 210 nm). It was shown that there was no significant interference from saline constituents present in ordinary natural fresh water. The major problem with the method was the interferences from dissolved organic carbon (DOC). For well waters this was negligible but for many river waters and sewage effluents a correction factor needed to be applied. This relied on the empirical basis that the absorption due to organic matter at 210 nm was four times as great as that at 275 nm. The latter wavelength was suitable because the absorption due to nitrate was extremely low. Hoather and Rackham (1959) looked at the absorption of nitrate in different types of water. In well water the agreement between the UV method and accepted chemical methods was high for hundreds of well water samples. There were about 50 anomalous results where the nitrate calculated from the UV method was higher than that calculated chemically. The results were attributed to abnormal seasonal conditions, nitrate being added to underground water when percolation starts after autumn rainfall.

A study by Rennie et al. (1979) also looked at the interferences that can occur in the measurement due to dissolved organic matter (DOM). They identified several solutions to the problem. One was to use the absorbencies at 210 nm and 275 nm and then apply a correction factor as mentioned before. The authors did not ascribe to this method, however, as they felt the correction factor did not work well when the nitrate concentration was low and the corresponding DOM concentration was high. Failures of the correction factor approach arose from the variability of the different organic compounds likely to be present in different types of water. Three other methods have also been proposed, these involve the chemical reduction of the nitrate ion, the coagulation of organic matter with aluminium sulphate and the adsorption of organic matter by activated carbon. Rennie et al. (1979) chose a variation of the activated carbon method. A filtration apparatus was set up consisting of 16 layers of 60 mm diameter active carbon papers sandwiched between two 60 mm diameter GF/C papers in a holder used in conjunction with a suitable vacuum source and a Büchner flask. This UV method was compared with accepted chemical methods between different laboratories and was generally found to be precise with good correlation with the results obtained by the automated method.

Thomas and Theraulaz (1994) proposed the use of UV spectrometry together with conductimetry to investigate the mineral and organic composition of water instantaneously to allow for representative sampling to take place. Electrical conductivity (EC) is related to the mineral content of the water and UV to DOC, suspended solids and nitrate. These authors were looking at the data qualitatively and found that information could be obtained for nitrates at 200 to 230 nm while 210 to 300 nm could be used for organic matter.

The problem of interferences caused by dissolved organic carbon (DOC) was investigated in depth by Edwards et al. (2001). Edwards et al. (2001) used KNO_3 to make up standard solutions of nitrate and polymaleic acid to make up DOC standards. Water samples were also collected and analysed using the standard chemical methods and by the UV method that had been developed. The absorption spectra of the nitrate and polymaleic acid both had their peak maximum at 200 nm. The organic matter spectrum showed a gradual decrease in absorption in the 200 to 300 nm range while the nitrate absorption decreased sharply beyond 210 nm. From the results it was decided that the nitrate measurement should be taken at 205 nm. Also it was advised that a measurement should be taken at a higher wavelength where nitrate does not absorb, but DOC does. Subsequently, calculation of the amount of absorption at 205 nm due to DOC could be described in a similar way to Hoather and Rackham (1959). The authors developed a relationship between the absorbance at 205 nm and the absorbance at 300 nm;

$$y \text{ (absorbance at 205 nm)} = 2.8414x \text{ (absorbance at 300 nm)} - 0.0126$$

To test the validity of the two wavelength approach Edwards et al. (2001) measured the recovery percentages by spiking nitrate standard solutions with various organic matter (polymaleic acid) concentrations. The absorbance was read at 205 nm and 300 nm and the absorbance contributed by organic matter at 205 nm was deduced using the equation above and then a traditional standard curve equation was used to calculate the nitrate concentration. The recovery of nitrate, based on three replicates for each treatment, varied from a minimum of 99.4% to a maximum of 103.9%, with an overall mean recovery of 100.6%. The recovery figures were not particularly affected by the concentration of DOM in the solutions. The minimum concentration of nitrate that could be detected was dependent on the spectrophotometer and the operational parameters (e.g. wavelength, absorbance range and optical path length).

The authors tested their method using 50 surface waters collected from various upland streams and rivers. The waters differed widely in their conductivity, pH and concentrations of DOC and nitrate. A comparison of the UV spectrophotometry method and the reference method were in good agreement, with a linear regression coefficient (r^2) of 0.990. For most of the waters, the nitrate concentrations determined by the two methods were within ± 2% of each other with the discrepancy arising mainly from samples with a lower than average DOC content. Further, the unknown nature of the DOC in these samples may have caused the problems. Edwards et al. (2001) decided that the UV method may not be particularly suitable for low nitrate upland waters especially if the nitrate concentrations are <0.1 mg N l^{-1}. However for concentrations above this, the method should be suitable, especially for lowland waters as they generally have nitrate concentrations >0.1 mg N l^{-1} and lower DOC than upland rivers. In conclusion, the authors decided that this two-wavelength approach compared well with the reference method and with further development would be suitable for *in-situ* field monitoring.

The UV measurement approach has also made it possible to measure other water quality parameters simultaneously. Dahlén et al. (2000) used UV spectra with partial least squares (PLS) regression to measure the concentrations of nitrate, chloride, total carbon (TC), inorganic carbon (IC), alkalinity and conductivity. However, they also tried sulphate, fluoride and pH but the results were poor. To do this, UV/Vis spectra were recorded in the range 990 to 1100 nm using a 10 mm path length quartz cell and capillary zone electrophoresis, while a TOC analyser and EC meter were used for the other parameters. Spectroscopic data and the data from the reference determinations were imported into the chemometrical software *The Unscrambler* where calculation of the PLS models were made.

Stanley et al. (1994) made a sensor based on fibre optics where a two-wavelength approach was again used. In this study, 210 and 275 nm were chosen as the optimal wavelengths and the sensor was compared with an amperometric sensor. They used fibre optics to reach the groundwater down a borehole with most of the equipment at the surface. The sensor was first tested with 40 m of optical fibre, 20 m between the source and the absorption cell and 20 m between the absorption cell and the detector. The effect of carbonates was investigated using a 300 mg l^{-1} carbonate and 4 mg l^{-1} $N\text{-}NO_3^-$ solution, which gave a 4.5 % positive error. The potential interference from iron was also tested and found not to be a problem unless it was present in very high concentrations. Humic acid was also tested using a 40 mg l^{-1} solution and found to produce a 10 % bias. However, this

concentration is much higher than the expected concentration of organic matter in groundwaters.

The use of UV to measure nitrate was also used by Simonsson et al. (2005) to measure NO_3^- and DOC concentration in forest floor leachates. They used partial least square (PLS) regression models, which were calibrated and validated using UV absorbance spectra and concentrations of DOC and nitrate in forest floor leachates. Also PLS models with specific absorbance (absorbance units per unit concentration of DOC) were tested as a means to estimate the ratio of hydrophobic to total DOC.

5.4.4 UV spectrophotometry of wastewater

As well as natural waters the method can also be used in wastewaters, which are likely to have higher concentrations of nitrate than natural waters. Karlsson et al. (1995) took samples from three different municipal waste water plants during a period of six months. The absorbance was measured at 220 and 275 nm as in previous studies and was analysed in a 10 mm quartz cuvette. Figure 4.13 shows the results from a scan from 190 to 820 nm. Due to the shape of the spectra of the unfiltered municipal waste water samples containing nitrate, it was impossible for the authors to correlate absorbance values at just one wavelength to the concentration of nitrate. An interfering phenomenon such as light scattering was observed. Absorbance maxima occur in the region 200 to 220 nm as expected for samples containing nitrate, however, the presence of UV absorbing species other than nitrate could not be excluded which added to the complexity of calibration and evaluation of results. This meant that a multivariate data analysis approach was required using as many absorbance values as possible. Several different approaches were tested, classical multiple linear regression (MLR) using all wavelengths failed completely. However, when using a selection of 5 wavelengths for classical and inverse MLR useful results were obtained.

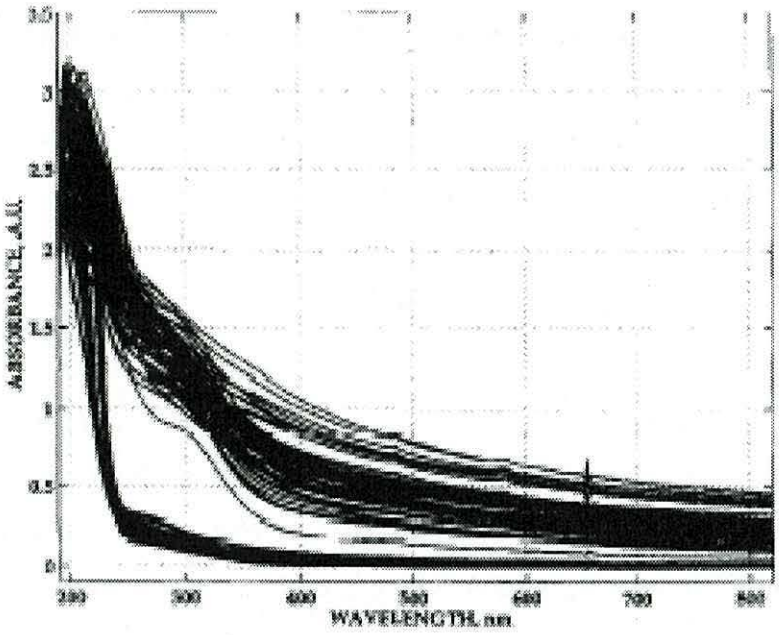

Figure 5.13: Absorbance spectra of 114 unfiltered municipal wastewater samples from 190 to 820 nm (Karlsson et al., 1995).

Partial least squares regression (PLS) was also tested and compared with the MLR models. It was found that PLS gave better predictions of nitrate and it offered the useful additional advantages of being self-diagnostic and able to detect outliers. The accuracy of the PLS regression can be improved by pre-treatment of the data to take into account factors which can affect Beer-Lamberts law such as turbidity. Using this method it was found that the working range for nitrate determination was 0.5 to 13.7 mg l^{-1} and the relative standard deviation was 3.4 % (Karlsson et al., 1995).

Chevalier et al. (2002) looked at a new UV analyser to measure water quality parameters including 5-day biochemical oxygen demand (BOD_5), total organic carbon (TOC) and total suspended solids (TSS). This UV analyser is based on the principle that the spectrum of any water sample is a composite of the spectra of its constituent parts, including BOD_5, COD, TSS, TOC, nitrates and surfactants. The UV analyser uses a deterministic approach to analyse the sample's spectrum by comparing it with a series of historical reference spectra. The comparison uses an internal library with approximately 100,000 reference spectra and the corresponding chemical analyses. The UV analyser determines the contribution coefficient for each reference spectrum and using the reference data stored in its internal UV-base software estimates the spectrum of the whole sample. Once the system has selected the contribution coefficients that yield the spectrum of the

sample test, the concentrations are computed and the results are displayed. This system offers several potential advantages over conventional water quality procedures. These include: real-time results collection, ease of operation, no requirement for reagents, portability, operation variability, measurement of a wide range of parameters, data storage and a PC interface (Chevalier et al., 2002).

5.4.5 UV spectrophotometry and DOC

Dissolved organic carbon is found in surface waters (Edwards and Cresser, 1997), wastewaters (Servais et al., 1999), and groundwater (Jacinthe et al., 2003). In the nitrate measurement using UV spectrophotometry, DOC can be an interference, however, this also means that it can be measured using this method as well and so in this case nitrate would be the interfering species. Wang and Hsieh (2001) measured natural organic matter in water using UV spectrophotometry using three commercial humic acids, nitrate solutions, formazine and kaolin to assess the effects on the measurement of organic matter in water with interferences caused by nitrate and turbidity.

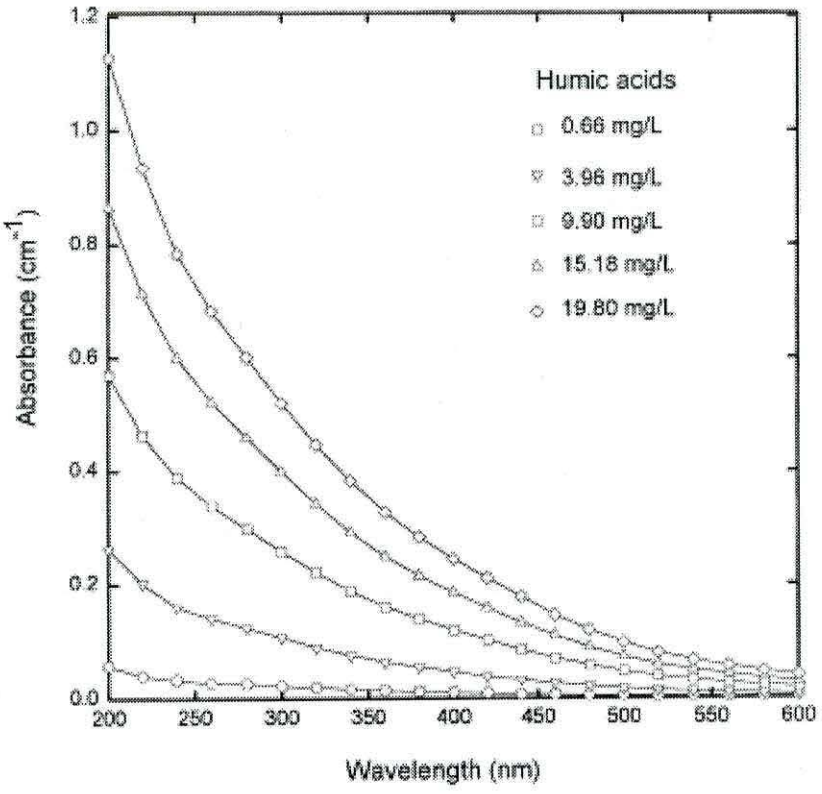

Figure 5.14: The absorbance of commercial humic acids in water at different concentrations of humic acids (Wang and Hsieh, 2001).

Figure 5.14 shows that the humic acids are absorbing at the same wavelengths as nitrate and so this is why the interference occurs with nitrate when using UV spectrophotometry. 254 nm (A_{254}) is frequently used in the monitoring of DOC in the water treatment industry, once the correlation between DOC and A_{254} has been established for the water of interest (Korshin et al., 1997, MacCraith et al., 1994).

There have been efforts to produce an in-situ instrument to measure DOC. MacCraith et al. (1994) used a combination of absorbance at 254 nm and fluorescence to investigate the use of fibre optic based instrumentation in the field. They reported a strong correlation between TOC and UV adsorption and so the potential to develop the method further. Thomas et al. (1999) recommended that the best method for wastewater measuring is to complement the use of a portable UV spectrophotometer with a TOC analyser as both methods have advantages and disadvantages.

5.5 Turbidity

Turbidity is defined as the reduction of transparency of a liquid caused by the presence of undissolved matter (EN ISO 7027: 1999). The term turbidity is often confused with suspended solids, however, these particles are generally bigger and if a scoop of sand is added to water there would initially be water, turbidity and suspended solids. If this was left for a few hours there would be water, turbidity and sand particles in the bottom that due to their mass and nature have settled out. The other particles, which are smaller in size and mass, have remained in suspension. These are referred to as turbidity particles, which contribute to the solutions turbidity properties. It is generally accepted that particles in excess of 100 μm are suspended solids and those less than 100 μm are turbidity particles. (Lee, 1982).

5.5.1 Methods of turbidity measurement

The first quantitative method widely used was the Jackson Candle turbidimeter. This consisted of a special candle and a flat-bottomed glass tube. The tube was calibrated using different dilutions of fuller's earth suspension in distilled water. The sample was poured slowly into the tube and the point at which the candle flame diffused to a uniform glow was recorded. The measurement was made in Jackson Turbidity Units (JTU) and the turbidity caused by 1 mg l^{-1} of suspended silica was defined as 1 JTU (Murren, 1993). There were problems with this method due to the reliance on the accuracy of the operator

of the turbidimeter and also the Jackson Candle turbidimeter is not sensitive to turbidities below 25 JTU, which would be the expected range for natural water and treated wastewater (APHA et al., 1975).

To overcome these problems new methods were developed. Measurements are based on the absorption and scattering of light by the turbidity particles. In the scattering method, the scattered light level is proportional to the particulate concentrations and can be measured by an electronic photodetector (Murren, 1993). Instruments based on this principle are called nephelometric turbidity meters.

The design of these nephelometric turbidity meters has to take into account the physics of scattered light and also several interacting variables, which contribute to the complexity of the problem. The size of the undissolved particles affects the intensity distribution of the scattered light. Small particles (less than one tenth of the light wavelength will scatter light uniformly in both forwards and backwards directions (Murren, 1993). This occurs by the particle absorbing light and then re-radiating the light (Sadar, 1998). As the particle size approaches and exceeds the wavelength of the incident light the light scattered from different points of the sample particle create interference that are additive in the forward direction. This results in forward scattered light of a higher intensity than light scattered in other directions (Fig. 5.15; Sadar, 1998).

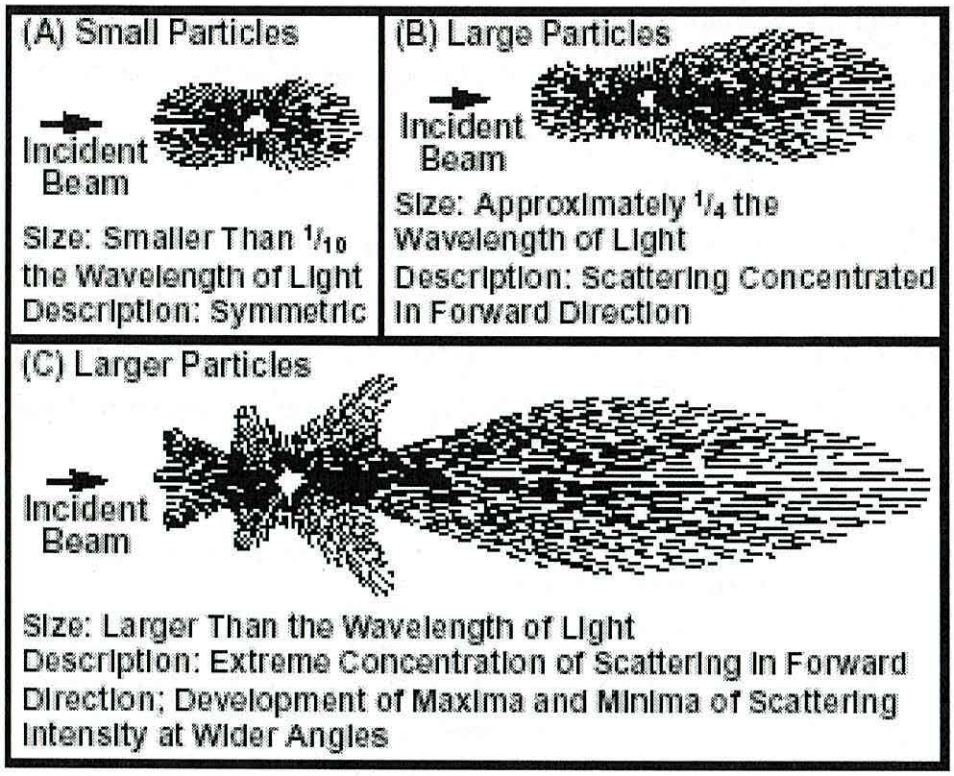

Figure 5.15: The patterns of light scattering caused by particles of three sizes. (A) small particles, (B) large particles, (C) larger particles (Sadar, 1998).

The characteristics of the scattered light are also affected by the particle shape and refractive index. A spherical particle will exhibit a larger forward-to-back scatter ratio than coiled or rod shaped particles. The refractive index is a measure of how a particle redirects light passing through it from another medium such as the suspending fluid. For the particle to scatter light its refractive index must be different from the refractive index of the sample fluid. The colour of the suspended solids and samples fluid are significant in the scattered light detection. A coloured substance absorbs light energy in certain bands of the visible spectrum. This changes the character of both the scattered light and the transmitted light, so preventing a certain portion of the scattered light from reaching the detection system. The nephelometric method works on the principle that the light scattering will increase as the particle concentration increases. However, as the scattered light hits more and more particles, multiple scattering occurs and absorption of light increases. When particulate concentration increases beyond a certain point, detectable levels of scattered and transmitted light drop rapidly, marking the upper limit of measurable turbidity by the nephelometric method (Sadar, 1998).

In the European Standard for turbidity measurement, (EN ISO 7027:1999) the scattered light must always be measured at 90° to the incident light. The wavelength of the light used to make the measurement has been set by industry at 400 to 600 nm in the visible region or 800 to 900 nm in the infrared region.

There are also other methods that have been employed in the measurement of turbidity such as remote sensing of natural waters (Fraser, 1998).

A problem that can occur in the measurement of turbidity is deposition on the optical cell surface windows. Lui and Dasgupter (1996) solved this problem by performing the nephelometric method on a single drop of solution as shown in Figure 5.15 where it can be seen that an optical fibre is used to collect the scattered light.

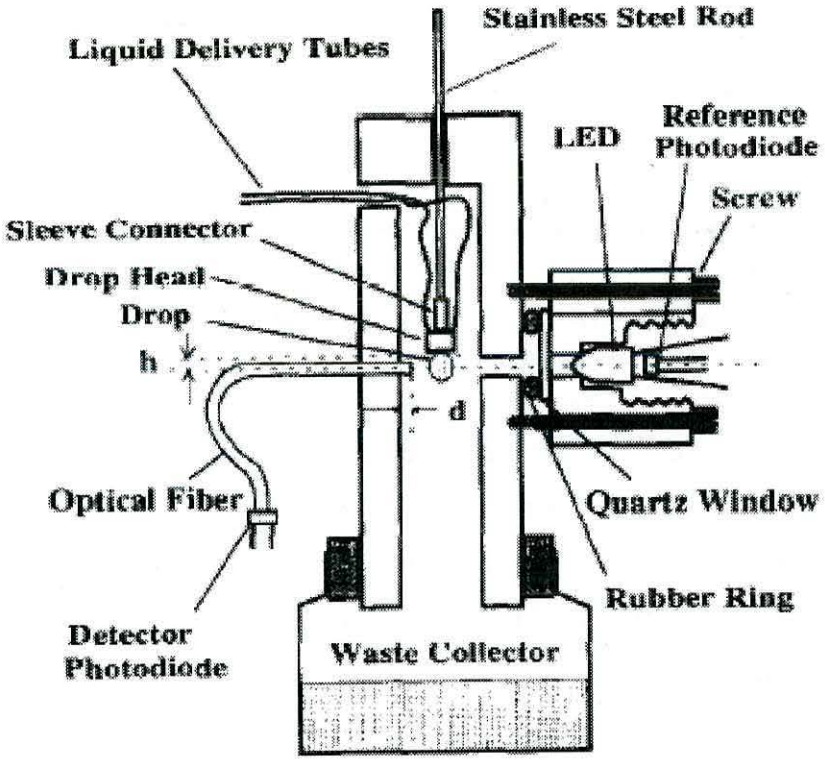

Figure 5.16: The method of turbidity measurement proposed by Lui and Dasgupter (1996) using a drop of solution.

5.5.2 International standards and units

There are two standards for the measurement of turbidity in common use worldwide. Until the mid- nineties most instruments were constructed to meet the U.S. standard (APHA et al., 1992). A revised procedure has been published by The International Organization for Standardization (ISO7027:1999) and in 1994 was adopted by the European Committee for

Standardization (Hongve and Åkesson, 1998). The main difference between these two methods is the light source that is used. In the US standard a tungsten filament lamp is operated at a temperature in the range of 2200 to 3000 K and with a spectral response of 400 to 600 nm. In the European standard the wavelength of the light is required to be 860 nm with a spectral bandwidth of less than 60 nm. Tungsten light sources are more sensitive to small particles but sample colour can be an interference whereas the 860 nm light source is less sensitive to small particles but there is less interference from colour (Sadar, 1998).

The units of turbidity measurement are especially confusing. The units in common use are summarised in Table 5.1.

Table 5.1: A summary of the frequently used turbidity units around the world.

Unit	Definition	Method of measurement
NTU	Nephelometric Turbidity Unit	Measured using US standard method (tungsten light source, scattering measured at 90 °, formazine calibration)
FNU	Formazine Nephelometric Unit	Measured using ISO 7027 method (LED at 860 nm, scattering measured at 90°, formazine calibration, for turbidities < 40 FNU)
FTU	Formazine Turbidity Unit	Measured using tungsten light source with formazine calibration. Used in water treatment.
FAU	Formazine Attenuation Unit	Absorbance measurement using LED at 860 nm with formazine calibration light scattering at 0° (for turbidities > 40 FNU)
JTU	Jackson Turbidity Unit	Out of date method of turbidity measurement using a Jackson Candle Turbidimeter.

There are also more specialised units for the nephelometric method used by industry including: EBC (European Brewery Convention, used in European breweries), ASBC (American Society of Brewing Chemists, used in American breweries) and Nephelo (used in the pharmaceutical industry). In practice there appears to be a lot of confusion about which unit should actually be used. For example, the instruction manual for the Oakton Turbidimeter T-100 (Oakton Instuments, 2003) specifies that the turbidimeter follows the ISO 7027(1999) method but the results are measured in NTU.

5.5.3 Standards for measurement

There have been many different standards used over the years for the measurement of turbidity. The method that is currently accepted for use by the American (APHA et al., 1992) and international standards (ISO 7027:1999) is formazine. Formazine is prepared

from an aqueous solution of hydrazine sulphate combined with an aqueous solution of hexamethylenetetramine and left to stand for 24 hours at 25 °C (Figure 4.17). This produces a stock solution of 4000 NTU, which can be further diluted to produce solutions of the required turbidity (Rice, 1976). The internationally accepted methods of measurement state that to dilute the stock solutions filtered water should be used (APHA et al., 1992, ISO 7027:1999). However, this assumes that these diluted solutions will give the same signal from the turbidimeter as a standard diluted using filtered supernatant fluid of the stock preparation. Rice (1976) found that this was not true and that turbidity readings in NTU when water was used to dilute the standard were 8 % higher than the comparable standards diluted using the supernatant. It was also found that the standards prepared with filtered supernatant solution were more stable than the ones prepared with water and gave constant NTU values for at least one month after preparation (Rice, 1976).

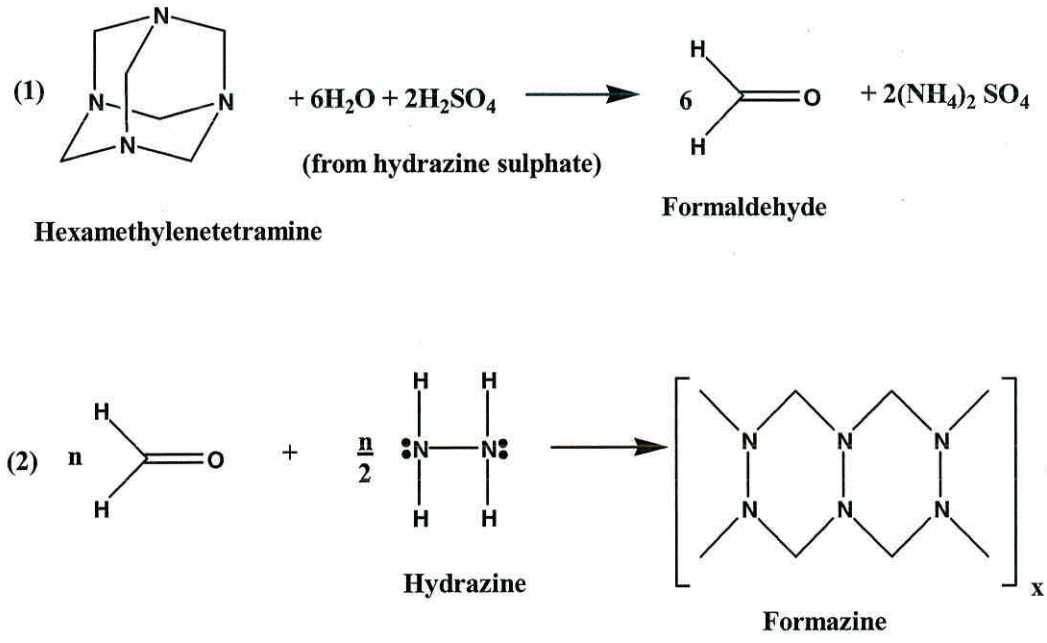

Figure 5.17: The formation of formazine from hexamethylenetetramine and hydrazine sulphate.

Formazine has several desirable characteristics, which make it suitable for turbidity measurement. It can be reproducibly prepared from assayed raw materials and the formazine polymer consists of chains of several different lengths, which fold into random configuration. This results in a wide range of particle shapes and sizes ranging from less

than 0.1 μm to over 10 μm, which yields statistically reproducible scatter for all makes and models of turbidimeters (Buzoianu, 2000). However, there are limitations to the use of formazine. The starting materials are hazardous and therefore formazine can only be made in a laboratory with the required safety equipment. Also, although the higher turbidity standards (>400 NTU) are stable for up to one year, the lower concentrations e.g. 2 to 20 NTU are only stable for 24 h. Both of these factors can lead to problems with the everyday use of formazine, especially in the field. Figure 5.18 shows SEM images of a kaolin and formazine solution. Different manufacturers produced the two kaolin solutions. Kaolin A solution contained many flake particles of about 2 μm in size. The kaolin B solution also had these particles and also many particles about 5 μm. There are a higher total number of particles in the kaolin A solution than in the kaolin B solution. In contrast, in the formazine solution there appears to be particles of 2 μm or smaller which have aggregated into a variety of complex shapes (Ebie et al., 2006).

The UV absorption of carbonate and kaolin suspensions from 200 to 800 nm were investigated by Berho et al. (2004) who found that the absorbance values decreased according to wavelength (Fig. 5.19). Generally the absorbance values of the carbonate spectrum are higher than the kaolin spectrum ones. This could either be due to the presence of finer colloids (<0.45 μm) in the carbonate suspension, which diffuse more light, or to a different shape of particles that influence the diffusion of light. A fractionation at 0.45 μm of these suspensions showed that the carbonate suspension contained more of the very small colloids than the kaolin one, which validated the first hypothesis.

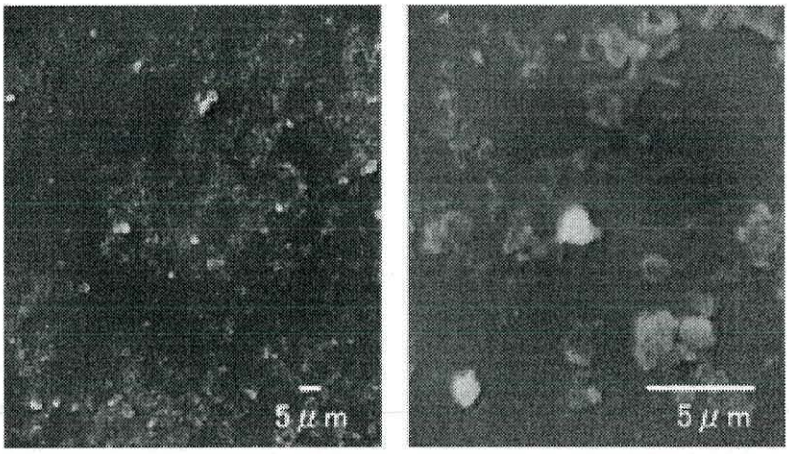

Photo. 1 – SEM images of kaolin A standard solution.

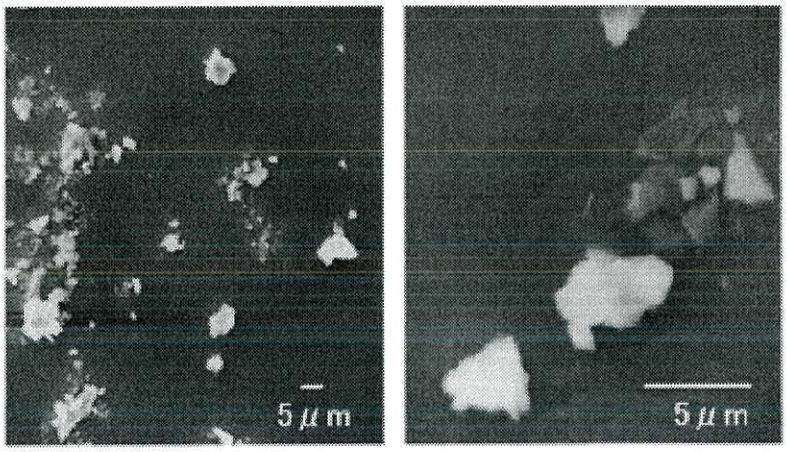

Photo. 2 – SEM images of kaolin B standard solution.

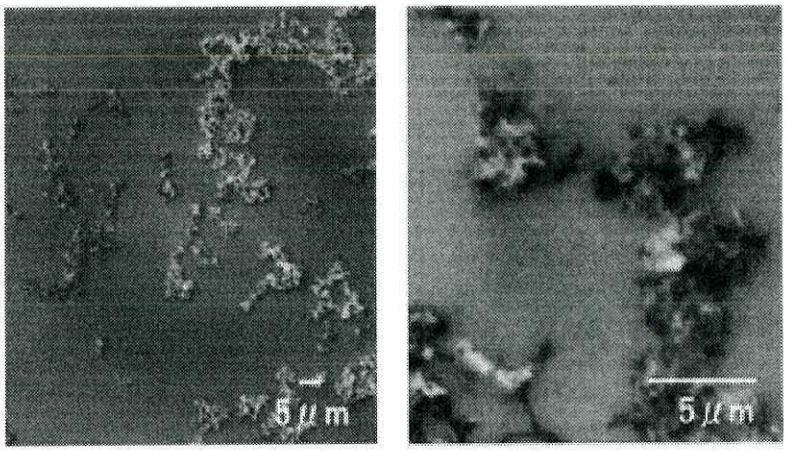

Photo. 3 – SEM images of Formazine standard solution.

Figure 5.18: SEM images of two types of kaolin and formazine turbidity solution (Ebie et al., 2006).

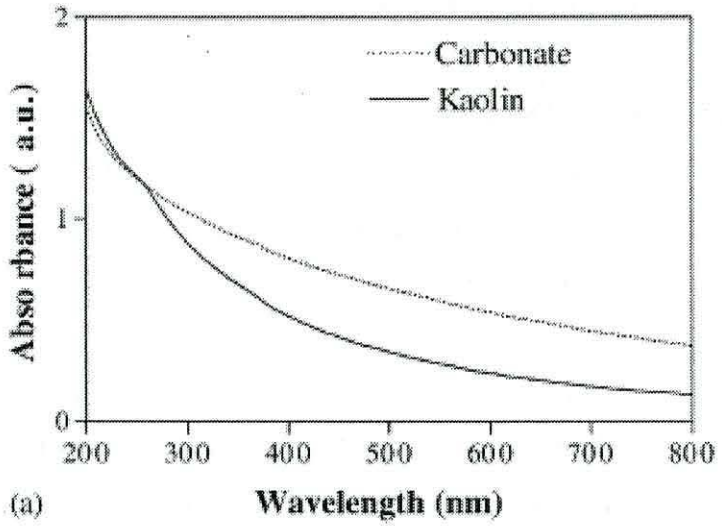

Figure 5.19: The UV absorption of carbonate (206 mg l^{-1}) and kaolin suspensions (207 mg l^{-1}) from 200 to 800 nm (Berho et al., 2004).

Due to the problems with formazine, secondary standards have also been developed which include latex suspensions, styrene divinylbenzene beads, metal oxide gels or stabilised formazine (Buzoianu, 2000). However they cannot be used in the standard methods unless they are verified as the correct turbidity by formazine standards.

5.5.4 Uses of turbidity measurement

Turbidity can indicate the presence of microbes, which may or may not be pathogens (Pronk et al., 2006) and also that transport is occurring as the microbes can be transported attached to suspended particles (Mahler et al., 2000). Karst aquifers are especially useful for studying the behaviour of bacteria and turbidity and are also vulnerable to bacterial contamination. Mahler et al. (2000) examined the transport of bacteria in karst systems and found that at various times up to 100 % of the bacteria in groundwater were associated with suspended sediments. However, there are a wide range of colloids and particles in groundwater, which have different origins and properties (Atteia et al., 1998). In addition, turbidity may either come from outside the aquifer or from the remobilisation of sediments inside the aquifer. In contrast, bacteria normally come from outside the aquifer. This means that turbidity is not always a good indicator of bacterial abundance (Pronk et al., 2006). In one study where turbidity and bacteria in a karst system were compared during a

storm rainfall event it was found that high turbidity always coincided with high contents of bacteria but that high contents of bacteria did not always coincide with high turbidity (Pronk et al., 2006).

As well as the common use of the nephelometric method in the analysis of turbidity in water there are also many other applications where it can be used. A few examples are in the production of olive oil (Mignani et al., 2003), the determination of detergents (Rajakumari et al., 2006), the *in-situ* monitoring of the growth stages of protein crystallisation (Dao et al., 2000) and a study looking at the texture of custard (Wijk et al., 2006). Turbidity measurement is frequently used in microbiology to determine population densities in solution (Sawai et al., 2006; Lindgvist, 2006; Stringer et al., 2005).

Turbidity in the environment is not only measured as a water quality factor but also because of its effects on organisms in that environment. Weiffen et al. (2006) studied how turbidity affected the behaviour of harbour seals and Spier and Heidinger (2002) looked at the effect of turbidity on the growth of different species of fish.

5.5.5 Turbidity and concentration of suspended solids

Many researchers have investigated the dependency between absorption or scattering of monochromatic light and the nature of suspended particles in order to establish a relationship between the behaviour of the light and the mass or volume concentration of the particles present in the sample. As in section 4.2.1 the behaviour of the scattered or absorbed light is dependent on the particles size, shape and refractive index. Studies to determine the effects of grain size (Baker and Lavelle, 1984), the shape of particles (Pak et al., 1970) and the refraction properties of the particles (Zaneveld et al., 1974) have been carried out but normally a calibration is still required in each ecosystem. McCarthy et al. (1974) proposed a method to measure turbidity in an estuarine system taking into account the ecological effects of turbidity but they state that this method could not be used in other areas or with other instruments without prior calibration at that site.

Other methods have been investigated to help identify the different turbidity fractions in natural waters. One method is field flow fractionation (FFF), which is a chromatography-like separation method. Normally a UV absorption detector is used with this technique but there have been investigations into using a nephelometric method of detection (Kammer et al., 2005).

5.5.6 Turbidity in the environment

Turbidity is now seen as a key pollutant. Contaminants are often associated with suspended sediment through complex binding effects. Washburn et al. (2003) used turbidity as a visual indicator of storm water plumes entering the sea. Associated with these plumes were toxic pollutants along with pathogenic bacteria and viruses resulting in closure of public beaches (Washburn et al., 2003).

Lawler et al. (2006) were interested in urban river water quality dynamics, especially during storm events, and found that surprisingly little had been published when compared with rural river basins. Turbidity was measured in this river study using an in-line probe housed in a riverside measurement facility. The optics were cleaned automatically 5 times every hour with mechanical wiper blades and calibrations performed monthly. The authors identified several important features of the storm event turbidity response,

- A sluggish early turbidity response, followed by a turbidity rush
- Quasi-coincident flow and turbidity peaks
- Increases in peak turbidity levels through storm sequences
- Initial micro-pulses in turbidity

Turbidity is also variable in lakes as a result of many different factors. A study of 20 Finnish lakes found that in summer the range of turbidities was from 2 to 52 FTU and in winter was 0.5 to 64 FTU over a six-year period (Ekholm and Mitikka, 2006). In summer, this turbidity was associated with chlorophyll and so the turbidity was partly attributed to algae and in winter may have been more likely to be caused by soil particles.

High turbidity and associated suspended solids also have important ecological impacts including light suppression effects and contribution to BOD levels (Lawler et al., 2006). Thorpe and Lloyd (1999) found that turbidity had a significant effect on macro-invertebrate communities. Walters et al. (2003) found that urbanisation of a river system caused the turbidity to increase. They hypothesised that likely causes of this included the baseflow transport of fine bed materials introduced during flood flow events and persistent near stream disturbances such as house and road construction. These higher baseflow activities may impact on the trophic pathways and spawning success of endemic species of fish (Walters et al., 2003).

5.5.7 Turbidity and groundwater

The turbidity present in groundwater systems is dependent on many factors, one of the most important being the rainfall that occurs at the surface. The relationship between rainfall and turbidity can be difficult to interpret. Figure 4.20 shows the relationship between rainfall, turbidity and discharge in a groundwater system in the Atlas Mountains (Bouchaou et al., 2002).

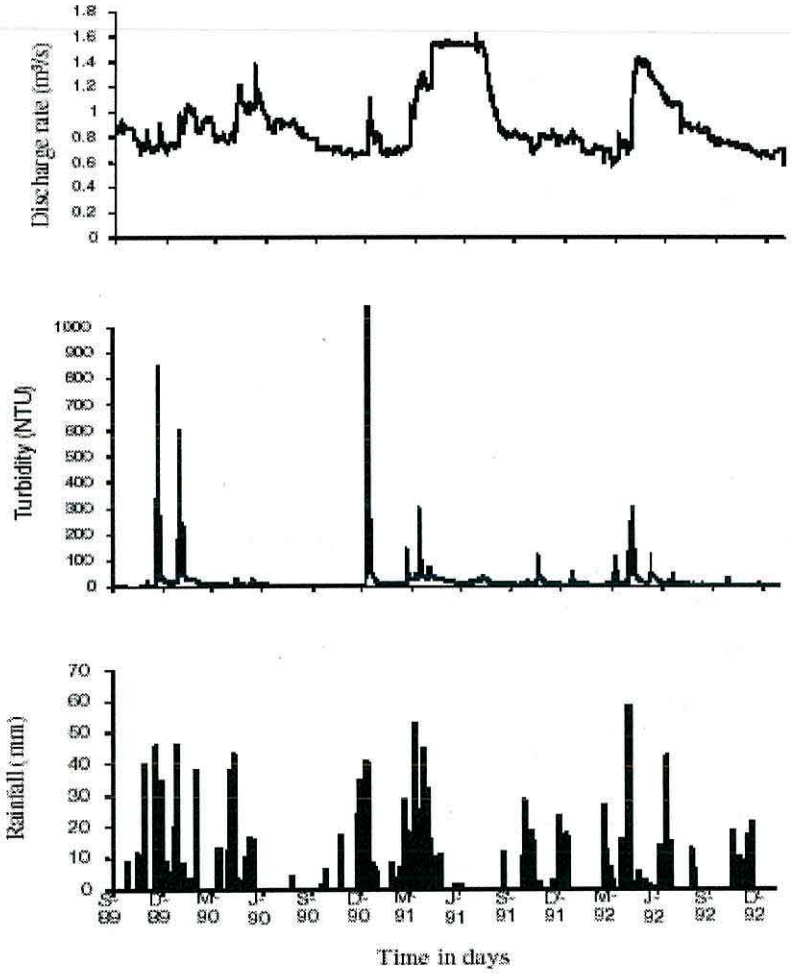

Figure 5.20: The relationship between rainfall, turbidity and discharge over time from a spring in the Atlas Mountains (Bouchaou et al., 2002).

Figure 5.20 shows many peaks of turbidity and discharge rate that were caused by each rainfall event. After further analysis of the results it was concluded during the discharge rate increase there is a sudden "unclogging" of particles from where they have filled

fissures and the sediment is evacuated towards the outlet in waves of turbidity (Bouchaou et al., 2002).

In groundwater systems turbidity can originate from settled sediments in the groundwater system or those which enter the system from the surface (Massei et al., 2003). The occurrence of turbidity following storm events is a common feature of karstic springs (Amraoui et al., 2003). The weathering of carbonate rocks over the watershed outcrops and within the aquifer yields arigillaceous minerals that form a residue (decalcification red clays). These residues fill the low topographic points, which are then moved when a strong surface flow occurs during a storm event. Water loaded with these sediments flows towards the various sinkholes and infiltrates into the aquifer. These can either remain in suspension or become clogged in the cracks in the aquifer. During fast infiltration, a flushing effect can occur, causing the re-suspension of settled sediments and clogged cracks. This internal turbidity is probably evacuated from an outlet first during a storm event and also becomes mixed with the external turbidity brought into the aquifer (Amraoui et al., 2003).

The level of turbidity in wells and boreholes will be affected by the presence of pumping. Ivahnenko et al. (2001) measured the turbidity in wells that were being pumped. They waited until the turbidity had become stable before taking the measurements. The stabilisation of the turbidity varied from well to well but generally turbidity stabilised 45 to 188 min after pumping commenced. Eleven out of the twelve wells measured had turbidity below 2 NTU and the other one was 9 NTU. The authors recommended that a minimum of one hour of pumping is required until the particulates that are artificially mobilised by the surging action of the pump and the increased groundwater flow are removed and a stable level of turbidity is reached.

5.6 Conclusions

The dependence on groundwater for water supplies is likely to increase in the future and therefore we need to better understand and monitor the processes occurring in these aquifers. UV spectrophotometry has been used successfully in similar situations in surface freshwaters and marine environments and therefore there is potential for it to be applied in a groundwater situation. The monitoring of aquifers is likely to increase as different aspects of the Water Framework Directive take effect and so the development of new technology that can be used in-situ down boreholes is important.

Interferences caused by dissolved organic carbon and turbidity are problems which can potentially be overcome. They can also become the parameters of interest, in which case nitrate is the interfering species. UV spectrophotometry has been applied to portable sensors that can be used in-situ in aqueous systems, which is not the case for the other methods and so future research is required to develop these methods further and apply them in everyday measurements of groundwater quality.

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CHAPTER 6

Development of a UV method for nitrate determination in groundwater

6.1 Introduction

This chapter describes the development of a UV spectrophotometric-based method for quantifying nitrate concentration in groundwater. UV spectrophotometry was chosen over other possible analytical methods such as Near IR spectroscopy, nitrate selective electrodes and colorimetric chemical methods as it possesses the most potential to be used in the field. The overall aim was to produce a sonde capable of measuring changes in nitrate concentration during passage of the sonde down a borehole. In particular, one potential application is in boreholes containing high nitrate concentrations. The water pumped to the surface constitutes a mixture of water that has entered the borehole at different depths/geological layers. If a nitrate sonde is lowered down a borehole during pumping it can potentially identify the location of the high nitrate water ingress, allowing localised measures to be installed to close off that part of the well. Pinpointing the inflow location may also help identify the original source of the nitrate pollution and allow mitigation measures to be put in place (e.g. if it is largely from surface water ingress).

The initial method proposed for the measurement of nitrate in groundwater was Near IR. However, after initial investigation of the method it was realised that it was impossible to make this work due to the strong absorbance of water in the region of the spectrum of interest. Many previous studies have identified a strong nitrate absorption peak in the region of 200 nm (Buck et al., 1954; Edwards et al., 2001; Stanley et al., 1994). In addition to nitrate, other constituents of groundwater may also potentially interfere with the measurements (Freeze and Cherry, 1979; Table 6.1). A major aim of this chapter was therefore to investigate and account for interference from other ions during nitrate measurement.

Table 6.1: The major and minor inorganic constituents in groundwater (Freeze and Cherry, 1979).

Major constituents in groundwater (> 5 mg l^{-1})	Minor constituents in groundwater ($0.01 - 10$ mg l^{-1})
Bicarbonate (HCO_3^-)	Boron
Calcium	Carbonate (CO_3^{2-})
Chloride (Cl^-)	Fluoride
Magnesium	Iron
Silicon	Nitrate (NO_3^-)
Sodium	Potassium
Sulphate (SO_4^{2-})	Strontium

From previous studies on marine- and fresh-waters it is clear that dissolved organic carbon (DOC) can represent a major interference in the measurement of nitrate by UV spectrophotometry (Edwards et al., 2001; Hoather and Rackham, 1959; Rennie et al., 1979). Turbidity may also affect nitrate absorbance in the UV region of the spectrum as a result of suspended particles scattering or absorbing the light. This chapter will therefore also address potential interference from these sources.

6.2 Materials and methods

6.2.1 Initial investigation of nitrate absorbance

A series of solutions with different nitrate concentrations were produced using $NaNO_3$ and distilled water. Solution absorbance was measured from 200 to 400 nm on a UV spectrophotometer (Unicam UV1, Thermo Electron Corporation, Cambridge, UK) using quartz cuvettes with a 10 mm pathlength. The spectrophotometer uses a deuterium lamp as the light source from 200 to 325 nm and then a tungsten bulb for wavelengths longer than this. During the scan, the lamp switch happens automatically at 325 nm and sometimes results in a slight aberration in absorbance. All measurements were carried out in triplicate with distilled water used as a blank (control).

Throughout the thesis the units used to describe nitrate concentration are mg l^{-1} of NO_3^- as it is the more commonly used unit by UK water companies (as the EU drinking water limit is set at 50 mg NO_3^- l^{-1}). The sonde is designed to be operated by water industry personnel interested in these guidelines.

6.2.2 Interferences from other substances found in groundwater

Alongside a 10 mg l^{-1} NO_3^- solution, absorbance comparisons from 200 nm to 600 nm were also made with solutions containing 10 mg l^{-1} Cl^- (as NaCl), 10 mg l^{-1} HCO_3^- (as $KHCO_3$), 10 mg l^{-1} SO_4^{2-} (as K_2SO_4), 10 mg l^{-1} CO_3^{2-} (as K_2CO_3), and 10 mg l^{-1} NO_2^- (as $NaNO_2$). Absorbance scans were also made with solutions containing 10 mg l^{-1} of NO_3^- and other anions simultaneously.

6.2.3 Dissolved organic carbon

Commercial humic acid sodium salt (H16752, Aldrich, Sigma-Aldrich Co Ltd., Gillingham, UK) was dissolved in distilled water to produce humic acid solutions ranging from 0.1 to 50 mg l^{-1}. This reflected typical concentrations found in the natural environment. The humic acid solutions were subsequently centrifuged (8000*g*, 20°C, 10 min) to remove particulates. The absorbance of the solutions was analysed from 200 nm to 300 nm using the method described previously. The DOC concentration of the humic acid solution was also measured using a TOC-V analyser (Shimadzu Corp., Kyoto, Japan).

6.2.4 Turbidity

Initially, the effect of solution turbidity on NO_3^- absorbance was simulated using two clay mineral suspensions (kaolinite and chlorite) similar to those carried out by Berho et al. (2004). Suspensions of kaolinite and chlorite were prepared in distilled water (25 to 500 mg l^{-1}) and their absorbance measured from 200 to 1000 nm. The absorbance of a standard turbidity solution was also determined using formazine solutions with predefined turbidities of 2.5, 5, 10, 20, 30, 40 and 50 NTU and the absorbance measured at 203nm and 220 nm.

6.3 Results

6.3.1 Initial investigations of nitrate absorbance

Figure 6.1 shows the results of the wavelength scan from 200 nm to 300 nm using solutions containing different NO_3^- concentrations. It shows that it is impossible to determine NO_3^- concentrations at 50 mg l^{-1} in the 200 nm region due to signal saturation. The peak maximum described in previous studies can be seen at 200 nm, which then decreases to zero absorbance by 250 nm. The results presented in Figure 6.2 show that it is possible to resolve NO_3^- concentrations in the range from 0 to 10 mg l^{-1}. NO_3^- absorbances at different concentrations were plotted at two fixed wavelengths (203 nm and 220 nm; Figs 6.3-6.6). Figure 6.3 shows that absorbance is linear up to NO_3^- concentrations of ca. 20 mg l^{-1} above which it becomes non-linear. At 220 nm, however, the absorbance is linear up to 50 mg l^{-1} after which it also yields a non-linear response (Fig. 6.5). Regression analysis from 0 to 20 mg l^{-1} at 203 nm and 0 to 50 mg l^{-1} at 220 nm gave high r^2 values indicating their suitability as a potential analytical method (Figs. 6.4-6.5).

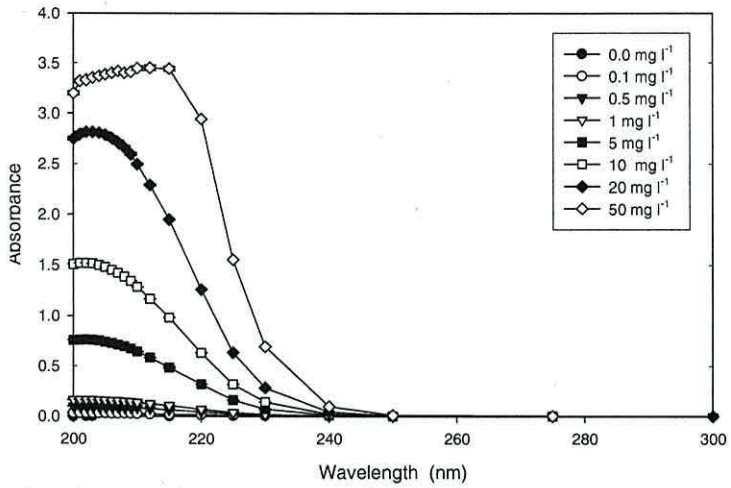

Figure 6.1: The absorbance of nitrate solutions with concentrations from 0.1 to 50 mg l^{-1} from 200 to 300 nm. All values represent means ± SEM (n = 3).

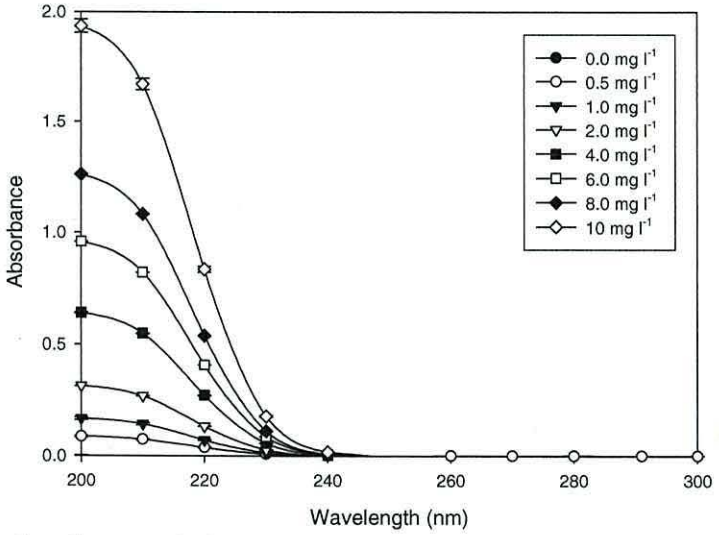

Figure 6.2: The absorbance of nitrate solutions with concentrations from 0 to 10 mg l^{-1} from 200 to 300 nm. All values represent means ± SEM (n = 3).

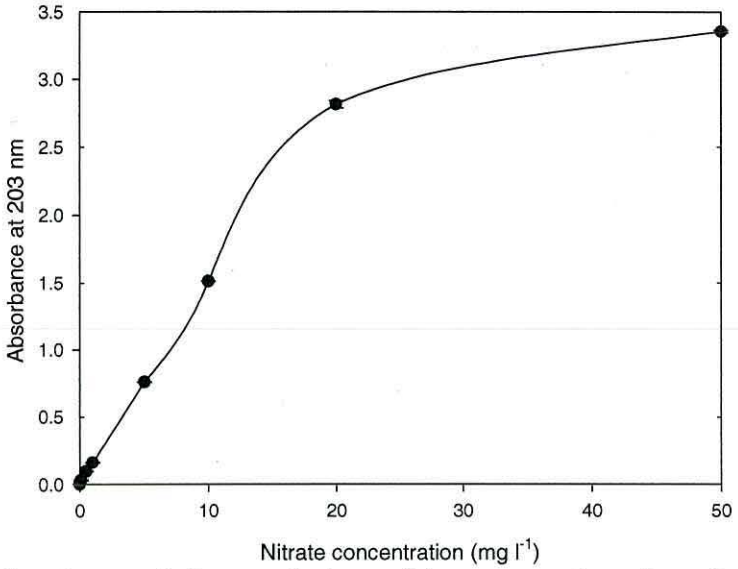

Figure 6.3: The absorbance of nitrate solutions with concentrations from 0 to 50 mg l^{-1} at 203nm. All values represent means ± SEM ($n = 3$).

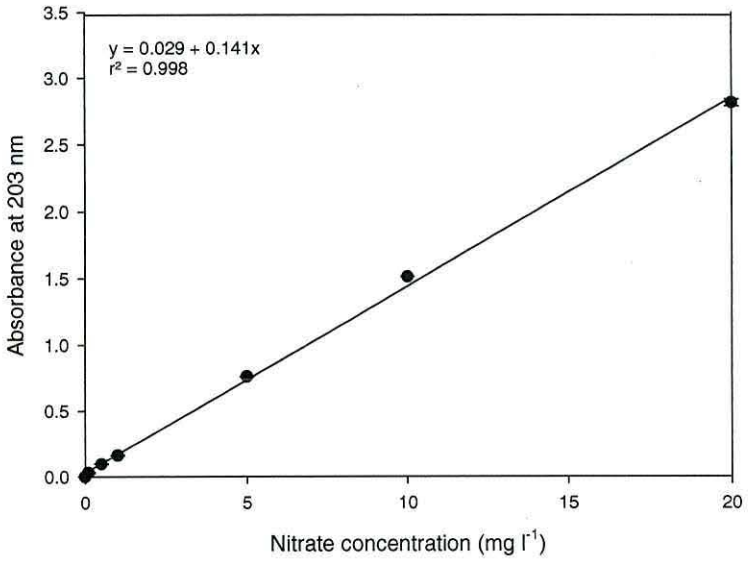

Figure 6.4: The absorbance of nitrate solutions with concentrations from 0 to 20 mg l^{-1} at 203 nm. All values represent means ± SEM ($n = 3$).

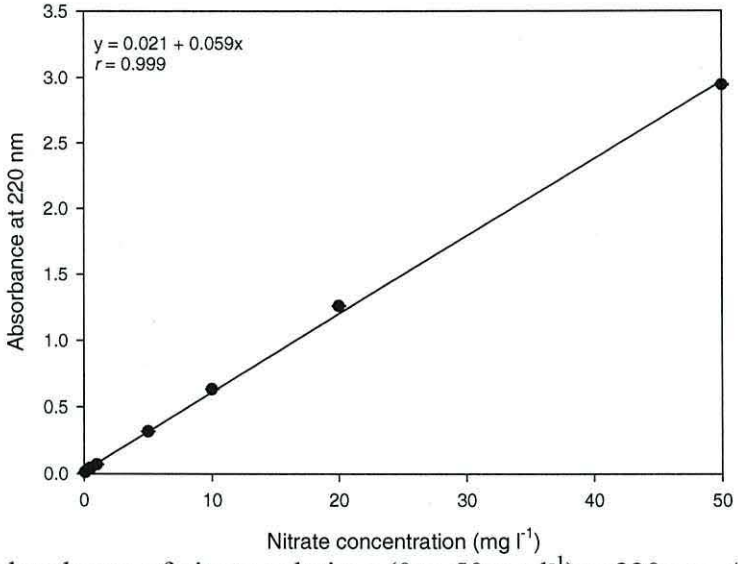

Figure 6.5: The absorbance of nitrate solutions (0 to 50 mg l^{-1}) at 220 nm. All values represent means ± SEM (n = 3).

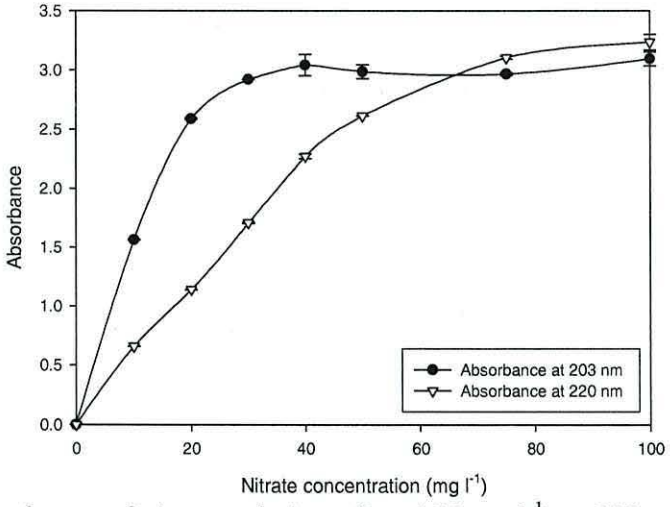

Figure 6.6: The absorbance of nitrate solutions (0 to 100 mg l^{-1}) at 203 and 220 nm. All values represent means ± SEM (n = 3).

6.3.2 Interferences from other substances found in groundwater

Figures 6.7 to 6.11 show the wavelength scans from 200 to 600 nm for a range of potentially interfering anions. Comparative scans for NO_3^- and a combined solution of both anions are also shown. For all anions, except nitrite, there presence had no significant effect on nitrate

absorbance. In the nitrite experiment there was a large effect on the nitrate absorbance as indicated by the clear $NaNO_2$ peak and an additive effect when NO_3^- and NO_2^- were present in solution together.

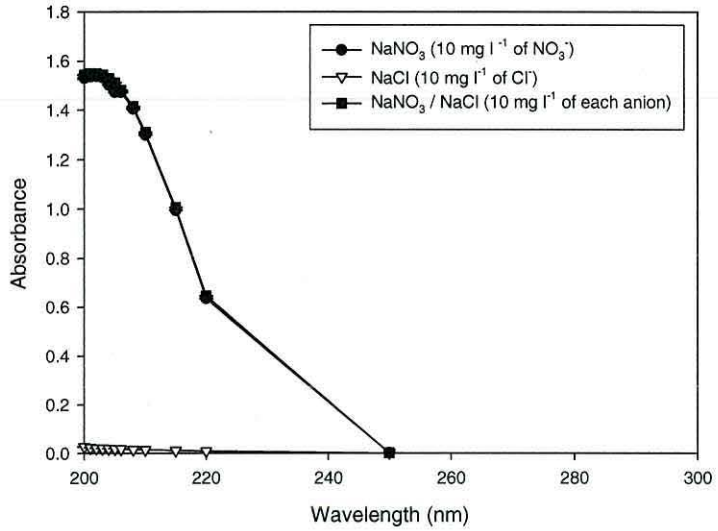

Figure 6.7: The absorbance of three different solutions containing NO_3^- and Cl^- from 200 to 300 nm. All values represent means ± SEM (n = 3).

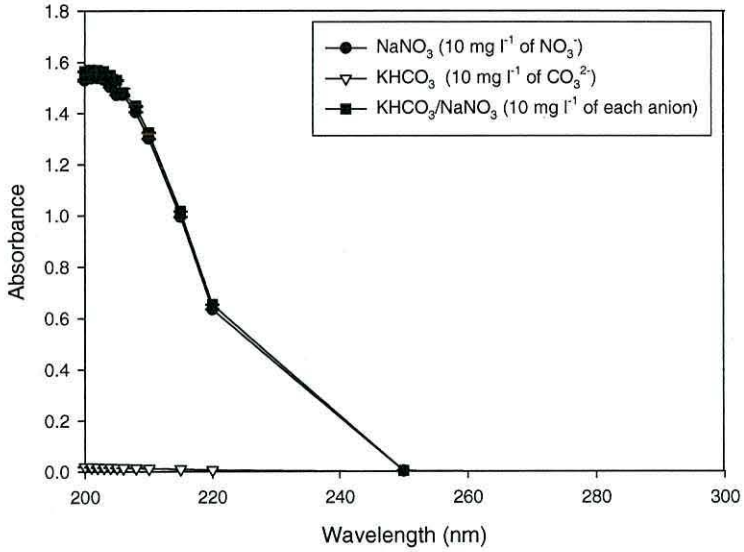

Figure 6.8: The absorbance of three different solutions containing NO_3^- and CO_3^{2-} from 200 to 300 nm. All values represent means ± SEM (n = 3).

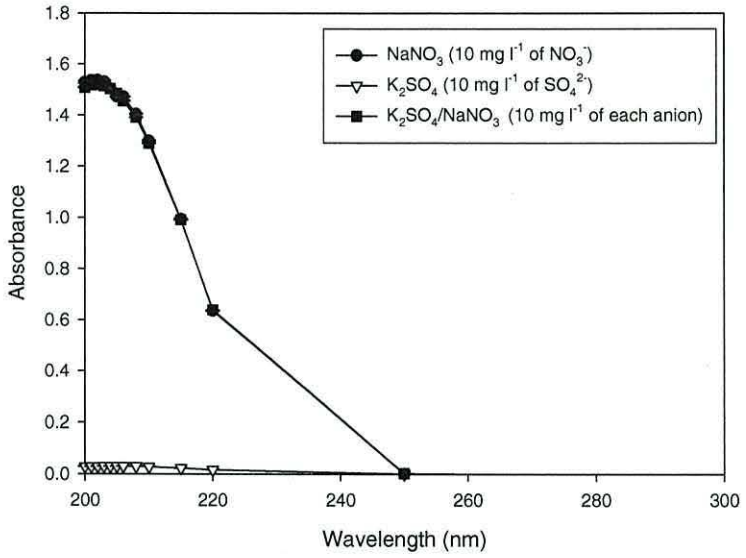

Figure 6.9: The absorbance of three different solutions containing NO_3^- and SO_4^{2-} from 200 to 300 nm. All values represent means ± SEM (n = 3).

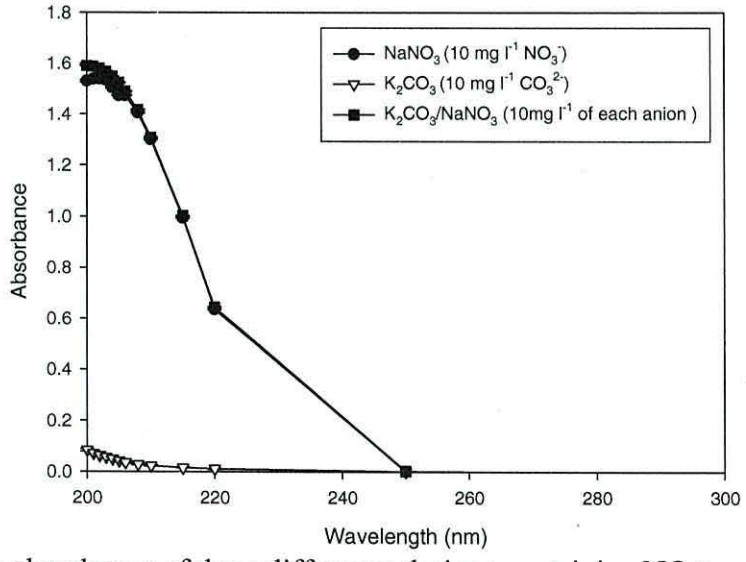

Figure 6.10: The absorbance of three different solutions containing NO_3^- and CO_3^{2-} from 200 to 300 nm. All values represent means ± SEM (n = 3).

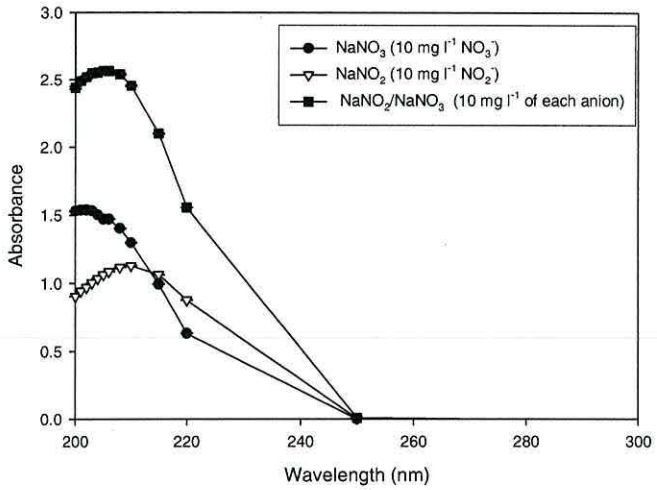

Figure 6.11: The absorbance of three different solutions containing NO_3^- and NO_2^- from 200 to 300 nm. All values represent means ± SEM (n = 3).

6.3.3 Dissolved organic carbon

Figure 6.12 shows the absorbance of different concentrations of humic acid. The wavelength scans show a high absorption in the 200 to 300 nm region where nitrate also has its maximum absorption. The DOC concentration for each of the humic acid standards is presented in Table 6.2. As with the nitrate standards, the absorbencies for different humic acid standards were examined at 203, 220 and 300 nm (Figs 6.13). All three humic substances show a linear relationship between absorbance and humic acid concentration up to 50 mg l^{-1}.

Table 6.2: The concentration of DOC in the humic acid standards. All values represent means (n = 3)

Concentration of humic acid (mg l^{-1})	Concentration of DOC (mg l^{-1})
0.1	0.34
0.5	0.43
1	0.52
5	1.85
10	3.63
25	8.41
50	18.2

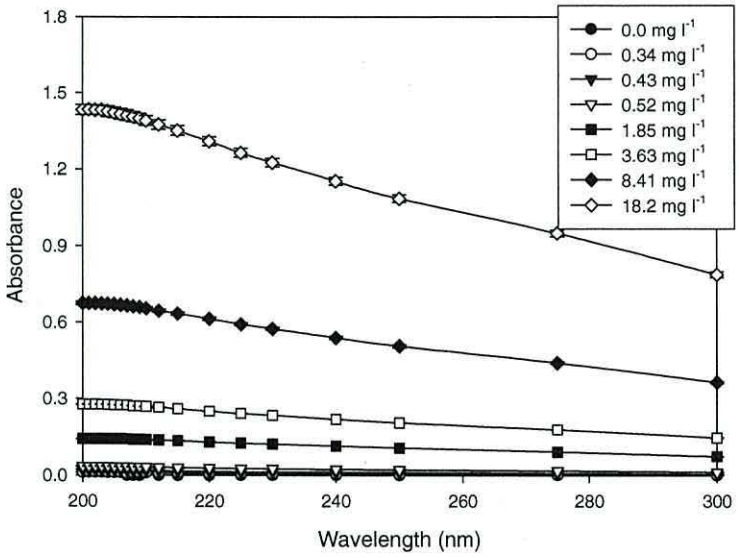

Figure 6.12: The absorbance of Aldrich humic acid standard at different concentrations of DOC. All values represent means ± SEM (n = 3).

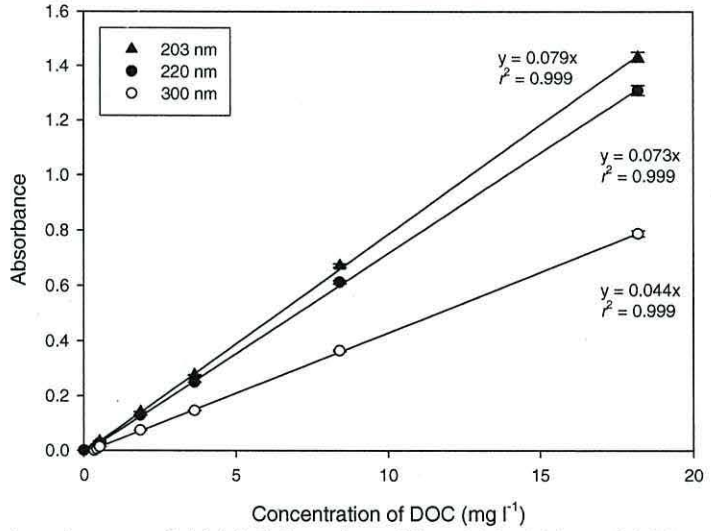

Figure 6.13: The absorbance of Aldrich humic acid at 203, 220 and 300 nm. All values represent means ± SEM (n = 3).

6.3.4 Effect of turbidity

Figures 6.14 and 6.15 show the absorbance from 200 to 1000 nm for different concentrations of kaolinite and chlorite. In both cases, the highest absorption occurs in the 200 nm region of the spectrum with absorbance progressively declining with increasing wavelength. Figure 6.16

shows the absorbance of formazine, which is the accepted standard for the measurement of turbidity. The results show that solution of low turbidity (>10 NTU) will produce an absorbance in the target area for nitrate measurement. Consequently, the turbidity correction will be based on the absorbance of formazine.

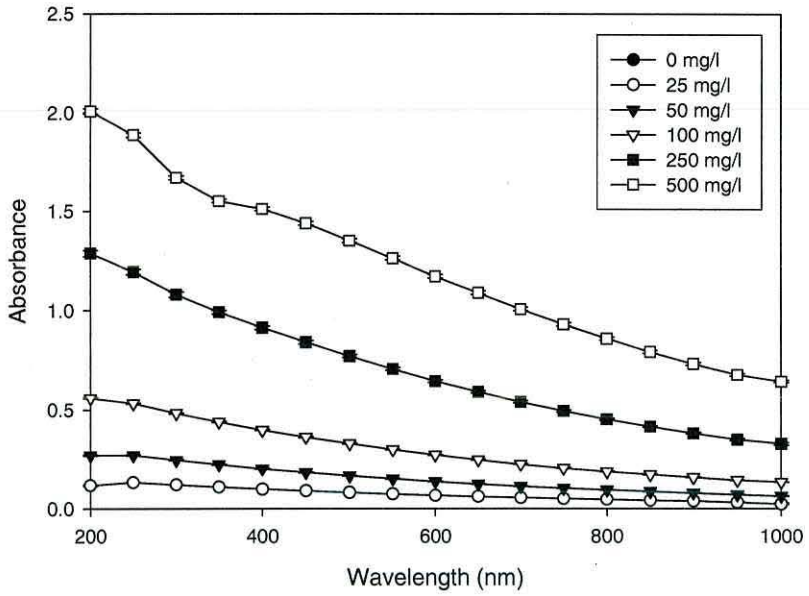

Figure 6.14: The absorbance of different concentration kaolinite suspensions from 200 to 1000 nm. All values represent means ± SEM (n = 3).

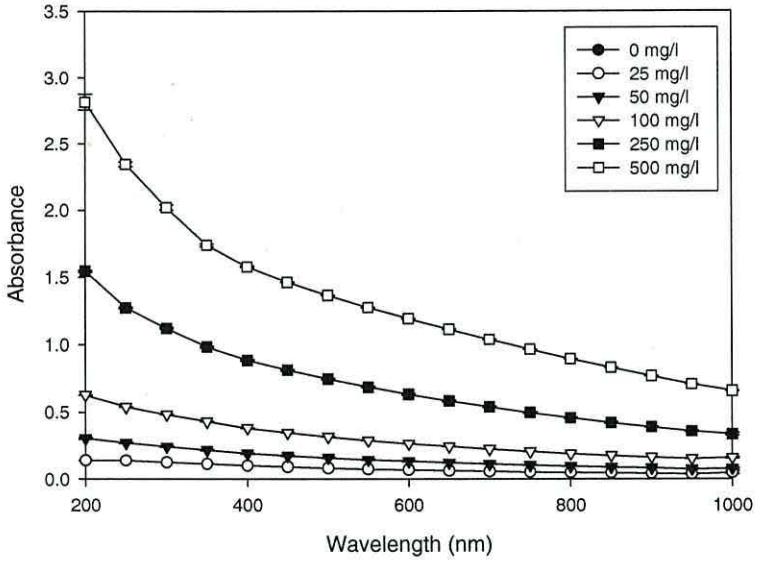

Figure 6.15: The absorbance of different concentration chlorite suspensions from 200 to 1000 nm. All values represent means ± SEM (n = 3).

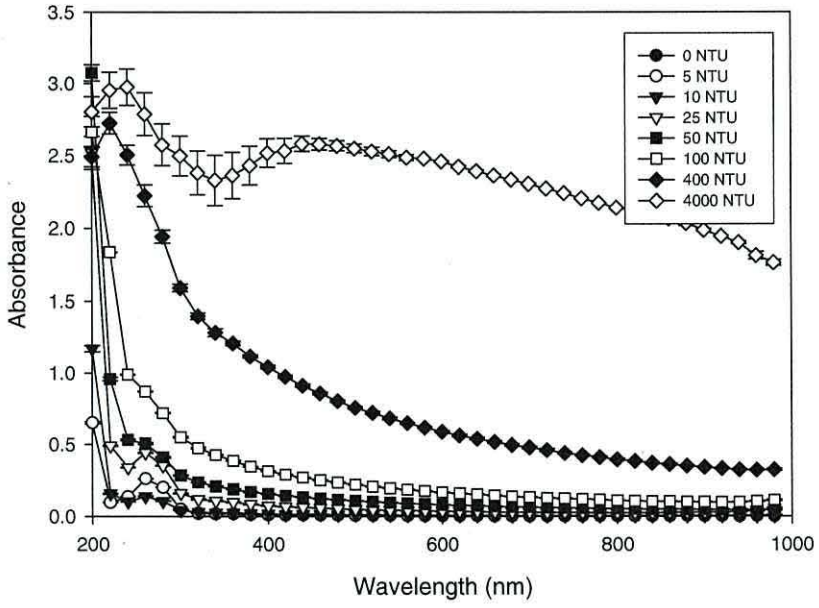

Figure 6.16: The absorbance of formazine solutions with different turbidities as measured on the UV spectrophotometer. All values represent means ± SEM (n = 3).

The turbidity associated absorbances caused by the formazine solutions at 203 nm and 220 nm are shown in Figures 6.17 and 6.18. The values from the equations of the lines fitted to the data will be used for the turbidity correction in the calculation of the nitrate concentration. The figures all show a linear relationship between turbidity and absorbance.

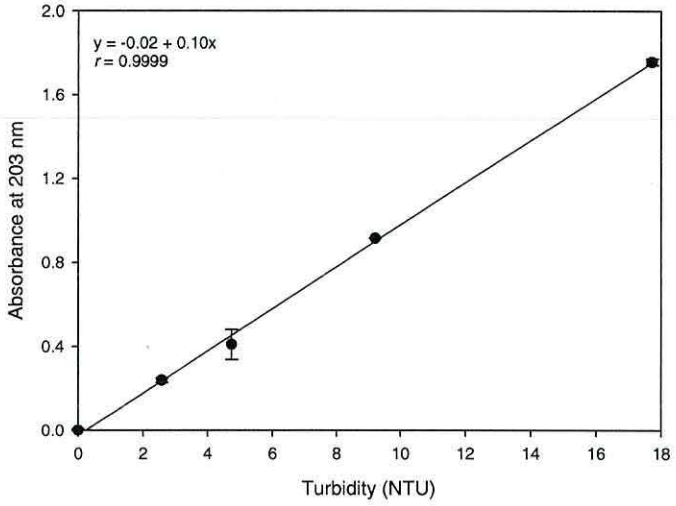

Figure 6.17: The absorbance of formazine solutions with different turbidities at 203 nm. All values represent means ± SEM (n =3).

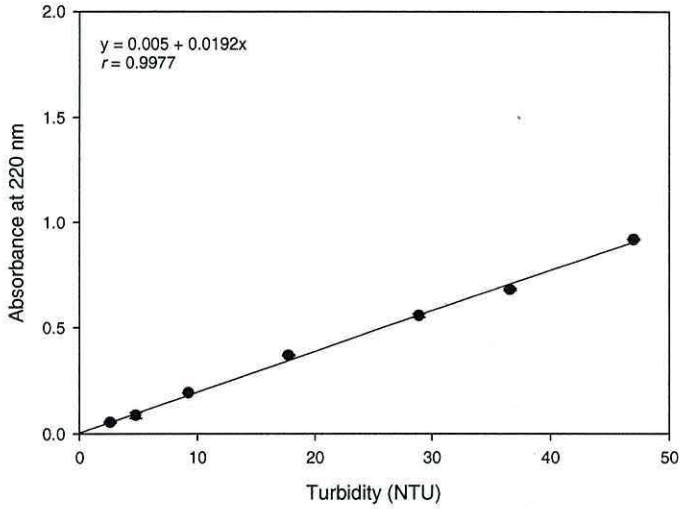

Figure 6.18: The absorbance of formazine solutions with different turbidities at 220 nm. All values represent means ± SEM (n = 3).

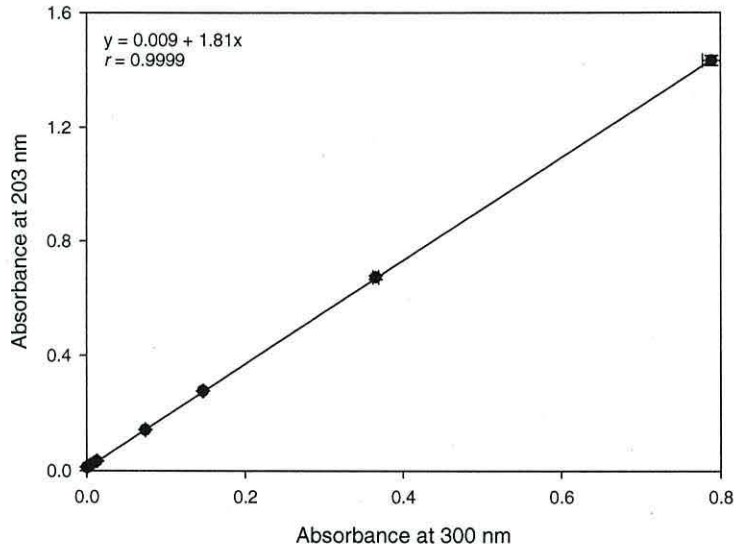

Figure 6.19: The relationship between the humic acid absorbance solutions at 203 nm and 300 nm. All values represent means ± SEM ($n = 3$).

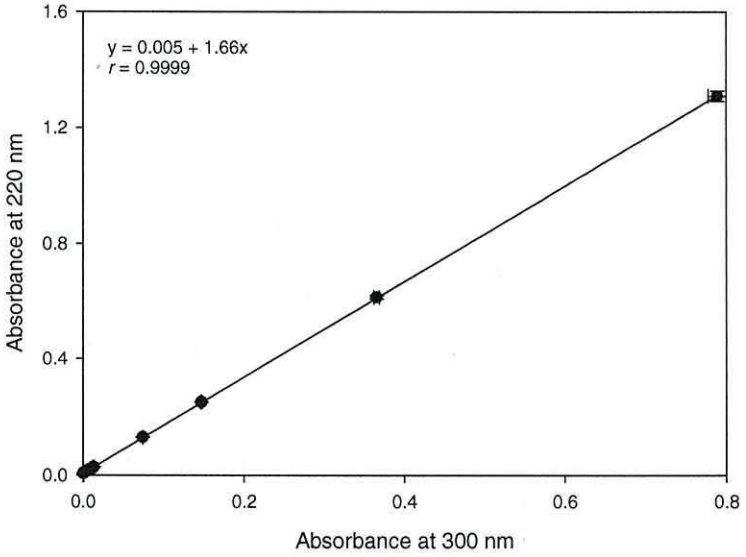

Figure 6.20: The relationship between the humic acid absorbance at 220 nm and 300 nm. All values represent means ± SEM ($n = 3$).

6.3.5 Calculation of nitrate concentration

Firstly, the relationship between DOC absorbance at 203 and 300 nm and also 220 and 300 nm was determined from Figures 6.19 and 6.20. Using the equations from these graphs, the amount of absorbance interference caused by DOC at 203 or 220 nm could be found by measuring the overall absorbance of the sample at 300 nm, where

DOC absorbance at 203 nm = 1.8079 × absorbance at 300 nm
DOC absorbance at 220 nm = 1.6654 × absorbance at 300 nm

The calculated value for DOC can then subtracted from the sample absorbance at 203 nm or 220 nm. If a turbidity correction were required the sample would need to be measured using a turbidity meter and the results used in the equations from Figures 6.17 and 6.18:

Absorbance at 203 nm caused by turbidity = 0.0985 × turbidity of the sample (NTU)
Absorbance at 220 nm caused by turbidity = 0.0194 × turbidity of the sample (NTU)

This value for absorbance was then subtracted from the sample absorbance at 203 nm or 220 nm as with the DOC correction. Finally, to find the absorbance due to nitrate at these wavelengths the equation from the nitrate standards (Figures 6.4 and 6.5) was rearranged and the absorbance values used to find the nitrate concentration whereby,

Nitrate concentration at 203 nm = corrected absorbance value at 203 nm / 0.1453
Nitrate concentration at 220 nm = corrected absorbance value at 220 nm / 0.0568

6.4 Discussion

6.4.1 Investigation of nitrate absorbance

The nitrate scans in Figures 6.1-6.2 are in agreement with those presented in previous studies (Johnson and Coletti, 2002; Ferree and Shannon, 2001). When plotting the results from the scans it was found that the best linear correlation was obtained at 203 nm. Figures 6.4 and 6.5 further show that 203 nm is the best region for monitoring low nitrate concentrations (< 10 mg l^{-1}). Above 30 mg l^{-1} the results become non-linear. The main reasons for deviations from Beer's Law fall into three categories; real, instrumental and chemical. In this case it is unlikely

to be chemical deviation as this normally involves an equilibrium reaction. The real deviation effect is caused because the absorptivity is not dependent on the concentration but actually the refractive index of the solution. The refractive index is essentially constant at low concentrations but changes at high concentrations (Willard et al., 1988; see Chapter 7). The instrumental deviation effect is linked to the relationship between the spectral slit width and the bandwidth of the absorption band. The effect of this relationship often becomes evident at high concentrations where on a plot of absorbance versus concentration, the curve bends towards the concentration axis as in Figures 6.4 and 6.6. Another possible effect is that the not all the incident light is absorbed at higher concentrations, i.e. the efficiency of absorption is lower.

It is a requirement that the commercial sonde be capable of measuring NO_3^- up to 50 mg l^{-1} as this is the area of greatest interest to the water industry. To allow higher concentrations to be measured another wavelength was chosen, in this case 220 nm. This reduces the sensitivity and so extends the linear range. Also this additional measurement could potentially be used to provide a secondary check on the results at 203 nm. Figure 6.6 shows that even at 220 nm the maximum concentration that can be measured using a 10 mm pathlength is 50 mg l^{-1}. To allow for measurement at higher concentrations would therefore require cuvettes with a shorter pathlength or to use a longer wavelength. This is something to consider when designing the commercial sonde, as it may be possible to vary the pathlength to enable different concentration range measurements to be made.

6.4.2 Interferences from other substances found in groundwater

The results indicate that interferences from other anions are unlikely to be a problem in the measurement of groundwater nitrate, with the exception of nitrite. However, in the many conventional methods used to measure nitrate, nitrite is also measured simultaneously and typically included in the nitrate value. Furthermore, nitrite typically represents only a minor component of groundwater soluble N (Freeze and Cherry, 1979) and tends to be short lived in water samples. As the UK Environment Agency frequently refer in their reports to Total Oxidisable Nitrogen (TON) which includes NO_3^- and NO_2^-, the need to separate NO_3^- from NO_2^- is not seen as being of high priority.

The absorbance of nitrate in the region of 200 nm occurs from the $\pi^* \leftarrow \pi$ transition (Tomišić et al., 2005; see Chapter 4). The other anions do not have an electronic transition in this wavelength area and so do not cause an absorbance and therefore there is no appreciable interference. Figure 6.11 also shows that maximum absorbance of nitrite occurs in a different place to nitrate. Rozan and Luther (2002) state that the maximum absorbance for nitrite occurs at 218 nm away from the 203 nm peak for NO_3^- although this may become an issue at the 220nm measurement.

6.4.3 Dissolved organic carbon

The results show that as expected, DOC absorbs strongly in the 200 to 300 nm region continuing up to 600 nm. The results also agree with spectra shown by others (Simonsson et al., 2005) and therefore DOC may represent a real interference problem when analysing NO_3^- in groundwaters. To prevent this, a correction factor needs to be applied, as in previous freshwater experiments (Edwards et al., 2001). A more detailed discussion of the effects of DOC can be found in Chapter 7.

6.4.4 Effect of turbidity

The results show that turbidity is likely to have a significant effect when determining NO_3^- in groundwater. However, the turbidity of groundwater samples is likely to be low (see Chapter 10) suggesting that this may only represent a minor problem. Further, the sonde could also be developed to measure nitrate and turbidity simultaneously. The correction factor shown here requires the use of a turbidimeter to measure the turbidity of the sample, which is then applied to a correction factor that was found using formazine standard solutions. It would also have been possible to use a correction factor purely based on the UV absorbance measurements as in the DOC correction. However, this is not an accepted method of measuring turbidity. Further, in the finished sonde the measurement will be carried out by an independent turbidity sonde using the nephelometric method of measurement and so for the correction factors applied at this stage it was better to use the actual turbidity of the sample.

6.4.5 Calculation of nitrate concentration

Overall, the method showed great potential to be used as an *in-situ* nitrate sonde. The method only requires a small amount of sample and no preparation was needed before measurement as in other methods such as the colorimetric method using hydrazine reduction (Downes, 1978). The correction factor calculations could also be easily incorporated into the existing sonde's software. Further development of the sonde is described in Chapters 7, 8 and 9.

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CHAPTER 7

The influence of different humic acids on the UV method of nitrate measurement

7.1 Introduction

The UV measurement of nitrate in natural waters is subject to interferences, the most significant of these being the interference caused by dissolved organic carbon (DOC) (Edwards et al., 2001; Rennie et al., 1979). This interference is due to the absorbance of DOC in the 200 nm region where nitrate also strongly absorbs. This interference can be utilised, however, to some advantage; 254 nm is frequently used in the measurement of DOC in the wastewater treatment industry (Dobbs et al., 1972; Korshin, et al., 1997) and there has also been investigation into the use of other wavelengths to quantify DOC such as 250, 330 and 350 nm (Hautala et al., 2000).

Absorbance of DOC in the UV region is caused by the molecules containing chromophores. In DOC they include aromatic groups with different types of substitution including phenols and various aromatic acids. The molar absorptivity of these substances is also dependent on the type of organic matter present. Chin et al. (1994) examined the spectroscopic properties of several aquatic fulvic acids, a commercial humic acid and unfractionated organic matter from four natural water samples. After measurements at 280 nm they found a strong correlation between molar absorptivity, total aromacity and the average molecular weights of all the humic substances. The absorbance of humic substances increases with decreasing wavelength, giving a similar pattern to nitrate absorption but extending over a larger range of wavelengths (Hautala et al., 2000). Natural waters that contain humic substances normally exhibit a yellow-brown colour. The measurement of this colour has frequently been used to measure the aquatic humus content and to estimate the humic properties. This measurement can be carried out using a comparator that compares the colour of the water visually to the colour of hexachloroplatinate and cobalt ions in solution to calibrated coloured glass disks (Hautala et al., 2000).

UV spectrophotometry has frequently been used for the measurement of DOC in natural waters. Foster and Morris (1971) investigated the use of UV absorption measurements for the estimation of organic pollution in seawater. The authors found that variation in the absorption over a year depended on the source of the organic matter such as land drainage and biological activity. This resulted in differences in the degree of absorption per unit of DOC and the relative absorption between different wavelength regions. This meant that only a poor correlation between UV absorption and DOC could be obtained.

There have been experiments carried out to assess the suitability of the technique when applied in-situ at water treatment plants. For example, Thomas et al. (1997) investigated the use of UV spectrophotometry for monitoring the TOC content of water leaving a water treatment plant. They achieved good quantification of DOC with the UV procedure having the advantage that the system could be connected to a fast sample loop with measurements taken thereby negating the need for physical sampling that could introduce errors.

DOC enters groundwater systems from a variety of natural and anthropogenic sources (Mertens et al., 2007; Yoo et al., 2006). Examples of DOC pollution in groundwater include groundwater contaminated with chlorophenols (0.7 to 32 mg C l^{-1}; Langwaldt et al., 2005), while landfill leachate is another major source of elevated DOC concentrations in groundwater (Lee et al., 2006). Groundwater DOC concentrations may also provide a good indicator of heavy metal pollution (e.g. As, Hg; Krabbenhoft et al., 1995; Anawar et al., 2003). Natural sources of DOC in groundwater include leachates from overlying organic soils (e.g. peats; Fraser et al., 2001).

Artificial recharge is the use of soil and aquifers as a natural filtration medium to provide good quality drinking water that requires less chemical treatment (Kortelainen et al., 2006). Reducing the concentration of DOC is one of the main challenges for the water industry and understanding the processes which gives rise to high DOC as well as effective removal measures remains a topical issue (Sharp et al., 2006; Murray and Parsons, 2006). Consequently, a sonde capable of quantifying DOC in groundwater may be of interest to water companies who wish to minimise its entry into drink water supplies (e.g. by closing off parts of the well to lower DOC and reduce subsequent treatment/purification costs).

7.2 Materials and methods

After the initial investigations into the effects of DOC using Sigma Humic Acid (Chapter 6), a range of humic acids and natural organic matter standards were obtained from The International Humic Substances Society (IHSS). These humic substances were supplied with information about their source (Table 7.1) and their composition (Table 7.2).

Table 7.1: Information supplied by the IHSS describing the source of the humic substances.

Humic substance	Description provided by IHSS
Leonardite humic acid	Produced by natural oxidation of exposed lignite, a low grade coal.
Elliot soil humic acid	Typical of fertile prairie soils of the states of Indiana, Illinois and Iowa. Sample collected from Joilet, Illinois. Consists of very deep, poorly drained soils on moraines and till plains. Mean annual precipitation is about 825 mm and mean annual air temperature is 10 °C.
Waskish peat humic acid	Collected in Pine Island Bog, Koochiching County, Minnesota. Sphagnum bog peat typical of temperate northern regions. Mean annual precipitation is about 560 mm. Mean annual temperature is 4 °C.
Suwannee River NOM	The Suwannee River rises in the Okefenokee Swamp in south Georgia and flows southwest. At its headwaters in the Okefenokee Swamp the river is a blackwater river with DOC concentrations ranging from 27 – 75 mg l^{-1} and pH values of less than 4.
Nordic Reservoir NOM	Obtained from a drinking water reservoir at Vallsjøen, Skarnes, Norway. The reservoir is at 225 m above sea level and has a maximum depth of 14 m. The sample was taken from a depth of 10 m with a pH of 5.6, temperature of 4 °C and a DOC concentration of 10.7 mg l^{-1}

Table 7.2: The elemental analysis of the different humic acids as provided by IHSS. H_2O is the % (w/w) content of H_2O in an air-equilibrated sample. Ash is the % (w/w) of the inorganic residue in a dry sample. nd = not determined.

Humic Substances	C	H	O	N	S	P	Total	H_2O	Ash
	% (w/w) of a dry ash free sample								
Leonardite humic acid	63.8	3.7	31.3	1.23	0.76	<0.01	100.8	7.2	2.6
Elliot Soil humic acid	58.1	3.7	34.1	4.14	0.44	0.24	100.7	8.2	0.9
Waskish Peat humic acid	54.7	4.0	38.5	1.47	0.36	0.31	99.4	6.9	1.6
Nordic Reservoir NOM	53.2	5.7	nd	1.1	nd	nd	nd	nd	41.4
Suwannee River NOM	48.8	3.9	39.7	1.02	0.60	0.02	101	8.2	7.0

The humic substances were dissolved in distilled water at different concentrations. Three drops of dilute NaOH were added to the Leonardite, Elliot soil and Waskish peat humic acids

to enable them to effectively dissolve. The humic substances were analysed on the UV spectrophotometer from 200 to 600 nm and the results plotted. The dissolved organic carbon and nitrogen contents of the humic substance solutions were measured on a Shimadzu TC-TNV analyser (Shimadzu Corp., Kyoto, Japan). The absorbance of a 50 mg l^{-1} solution of humic substance was measured and used to compare with the N, S and P content of the humic substance to check that the absorbance was caused by the C content and not other elements. The relationship between the humic substance absorbance at 203 and 300 nm was also plotted for each humic substance, as the equation was required during the DOC correction of the nitrate calculation.

To test the method, actual groundwater samples were used from two sites located in Abergwyngregyn, Gwynedd, North Wales (53°14'N, 4°01'W). The two sites were referred to as Gley Field boreholes and Morfa Mawr boreholes. The Gley Field boreholes contained shallow soil/groundwater from a maximum depth of 30 cm, the Morfa Mawr boreholes were located closer to the sea and from a depth of greater than 2 m. For further details and pictures see Chapter 9. The two sites were chosen for this experiment as it was known from initial investigations that they had a wide range of nitrate concentrations. Groundwater was collected at various times from these boreholes, centrifuged at 8000 *g* at 10°C to remove particulates, stored at 4°C and then analysed within 24 h. Nitrate in solution was determined with a Skalar San^{+} segmented flow auto analyser (Skalar UK Ltd., York, UK) and compared with the amount of nitrate determined using the UV spectrophotometer as described in Chapter 6. To decide which humic acid gave the best results when used as a correction factor, the calculation was repeated using the equation for Leonardite humic acid, Elliot soil humic acid, Waskish Peat humic acid, Nordic reservoir NOM, Suwannee river NOM, all of the results combined and Sigma humic acid. The calculation was also performed with no DOC correction to assess if the DOC correction was necessary.

7.3 Results

The absorbances for each humic substance obtained from the International Humic Substances Society are shown in Figures 7.1 and 7.2. Although the absorbance patterns were similar for all types of DOC, the absorbance of the humic acids was higher than the absorbance for the NOM solutions. Figure 7.3 shows a comparison of the absorbance of a 50 mg l^{-1} solution of

humic acid at 300 nm using a 50 mg l^{-1} solution with the percentage of N, P or S in the humic acid. This was to check that the carbon in the compound and not another chemical interference was causing the observed absorption.

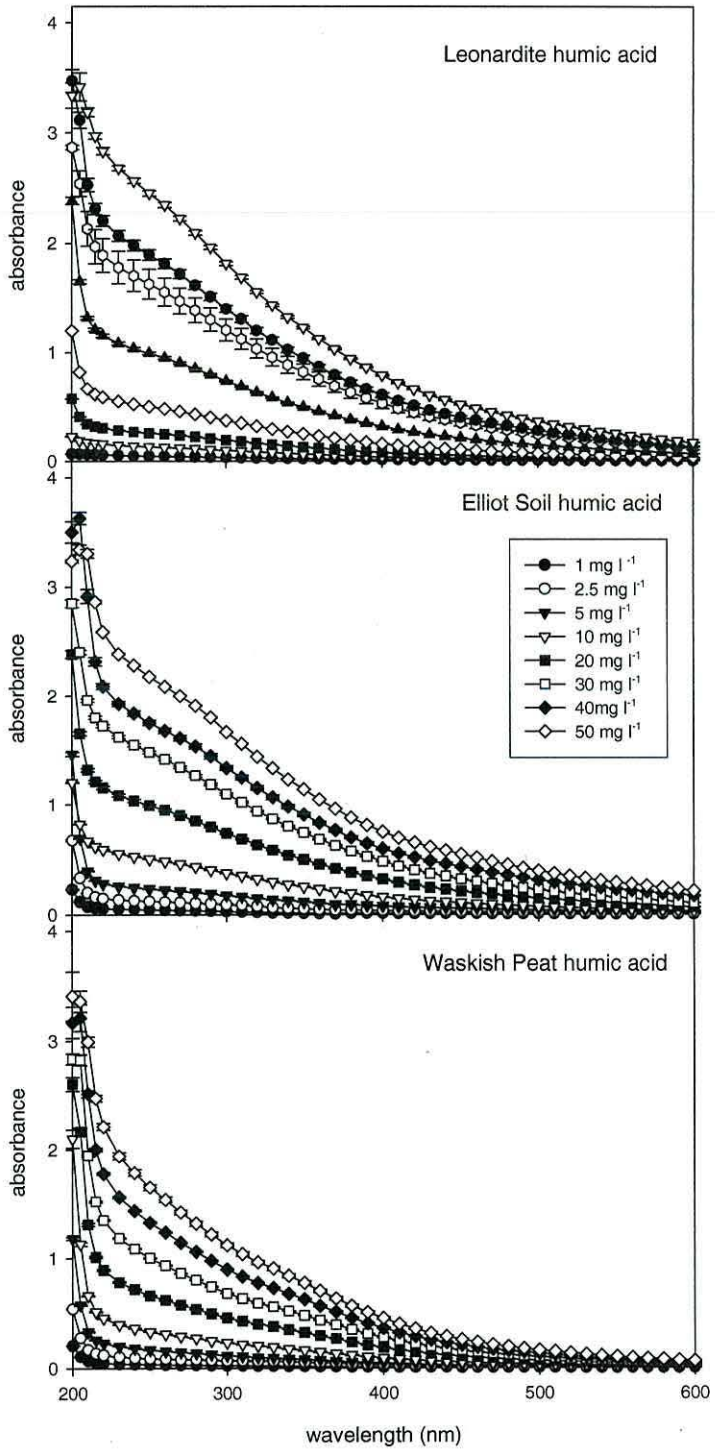

Figure 7.1: The absorbance of a range of concentrations of humic acid samples from the IHSS at wavelengths ranging from 200 to 600 nm. All values represent means ± SEM ($n = 3$).

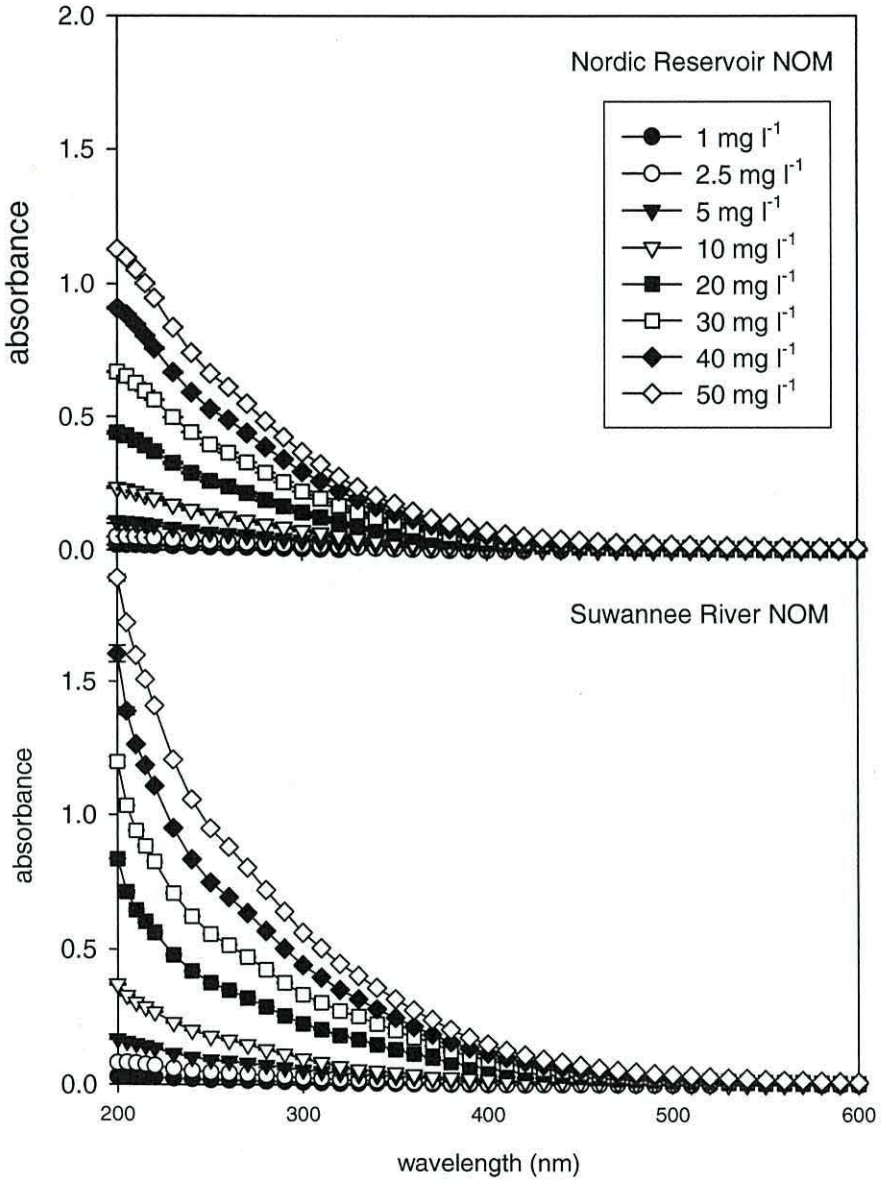

Figure 7.2: The absorbance of a range of concentrations of humic acid samples from the IHSS at wavelengths ranging from 200 to 600 nm. All values represent means ± SEM ($n = 3$)

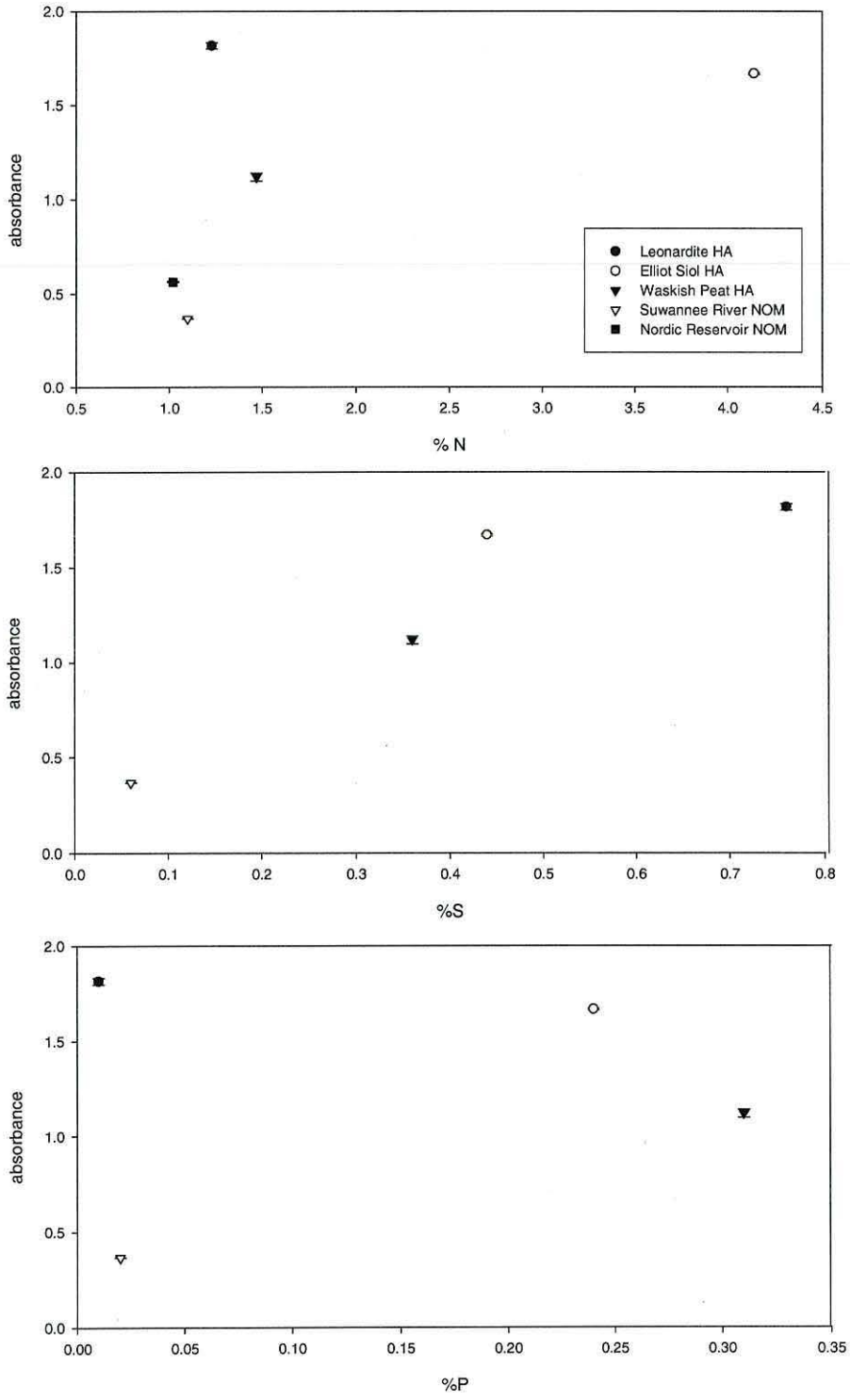

Figure 7.3: A comparison between the percentage of N, P and S in the humic substances as supplied by the IHSS and the absorbance of a 50 mg l^{-1} solution of the substance. (No data supplied for %S or %P for Nordic reservoir NOM). Values represent means ± SEM (n = 3).

The concentrations of DOC in each of the humic acid standards were measured using a TOC-V analyser. The results are shown in Table 7.3 as the known DOC concentration of 50 mg l^{-1} of each individual humic substance in distilled water.

Table 7.3: The concentration of dissolved organic carbon (DOC) and nitrogen (DON) in five humic substances solutions of 50 mg l^{-1}. Values represent means ± SEM (n = 3).

Humic Substance	DOC (mg l^{-1})	DON (mg l^{-1})	C:N ratio
Leonardite humic acid	40.1 ± 1.1	1.1 ± 0.1	40
Elliot Soil humic acid	32.5 ± 1.4	2.0 ± 0.1	16
Waskish Peat humic acid	31.0 ± 2.2	0.6 ± 0.1	51
Suwannee River NOM	23.3 ± 0.5	0.7 ± 0.1	33
Nordic Reservoir NOM	15.8 ± 0.5	0.3 ± 0.1	53

For the DOC correction to be calculated, the absorbance of the humic acids at 203 nm and 300 nm were plotted and this equation used in the calculation. The results for each of these are shown in Figure 7.4 and showed a big difference in the absorbance between the different humic acids, which led to differences in the nitrate concentrations which were subsequently calculated using these equations. For the different humic substances there were varying differences between their absorbance at 203 nm and 220 nm. For Waskish peat humic acid, Nordic reservoir natural organic matter and Sigma humic acid there was very little difference between the absorption at 203 nm and 220 nm. In contrast, for the other humic substances there was a larger difference between the absorption at 203 nm and 220 nm. Figure 7.5 shows all of the results from Figure 7.4 plotted onto the same graph with the data corrected as though the same amount of DOC was present in each solution. The equation was then calculated to see if a range of different DOC sources gave the most accurate equation to be used for the various groundwater samples.

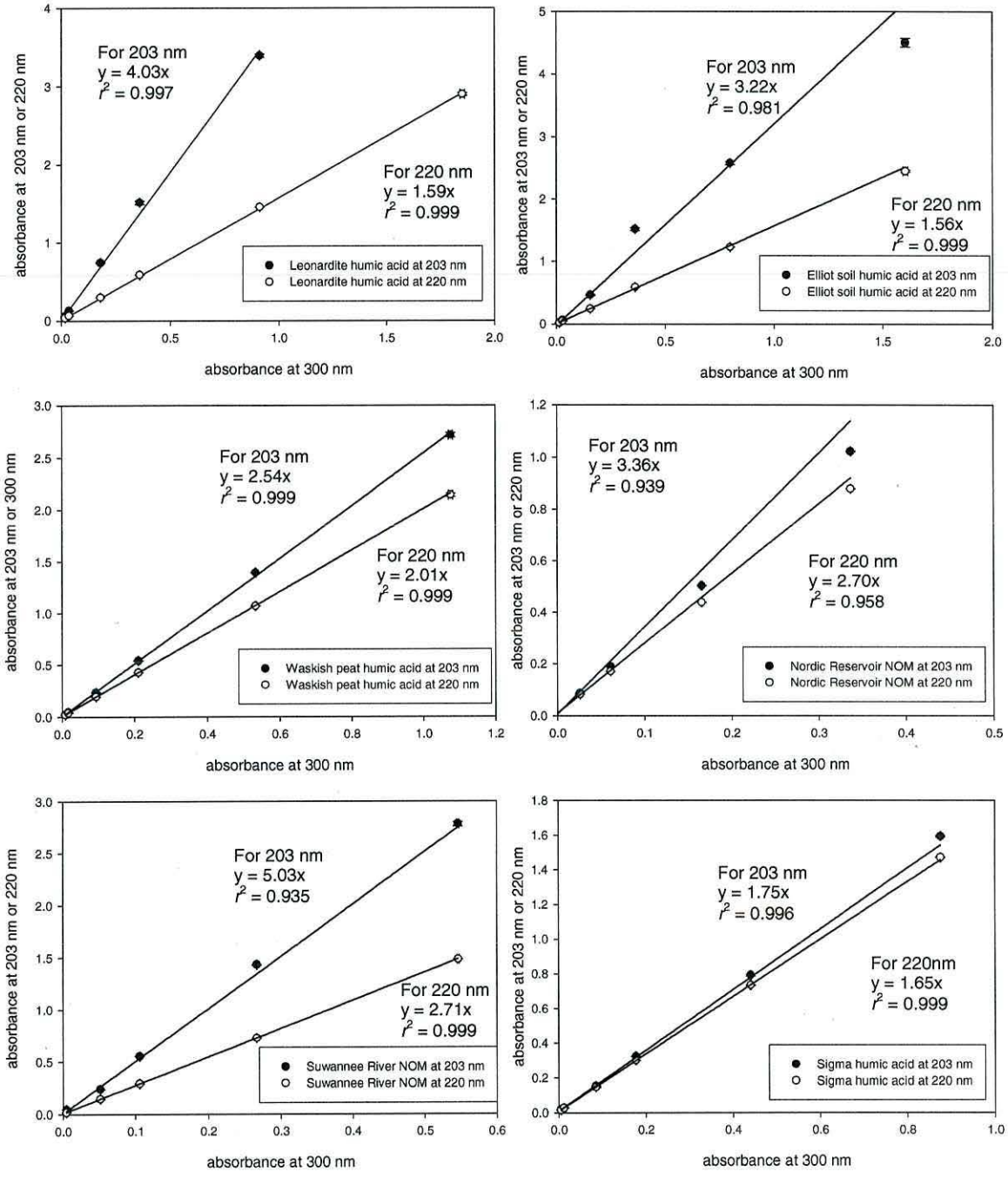

Figure 7.4: Plots of humic substance absorption at 300 nm versus that at either 203 nm or 220 nm. All values represent means ± SEM (n = 3).

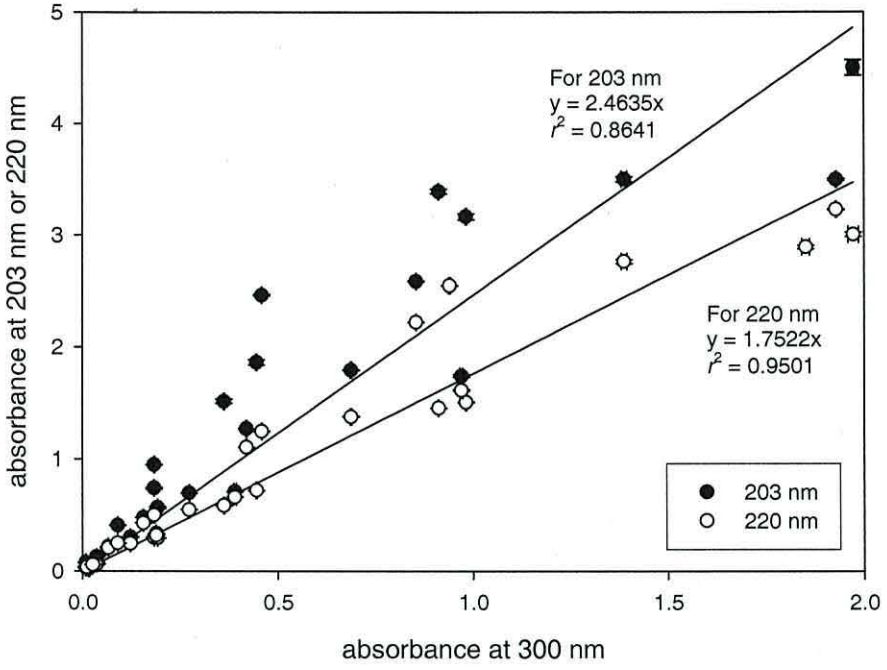

Figure 7.5: The combined absorbance of all of the humic substances at 300 nm versus that obtained at either 203 or 220 nm. All values represent means ± SEM ($n = 3$).

Figures 7.6 and 7.7 show the results of plotting the nitrate concentrations found using UV spectrophotometry and Skalar methods for the same natural samples. Figure 7.6 shows the results from the Gley Field boreholes that were measured at 203 nm. In summary, all of the samples except one had concentrations lower than 15 mg l^{-1} nitrate. In Figure 7.7 the measurements of nitrate concentration in the Morfa Mawr boreholes were made using 220 nm as they all had concentrations higher than 20 mg l^{-1}. The figures show that there was a much greater range of results caused by using the different humic acids at the lower concentrations of nitrate than at the higher concentrations. Table 7.4 shows the values for the equations of the lines shown in Figures 7.6 and 7.7. The results presented in Table 7.4 for the Gley Field boreholes suggests that the equation using all of the results combined together gave the gradient closest to one (i.e. y = 0.9983x) and had a good r^2 value of 0.916. For the Morfa Mawr boreholes the best results were obtained when no DOC correction was performed (y = 1.0043x, r^2 = 0.997). The data in Figures 7.6 and 7.7 were plotted on the same graph (not

shown) to see which gave the best fit between the two sets of results. In this case, the Sigma humic acid gave the best results (y = 1.0061x, r^2 = 0.916).

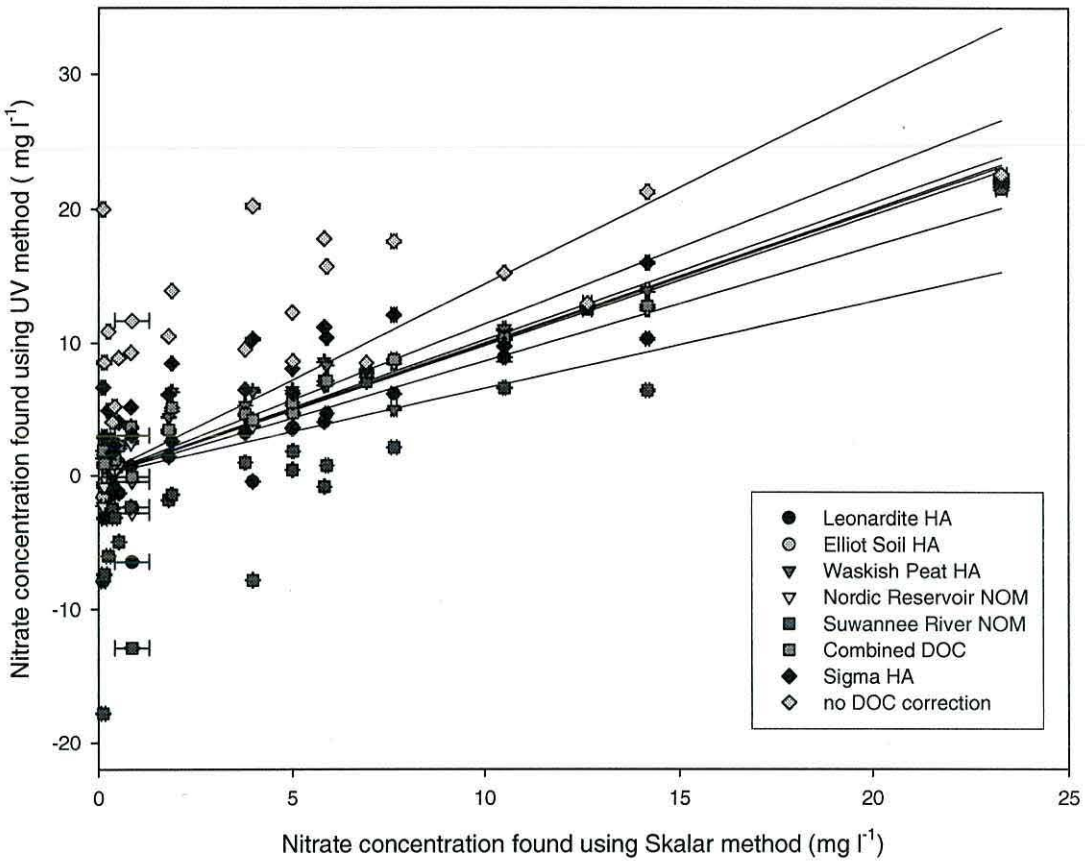

Figure 7.6: A comparison of the nitrate concentration results obtained for groundwater samples taken from the Gley Field boreholes on two separate occasions. The nitrate concentrations were obtained using the UV method at 203 nm and the Skalar hydrazine reduction method. The influences of different types of DOC were compared when used in the equation for nitrate concentration from the UV measurement. All values represent means ± SEM (n = 3).

Table 7.4: A comparison of the equations for the linear regressions performed in Figures 7.5 and 7.6. The nitrate concentrations were calculated using the equations from different humic acids and then compared with the results from the Skalar hydrazine reduction method.

	Gley Field Boreholes (measured at 203 nm)		Morfa Mawr Boreholes (measured at 220 nm)		Results from the Morfa Mawr and Gley Field boreholes plotted on the same graph (graph not shown)	
	Linear regression equation	r^2	Linear regression equation	r^2	Linear regression equation	r^2
Leonardite HA	y = 0.8617x	0.8158	y = 0.9907x	0.9968	y = 0.9767x	0.9767
Elliot Soil HA	y = 0.9914x	0.9388	y = 0.9911x	0.9968	y = 0.9911x	0.9911
Waskish Peat HA	y = 1.0226x	0.8673	y = 0.9871x	0.9959	y = 0.9909x	0.9909
Nordic Reservoir HA	y = 0.9777x	0.9394	y = 0.9817x	0.9941	y = 0.9817x	0.9817
Suwannee River HA	y = 0.6565x	0.3780	y = 0.9808x	0.9937	y = 0.9456x	0.9456
Combined DOC	y = 0.9983x	0.9163	y = 0.9891x	0.9965	y = 0.9894x	0.9894
Sigma HA	y = 1.1400x	0.4311	y = 0.9898x	0.9966	y = 1.0061x	0.9157
No DOC correction	y = 1.4374x	-1.8314	y = 1.0043x	0.9971	y = 1.0514x	0.2835

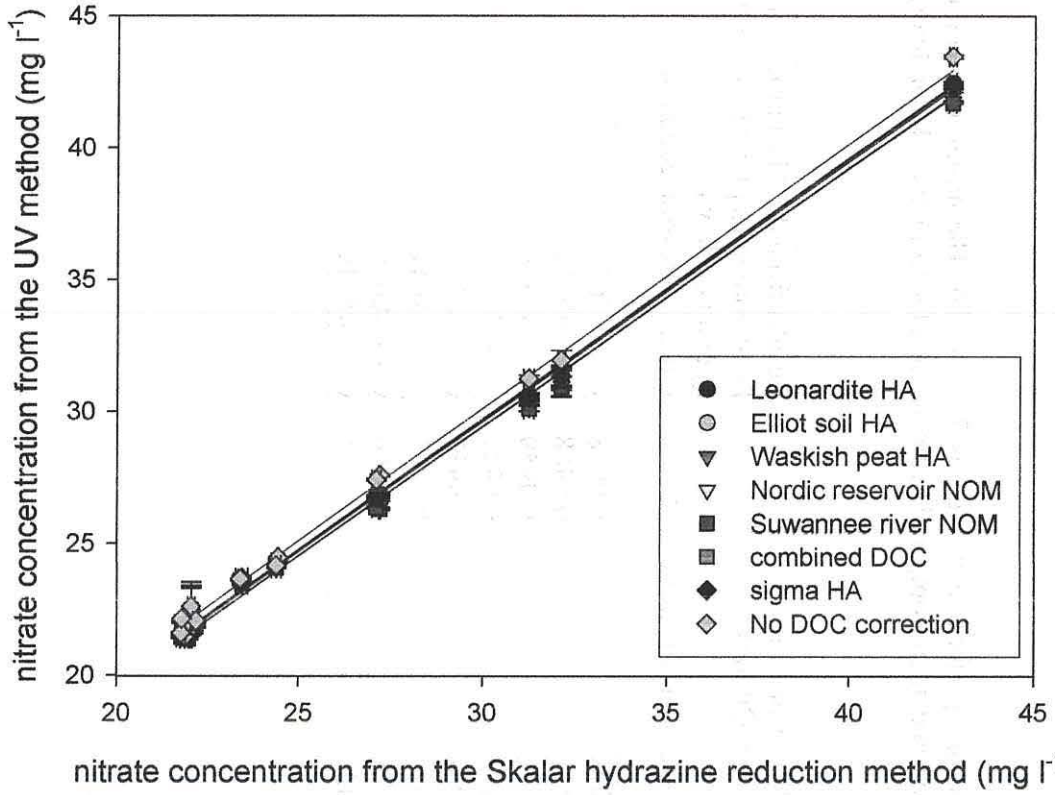

Figure 7.7: A comparison of the nitrate concentration results obtained for groundwater samples taken from the Morfa Mawr boreholes on three separate occasions. Nitrate concentrations were obtained using the UV method at 220 nm and the Skalar hydrazine reduction method. All values represent means ± SEM ($n = 3$).

7.4 Discussion

The absorbance of UV radiation is caused by the excitation of an electron in the molecule. The absorption usually arises from the excitation of bonding electrons and so this absorption can usually be correlated with different types of bond in the molecule (Skoog, 1985). Therefore the difference in absorption between the humic substances is caused by differences in their structure.

The structure and nature of humic substances has been widely debated and investigated (Koch and Dittmar, 2006; Keeler et al., 2006). Originally, the proposed structure of humic substances comprised of randomly coiled macro-molecules that had elongated shapes in basic or low-ionic strength solutions but became coils in acid or high ionic strength solutions (Stevenson, 1994). More recently, however, a new structure of humic substances has been

proposed which consists of supramolecular associations. This involves small and chemically diverse organic molecules forming clusters linked by hydrogen bonds and hydrophobic interactions (Sutton and Sposito, 2005).

The spectra presented in Figure 7.1 and 7.2 are consistent with other UV/visible spectra of humic substances (Jung et al., 2005; McKnight et al., 1997). They all show the interference with the nitrate measurement being maximal between 200 to 230 nm and then the declining absorbance down to 600 nm. Further, all the humic substances possessed different absorbances for the same amount of DOC in solution. A similar finding was reported by Foster and Morris (1971) when they tried to use UV absorbance to investigate and quantify the amount of DOC in water samples.

The humic substances all have different equations for their lines of best fit at 203 and 220 nm as shown in Figure 7.4. Consequently, they all have different values for the $y = mx$ equation and so each correction factor will ultimately yield different results for nitrate concentration. The varying amount of separation between the two lines in Figure 7.4 is likely to be due to the different arrangements of the bonds in the molecules, some of which absorb closer to 200 nm and some closer to 220 nm. These experiments were performed to determine the best correction factor to use in the measurement of nitrate in actual groundwater samples.

Table 7.4 shows the results of using the different correction factors for natural groundwater samples. For the Gley Field boreholes the best results were obtained when the data from the humic substances were plotted on the same graph and adjusted to give the same DOC concentration for each humic solution and the resulting equation was used. The worst results were obtained when no DOC correction was used and also using a correction factor derived from the Suwannee River humic acid. However, these shallow groundwater samples obtained within the soil's Bg horizon are likely to be unrepresentative of that present in deep groundwater boreholes (due to the high amounts of organic inputs in the Bg horizons from roots and microbial turnover etc). This is evidenced by the solutions obtained from the shallow boreholes (< 30 cm depth) being yellow in colour even after centrifugation. In contrast, the Morfa Mawr boreholes gave a better representation of the situations where the sonde will ultimately be used (> 2 m deep) and where the water shows no distinct colouration.

The results from the Morfa Mawr boreholes showed that it was best to have either no DOC correction at all or use the equation derived from the Elliot Soil humic acid. This

correlates with personal communications with Dave Buckley, Alexander Gallagher and Daren Gooddy of the British Geological Survey (unpublished) who stated that the levels of DOC in groundwater boreholes was usually between 1 and 3 mg C l^{-1} and the UK median was 2 mg C l^{-1} with humic substances commonly accounting for ca. 50% of this total. This has important implications for the development of the sonde, as it will need to be decided if it is actually necessary for the DOC correction to be carried out if there is only 2 mg l^{-1} DOC in the groundwater samples. The target end-users are also most interested in nitrate concentrations over 50 mg l^{-1} where interference from DOC is likely to be very small. It is noted, however, that the requirements of the sonde will be different for different customers and therefore its inclusion as a factor in calculating nitrate concentrations may ultimately prove useful for other scenarios where high DOC may occur (e.g. boreholes contaminated with landfill leachate or wastewater) or even in the measurement of DOC.

In conclusion, the humic acid results presented here show that complications can arise when attempting to obtain the most-reliable interference correction factors. Although it is likely that DOC will not be a factor of great importance in the measurement of groundwater boreholes in the majority of the UK, and particularly for drinking water aquifers, it is still important to investigate/quantify the effects of DOC. In the future there may be potential customers who will want to use the sonde in polluted boreholes with high DOC contents (e.g. near waste handling facilities). The results also indicate that it is possible that the method could be used on traditional laboratory-based UV spectrophotometry instruments for natural water samples from places such as upland rivers where the effects of DOC would be much greater (Edwards and Cresser, 1987). In these circumstances it would be vital to pick the best correction factor for the intrinsic conditions.

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Chapter 8

The development of the nitrate sonde

8.1 Introduction

The nitrate sonde involved many phases of development. This chapter explains some of the initial stages of development of the sonde, its preliminary testing in the laboratory and the results from an initial test run down a borehole. The chapter also highlights some of the potential problems that can be encountered when making a new piece of analytical equipment.

8.2 Materials and Methods

8.2.1 Initial testing

To check that the UV-based method could be replicated using components that could physically fit into a groundwater sonde, we produced a test model which contained the required electronics and the light source. The light source is referred to as a fibre light as it has a fibre optic attached to it to provide more flexibility in the component (Fig 8.1). The method was tested with a range of nitrate standards made from $NaNO_3$.

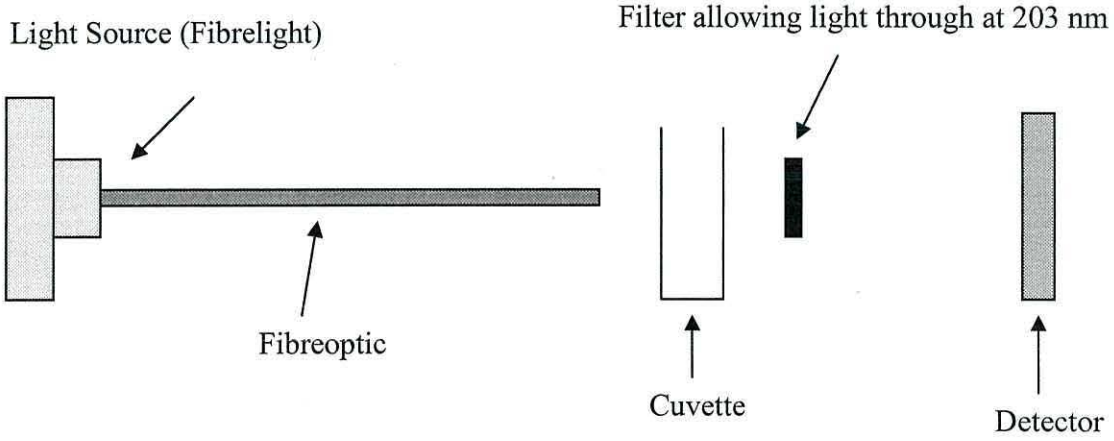


Figure 8.1: The test set up of the UV method showing the positions of the components.

8.2.2 Testing the sonde in the laboratory

To ensure that the sonde was manufactured correctly, the following essential requirements were defined:

- Capable of measuring absorbance in the 200–205 nm range to enable NO_3^- concentrations up to 20 mg l^{-1} to be measured accurately (i.e. to coincide with the maximum absorbance/sensitivity for nitrate).
- Capable of measuring absorbance at 220 nm to enable nitrate concentrations ranging from 20 to 50 mg l^{-1} to be measured. This secondary wavelength could also provide a secondary check for measurements made at lower wavelengths.
- Capable of measuring absorbance at 300 nm to enable determination and subtraction of the dissolved organic carbon (DOC) signal.
- The software needs to be able to correct the absorbance measurement made at 200–205 nm for the presence of DOC and for turbidity and to calculate NO_3^- concentration.

Possible extras for the sonde included:

- Potential to change the pathlength of the measurement to allow greater flexibility in the nitrate concentration range of measurement.
- Addition of pH, temperature and conductivity sensors.

Subsequently, the sonde was designed and constructed with GeoVista in line with these requirements and tested in the laboratory using $NaNO_3$ and humic acid standards.

8.2.3 Testing the sonde in a borehole

The sonde was taken to an exploratory groundwater borehole to check that it could physically withstand field borehole conditions and that it did not leak. The nitrate concentration in the borehole was unknown so the NO_3^- readings were not validated. The sonde was subsequently sent to the British Geological Survey in Wallingford where it is being tested in their boreholes and is likely to require further modifications before it can be sold commercially.

8.3 Results and Discussion

8.3.1 Initial testing

Initial testing of the prototype sonde proved largely unsuccessful with no results obtained matching the pattern of results presented in Chapter 6. Nitrate solutions in the range 0 to 1000 mg l^{-1} were tested and no apparent difference in light transmission was found between them. In an attempt to make the sonde work, many different aspects of the electronic circuitry and structure were changed to see if they were having a deleterious affect on the measurement.

These included: (1) changing the distance from the source to the sample and to the detector, (2) changing the filter type, and (3) placing the filter between the light source and the sample instead of between the sample and the detector. Even with these modifications no meaningful results were obtained. Finally, we directly tested the filter itself as it was deduced that this was not permitting passage of the correct wavelength of light (i.e. and therefore letting through a wavelength where nitrate had no effect on light transmission). To ensure it was transmitting the correct amount of light at the required wavelengths, the filter was removed from the prototype sonde and attached to a bench-top UV spectrophotometer (Unicam UV1, Thermo Electron Corporation, Cambridge, UK). As shown in Figure 8.2, it was shown that the filter transmitted approximately 10% of the incident light in the 200 nm region of the spectrum. Subsequently, another filter identical to the first, but accidentally heated during soldering, was tested. As the second filter gave identical results to the first filter it was concluded that the filter was not the source of the problem.

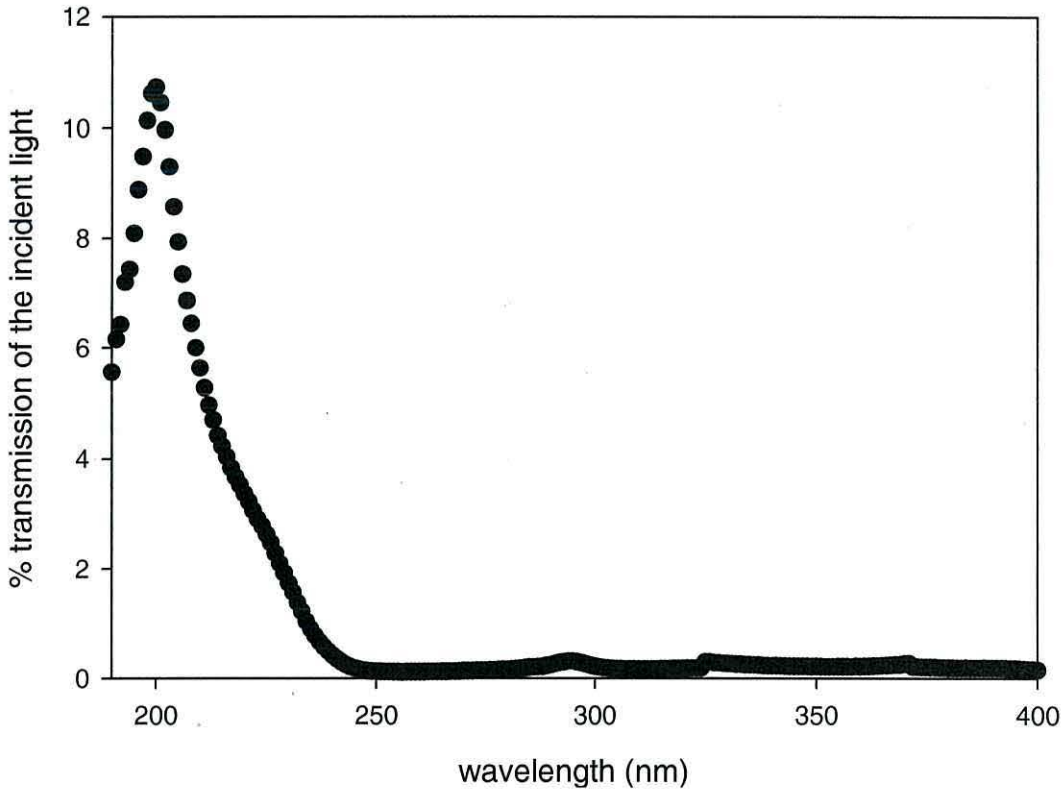


Figure 8.2: The transmission of light using the filter that had been tested in the experimental set up in the sonde.

Next the light source was removed and placed in the UV spectrophotometer to test for defects in this component. The existing deuterium lamp in the UV spectrophotometer was covered (to negate interference) and the fibre light attached into position with the fibre optic cable either attached or removed. Figure 8.3 shows that when the fibre optic was attached there was no light reaching the detector at the correct wavelength of 200 nm and therefore this would directly explain why there was no change in absorbance when different concentrations of nitrate were tested. When the fibre optic was removed it showed the expected transmission of light in the 200 nm range of the spectrum where the light was needed for the nitrate measurement. We therefore eliminated the fibre optic part from the system. Figure 8.4 shows that a strong correlation was achieved between nitrate concentration and absorbance in the revised system ($y = 0.0688x + 0.0183$; $r^2 = 0.995$). At this stage, we were confident that the method was working and consequently we constructed a sonde for field testing.

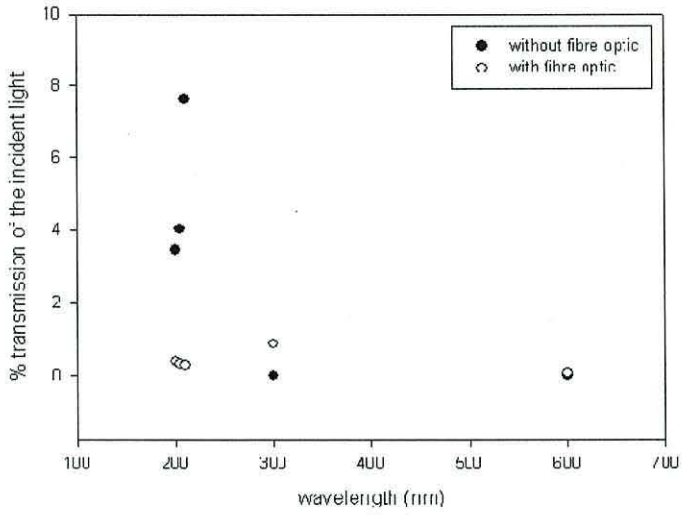


Figure 8.3: The percentage of light transmitted when the light source for the sonde was tested in the UV spectrophotometer with the fibre optic attached and removed

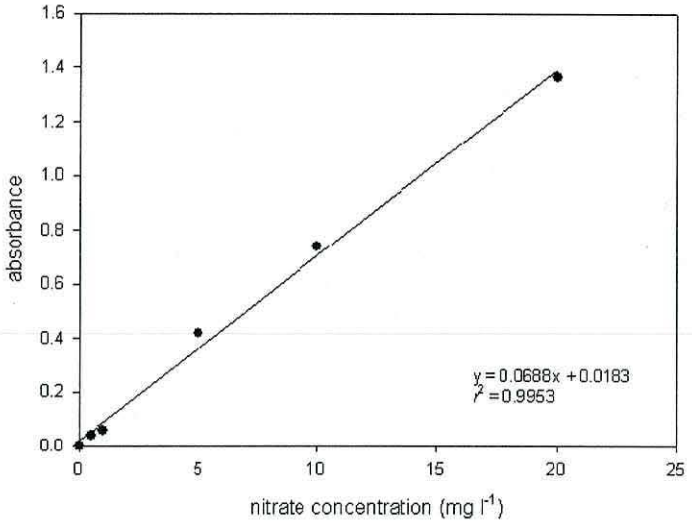


Figure 8.4: The absorbance of the light at different nitrate concentrations using the equipment in Figure 8.1 without the fibre optic.

8.3.2 Testing the sonde

Figures 8.5 to 8.10 show the nitrate sonde and some of the components contained within it. Figure 8.5 shows the size of the sonde (compared with a standard door for scale) while Figure 8.6 is a close-up of the aperture window where the measurements are made. Initially, this was closed off with black electrical tape to allow samples to be measured. Figures 8.7 and 8.8 show the detector part of the sonde with the mechanism for changing the filter in front of the photomultiplier tube. Figures 8.9 and 8.10 show the circuit boards in the sonde for the detector and the emitter parts of the sonde.

Figure 8.5: The nitrate sonde

Figure 8.6: Close up of the sonde showing the opening for the windows where the measurement takes place.

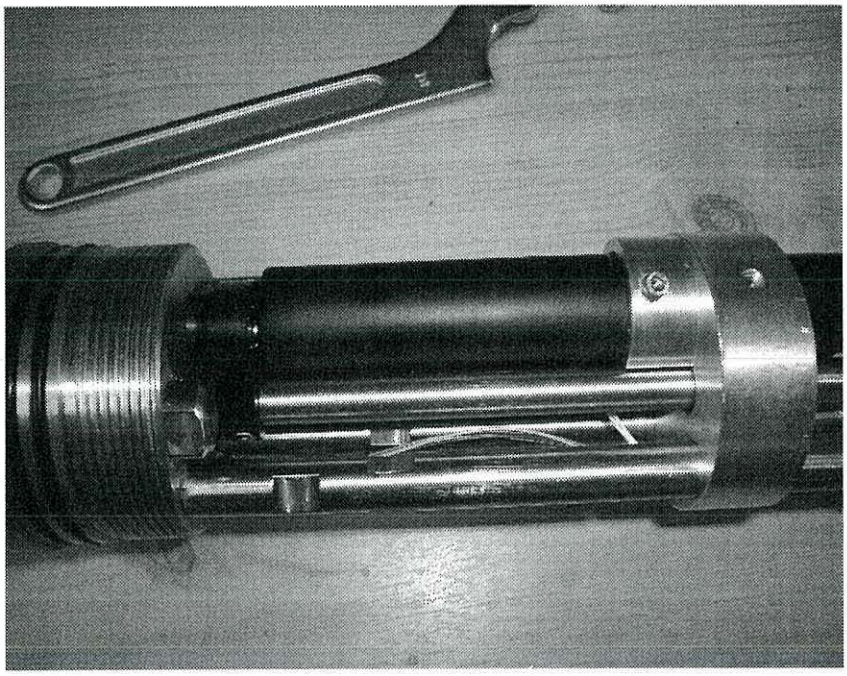


Figure 8.8: A side view of the sonde showing the photomultiplier tube in the detector.

Figure 8.7: The inside of the sonde showing the filter holder which moves the two filters (200 and 350 nm) in front of the photomultiplier tube in the detector.

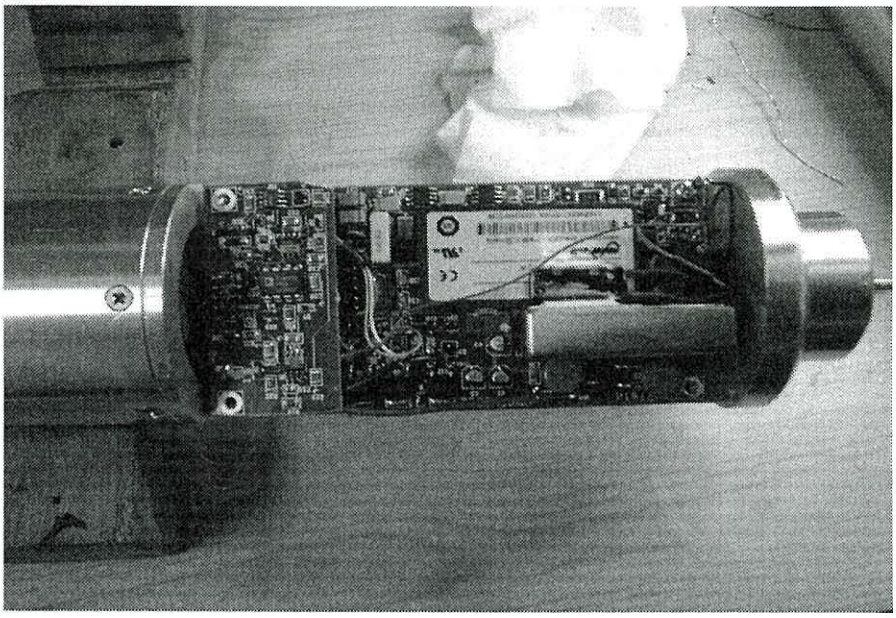


Figure 8.9: Close up of the circuit board for the detector in the sonde.

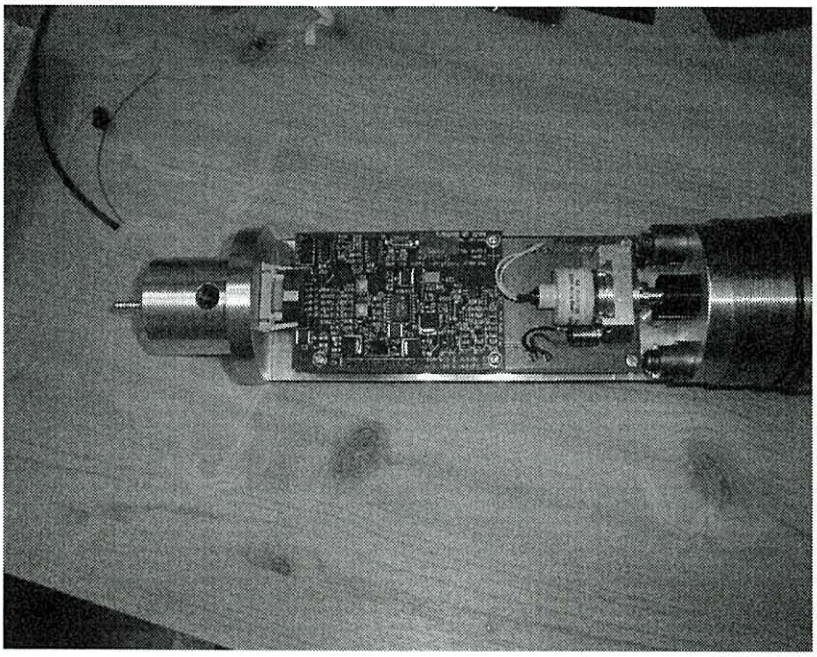


Figure 8.10: Close up of the sonde showing the circuit board and the light source for the emitter.

8.3.3 Testing the sonde in the field

To date, the sonde has only been tested in the field once to check that it could withstand the pressure conditions present in a borehole. The downhole log from this test is shown in Figures 8.10, 8.11 and 8.12. The nitrate concentration in the borehole was unknown, however, the log shows that the nitrate concentration stayed below 20 mg l^{-1} once the sonde had gone through the top layers of the water and the DOC concentration also stayed very low, < 5 mg l^{-1}, which is as expected. The sonde showed good potential to withstand the conditions and so further calibration and testing is ongoing with British Geological Survey.

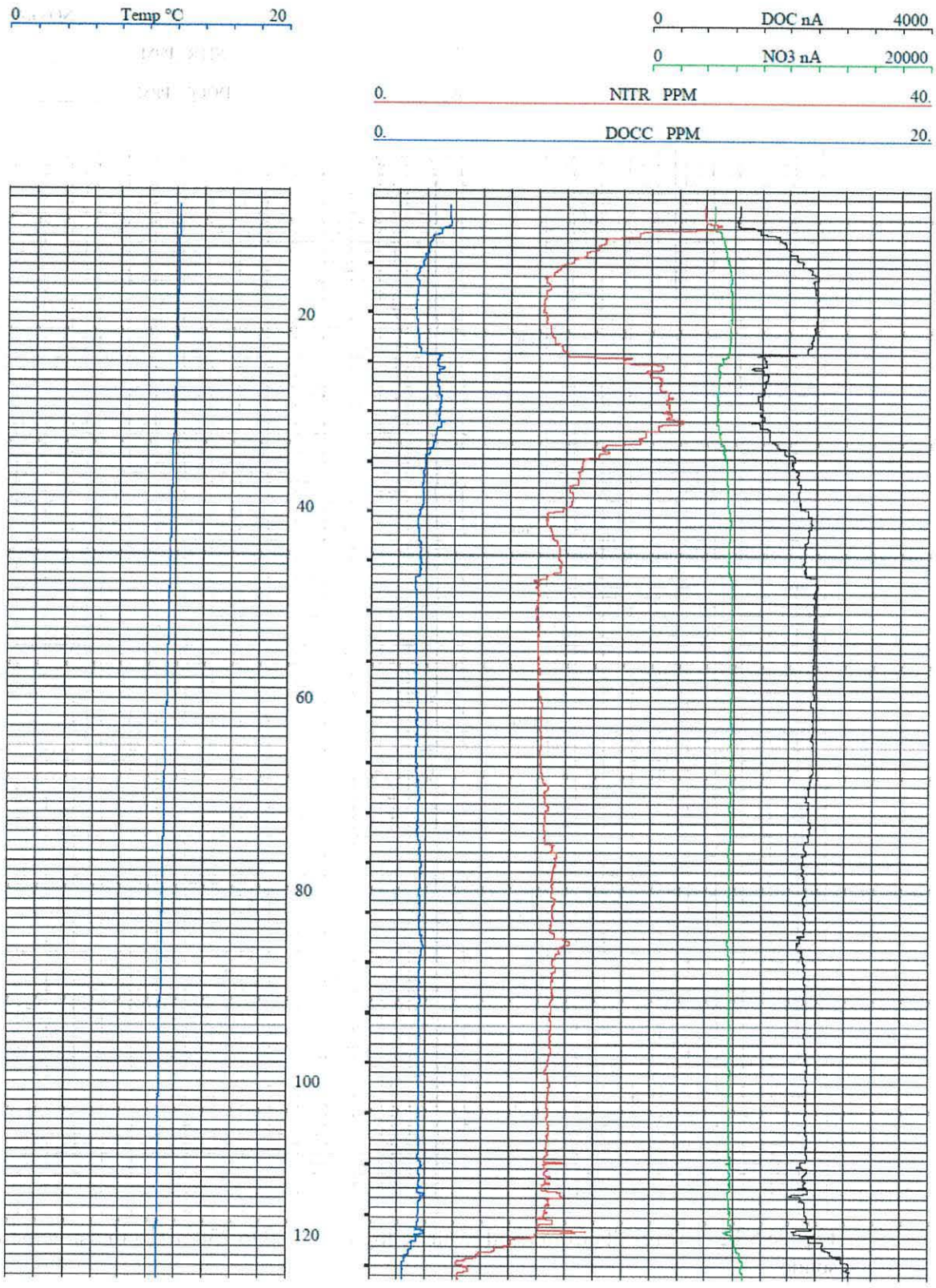


Figure 8.11: The results from the downhole log from 0 to 120 m depth using the nitrate sonde.

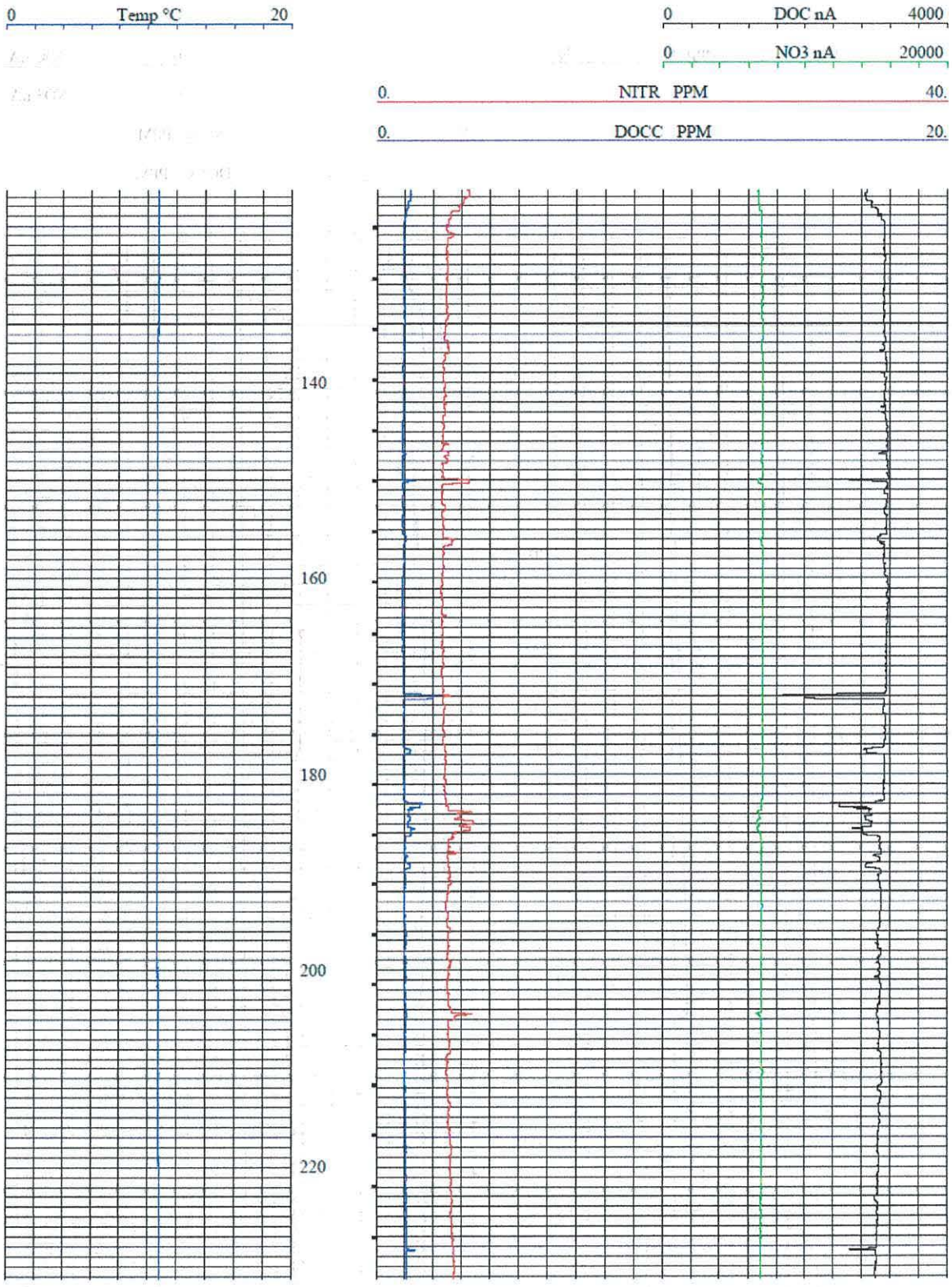


Figure 8.12: The results from the downhole log from 120 to 230 m depth using the nitrate sonde.

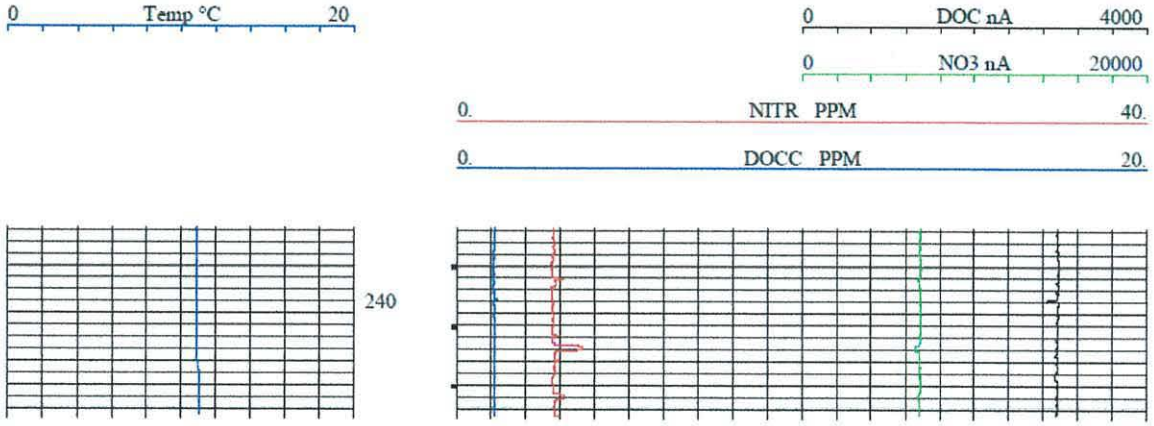


Figure 8.13: The results from the downhole log from 120 to 230 m depth using the nitrate sonde.

8.4 Conclusions

This chapter shows that the design and construction of a novel piece of analytical field equipment can encounter many problems. Getting a robust working model can involve frustration and significant redesign of components. GeoVista had to make many subtle changes to the electronics, software and design of the sonde, which are not documented in this chapter but were vital to the production of the sonde. There will be more decisions to make in the future such as: is the DOC measurement required and useful for the sonde customers and are other modifications required by customers?

CHAPTER 9

Analysis of nitrate in groundwater boreholes using UV spectrophotometry

9.1 Introduction

Groundwater is an increasingly important resource for water supply in Great Britain. It provides approximately a third of public water supplies in England and Wales with the total extraction each year being 2400 million m^3 y^{-1} (Downing, 1998). The main pollutant of this resource is nitrate (Freeze and Cherry, 1979), which due to the nature of groundwater is likely to remain in the aquifer for many years (Knapp, 2005). The contamination of ecosystems by nitrate is becoming increasingly widespread due to the increasing use of inorganic fertilisers, ploughing of old grassland and disposal of organic wastes. Withers and Lord (2002) reviewed the current state of groundwater in the UK. They found that typically, arable and managed grasslands in the UK receive on average 125 kg N ha^{-1} in inorganic fertilisers each year. In addition, inputs of manure must also be considered (e.g. deposited during grazing or applied as an organic fertiliser). The Water Framework Directive (WFD) requires the achievement or maintenance of good ecological status and therefore involves the preservation of good quality water as one factor. This can be achieved through the adoption of river basin management plans (Withers and Lord, 2002). As well as the Water Framework Directive, there are directives relevant to nutrient pollution, which include the Nitrates Directive targeted at drinking water and the Integrated Pollution Prevention and Control Directive targeted at large livestock enterprises (Withers et al., 2000).

The use of UV spectrophotometry in the measurement of nitrate in water has been investigated for use in the laboratory for marine (Foster and Morris (1971), freshwater (Rennie et al., 1979; Edwards et al., 2001) and wastewater samples (Karlsson et al., 1995; Chevalier et al., 2002). There has been more limited investigation into the use of the technique in-situ (Finch et al., 1998; Johnson and Coletti, 2002; Stanley et al., 1994). UV spectrophotometry is possible because nitrate has a strong absorbance in the 200 nm region of the spectrum (Buck et al., 1954). The problem with the measurement of nitrate by UV spectrophotometry is the interference of DOC as it also absorbs in the 200 nm region (Edwards and Cresser, 1997; MacCraith et al., 1994). Edwards et al. (2001) accounted for this interference by calculating the relationship between the absorbance of DOC at 205 nm with the absorbance at 300 nm, as nitrate does not absorb at this wavelength. The absorbance of DOC at 300 nm was then used to calculate the effect that DOC was having on the absorbance at 200 nm when a sample with unknown concentration of DOC was measured.

This chapter shows the development of a sonde to measure the groundwater nitrate concentrations. In particular, one potential application is in boreholes containing high nitrate concentrations. The water pumped to the surface constitutes a mixture of water that has entered the borehole at different depths/geological layers. If a nitrate sonde was lowered down a borehole it could potentially identify the location of the high nitrate water ingress, allowing localised measures to be installed to close off that part of the well. Pinpointing the inflow location may also help identify the original source of the nitrate pollution and allow mitigation measures to be put into place (e.g. if it is largely from surface water ingress). The development of the method, quality control experiments and the development of the sonde are all shown in this chapter.

9.2. Materials and methods

9.2.1 UV measurement of nitrate and DOC

The concentration of nitrate in groundwater was measured using a UV spectrophotometer (Unicam UV1, Thermo Electron Corporation, Cambridge, UK) as described previously. The absorbance at 203 nm was used for the measurement of nitrate concentrations in the range 0 to 20 mg l^{-1} and 220 nm was used for concentrations greater than this. The absorbance of DOC in the same region of the spectrum was calibrated using a Elliot Soil Humic acid standard obtained from the International Humic Substances Society. The humic acid was dissolved in water with a few drops of 1 M NaOH and analysed on the UV spectrophotometer as described previously. A correction factor was subsequently applied for the absorbance caused by DOC in the 200 to 220 nm region. This was achieved by measuring the absorbance of DOC standards at 203 or 220 nm and comparing them with the absorbance at 300 nm where nitrate does not absorb appreciably. This relationship was used to correct for interferences caused by the absorbance of DOC during nitrate measurement.

NO_3^- was determined with a Skalar San$^+$ segmented flow autoanalyser (Skalar UK Ltd., York, UK) using the hydrazine reduction method. This was compared with the NO_3^- concentrations in the samples found using UV spectrophotometry at wavelengths of 203 nm or 220 nm (Unicam UV1, Thermo Electron Corporation, Cambridge, UK) with a correction factor for DOC applied by simultaneously measuring the absorbance at 300 nm.

In many cases, the impact of turbidity was assessed by measuring UV profiles of the samples before and after centrifuging to remove particulate matter.

9.2.2 Groundwater samples

Groundwater samples were collected from a range of locations in England and Wales including: Gwynedd, Dorset, Dee Valley, Wirral, Lancashire and Cumbria. After collection, the groundwater samples were stored at 4°C until analysed.

9.2.2.1 Gley field boreholes

A series of shallow boreholes (< 40 cm deep) were installed at the Henfaes Research Farm, Abergwyngregyn, Gwynedd (53° 14'N, 4°01'W) where the water table is know to approach the soil surface (soil type is a Dystric gleysol of the Cegin Series). This was achieved by inserting plastic drainpipes with open bottoms into the soil as shown in Figure 9.1. After installation, a lid was screwed on and the water level allowed to rise. The insides of the boreholes were emptied and cleaned out three times over the next two weeks using a sponge and water from a nearby stream. This was done to remove soil left after the installation process. The boreholes were allowed to refill before samples were taken.

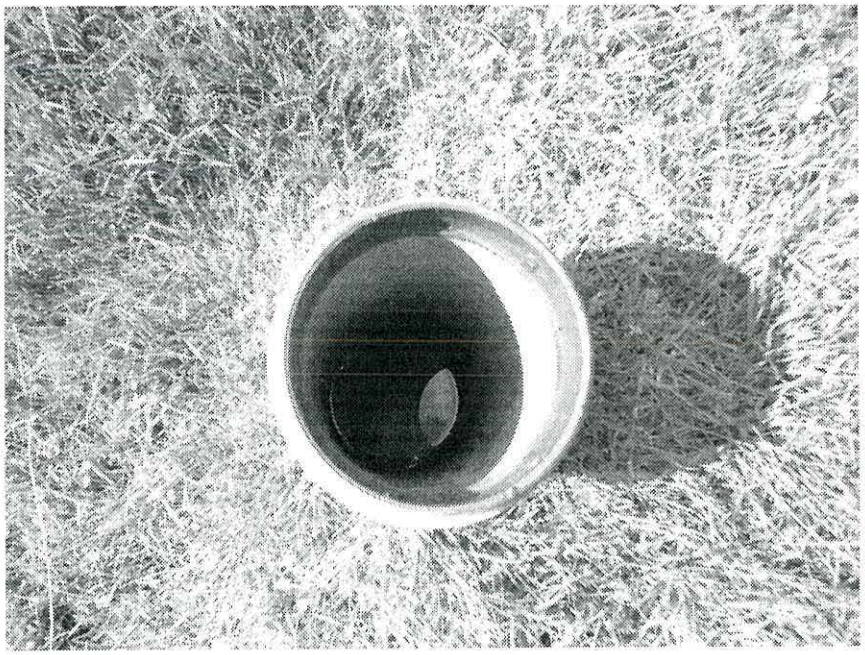


Figure 9.1: The inside of one of the gley field boreholes.

9.2.2.2 Morfa Mawr boreholes

These boreholes were also located at Henfaes and were in Morfa Mawr, a field located close to the sea (soil type is a Eutric cambisol of the Denbigh Series). There were five boreholes in total; the details of which are summarised in Table 9.1.

Table 9.1: Details of the boreholes located in Morfa Mawr field at Henfaes.

Borehole	Depth of borehole (m)	Depth of water table (m)	Further details
B10/1	7.9	Initially 2.6 m, rose to 1.2 m after 20 min.	At bottom of borehole 0.7 m screen, then 4 m of slitted pipe and then solid pipe up to ground level.
B10/2 (main hole)	7.8	1.1	Screen was put at bottom 3 m and then solid pipe up to ground level.
B10/2A	6.8	Initially 1.2, rose to 0.8 m.	Bottom 3 m encased in slitted pipe and rest with solid pipe.
B10/2B	7.4	Initially 4.8, rose to 0.4 m.	Bottom 3 m encased in slitted pipe and rest with solid pipe.
B10/D	7.0	Initially 2.0 m, rose to 1.4 m.	Bottom 4 m encased in slitted pipe and rest with solid pipe.

9.2.2.3 Bishops Court Farm boreholes

Two samples of groundwater were obtained from the British Geological Survey, from Bishops Court Farm, Shapwick, Dorset from a chalk aquifer. Two samples were received which both contained noticeable amounts of "chalky particles". A portion of each sample was filtered and used in the analysis. The raw samples were also used.

9.2.2.4 Bottom Barn borehole

These samples were obtained by the British Geological Survey from a chalk aquifer. Two samples were received. One was obtained 20 minutes into pumping of the well while the other was from the discharge approximately 1 week later.

9.2.2.5 Melling borehole

A groundwater sample was obtained from a sandstone borehole in Lancashire with the help of United Utilities.

9.2.2.6 Dee area boreholes

Groundwater was collected over three days from different boreholes in the River Dee area with the help of the Environment Agency's monitoring and data team. The locations of the

boreholes were Caldy Golf Course (Fig. 9.2), Chester Zoo (Fig. 9.3), Decoy Farm (Fig. 9.4), Hawarden Castle Fruit Farm (Fig. 9.5), Heswall Golf Course, Hill Farm (Fig. 9.6), Mostyn House School (Fig. 9.7), Ness Gardens (Fig. 9.8) and Rough Hill Farm.

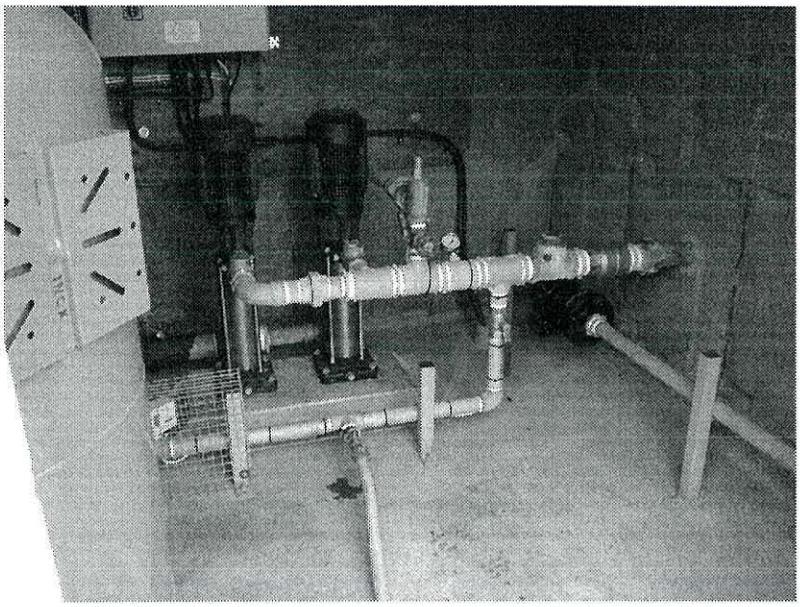


Figure 9.2: The tap at the top of the borehole at Caldy golf course.

Figure 9.3: The tap at the top of the Chester Zoo borehole.

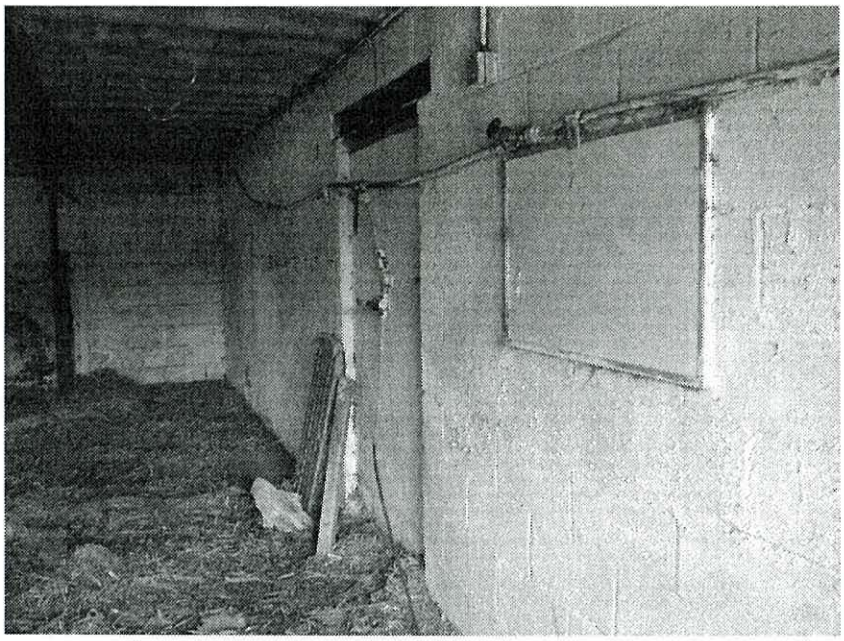


Figure 9.4: The tap at the top of the Decoy farm borehole.

Figure 9.5: The hosepipe and tap at the top of the Hawarden Fruit Farm borehole.

Figure 9.6: The tap and hosepipe for the borehole at Hill Farm.

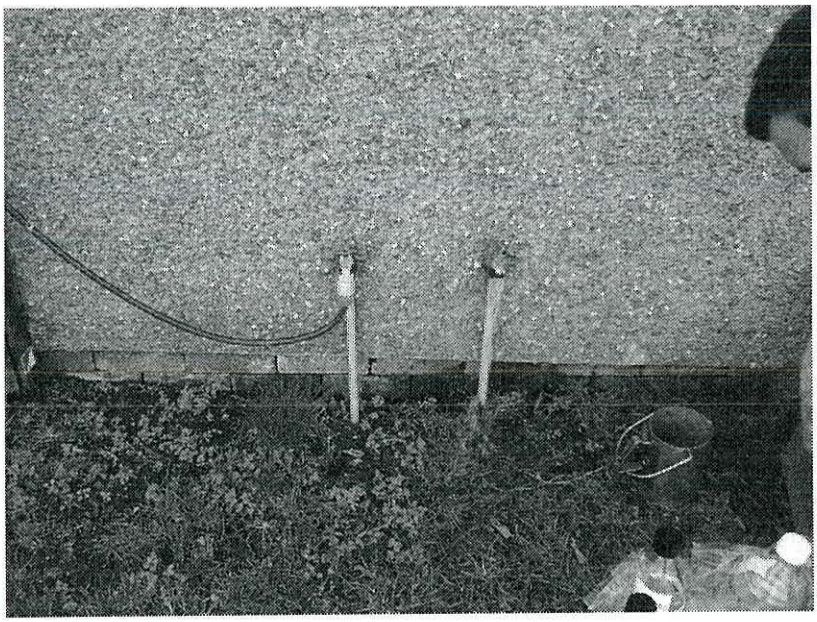


Figure 9.7: The taps at the top of the Mostyn House School borehole.

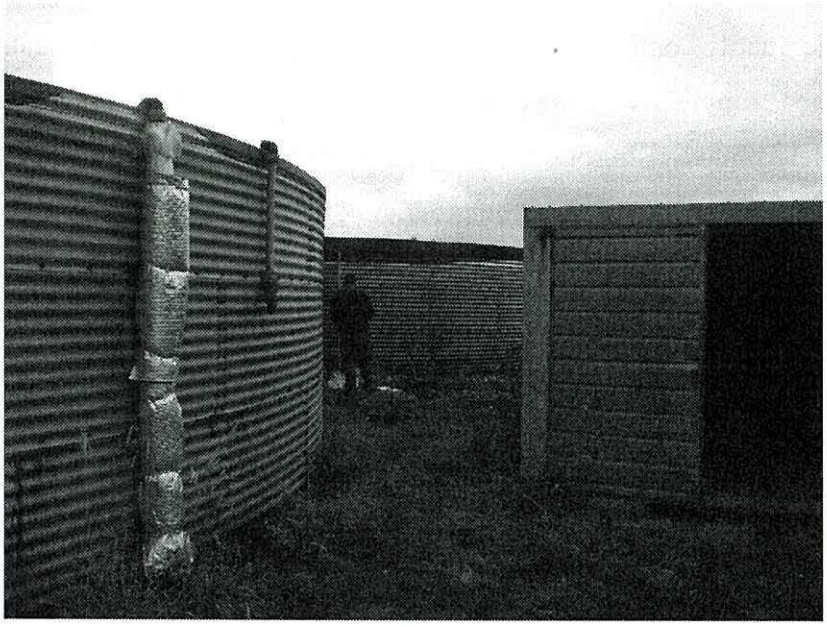


Figure 9.8: The water tanks that are filled using the Ness Gardens Borehole. The water is used to water the plants in the botanical gardens.

9.2.2.7 Langwathby Borehole

Groundwater was collected from Langwathby borehole (Fig. 9.9) during a trip with the British Geological Survey to test the turbidity sonde (Chapter 10). Water was collected from two depths, 12 m and 16 m, using a GeoVista sonde that collects water samples.

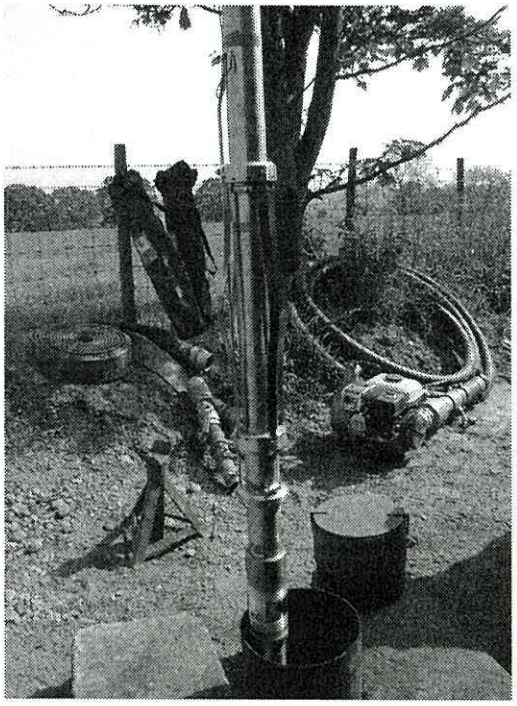


Figure 9.9: The Langwathby borehole with a camera sonde.

9.2.3 Quality control experiment

For the quality control experiments water samples with low concentrations of nitrate were collected from the Gley Field boreholes located in Abergwyngregyn. The NO_3^- in solution was determined using three methods namely: (1) UV spectrophotometry, (2) Skalar autoanalyzer, and (3) ion chromatography (IC; Dionex DX-120, Column: Ionpac AS4A, Eluant: 1.7 mM $NaHCO_3$ / 1.8 mM $NaCO_3$). IC measurements were made by the Centre for Ecology and Hydrology (CEH) in Bangor and performed independently by their technician.

9.2.4 Development and testing of the nitrate sonde

After initial experiments to finalise the design and electronics that would be included in the sonde, GeoVista made a working sonde to test. As the sonde could not physically access many of the groundwater boreholes (due to the presence of taps etc), nitrate concentrations were tested in the laboratory with the sonde by placement of groundwater into the measurement chamber aperture. The sonde was calibrated with solutions of $NaNO_3$ and Humic Acid. Initially, the testing was performed at 203 nm, however, later on the filter was changed to 228 nm (it was not possible to purchase a filter at 220 nm so an adjustment to the method had to be made) to allow higher concentrations of nitrate to be fully evaluated.

9.3. Results

9.3.1 The UV absorbance profiles of the water from the boreholes

The absorbance profiles of solutions taken from the gley field boreholes from Abergwyngregyn are shown in Figures 9.10 to 9.18. The absorbance profiles were measured either before (shaken) or after centrifuging to assess the significance of turbidity interference. The absorbance profiles of the groundwater samples obtained from the five Morfa Mawr boreholes from 200 to 400 nm are shown in Figure 9.19. Similarly, the absorbance of the water samples obtained from the BGS and Environment Agency monitoring sites are shown in Figures 9.20 to 9.31. The UV absorbance of the Langwathby borehole is shown in Figure 9.32.

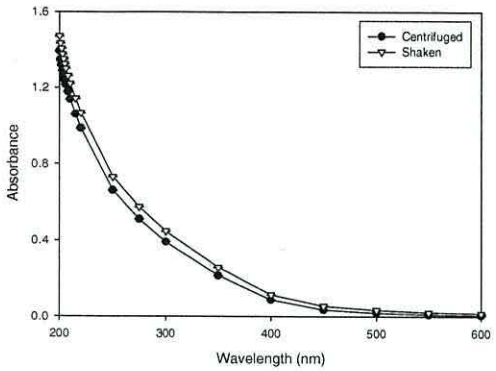


Figure 9.10: The centrifuged and shaken absorbance profiles from Gley Field borehole 1. Values represent means ± SEM (n = 3).

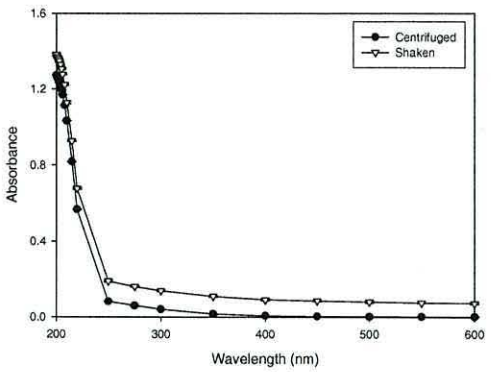


Figure 9.11: The centrifuged and shaken absorbance profiles from Gley Field borehole 2. Values represent means ± SEM (n = 3).

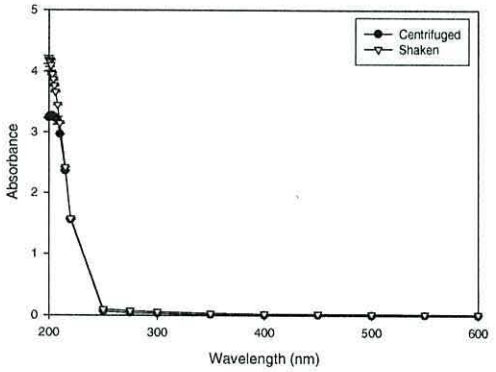


Figure 9.12: The centrifuged and shaken absorbance profiles from Gley Field borehole 3. Values represent means ± SEM (n = 3).

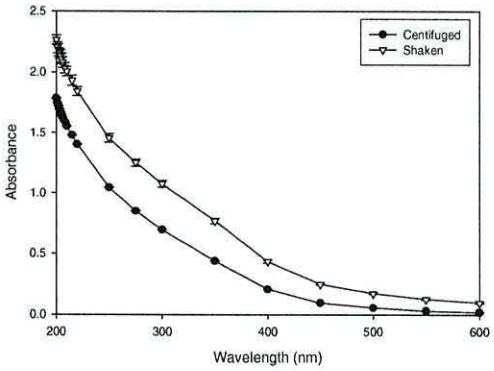


Figure 9.13: The centrifuged and shaken absorbance profiles from Gley Field borehole 4. Values represent means ± SEM (n = 3).

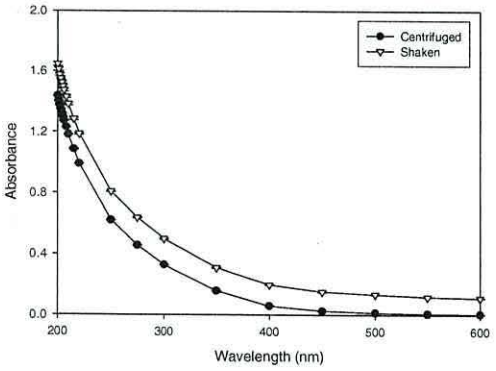


Figure 9.14: The centrifuged and shaken absorbance profiles from Gley Field borehole 6. Values represent means ± SEM (n = 3).

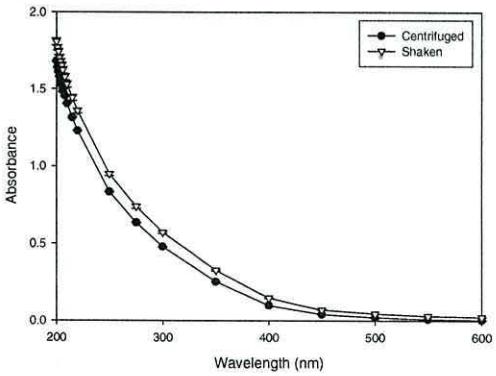


Figure 9.15: The centrifuged and shaken absorbance profiles from Gley Field borehole 7. Values represent means ± SEM (n = 3).

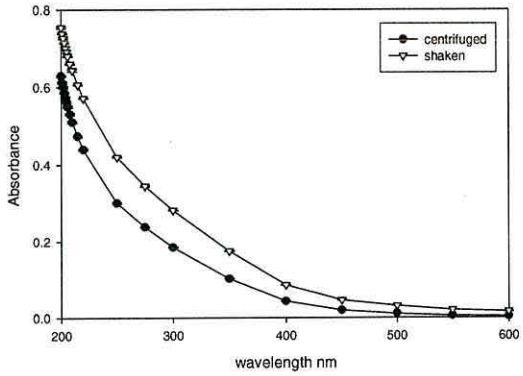


Figure 9.16: The centrifuged and shaken absorbance profiles from Gley Field borehole 9. Values represent means ± SEM (n = 3).

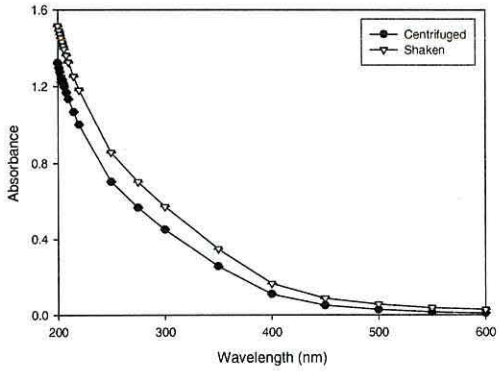


Figure 9.17: The centrifuged and shaken absorbance profiles from Gley Field borehole 11. Values represent means ± SEM (n = 3).

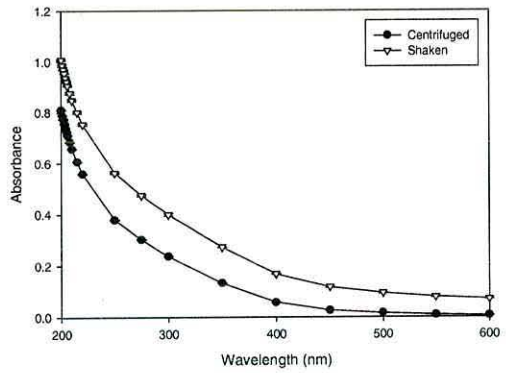


Figure 9.18: The centrifuged and shaken absorbance profiles from Gley Field borehole 10. Values represent means ± SEM (n = 3).

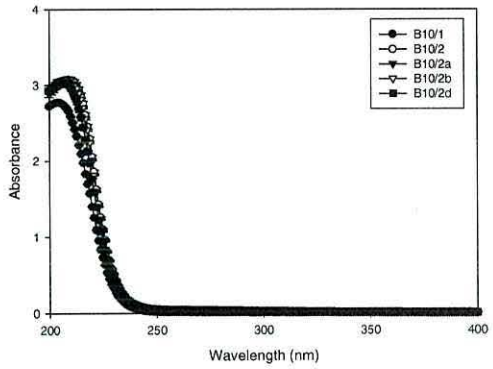


Figure 9.19: The absorbance of the five Morfa Mawr borehole samples from 200 to 400 nm. All values represent means ± SEM (n = 3).

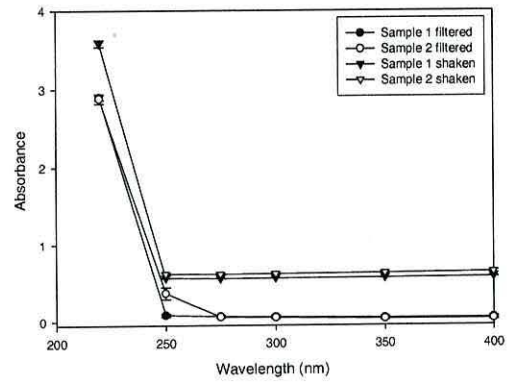


Figure 9.20: The absorbance of the Bishops Court Farm borehole samples from 200 to 400 nm. The results for the filtered sample and the agitated sample are shown. All values represent means ± SEM (n = 3).

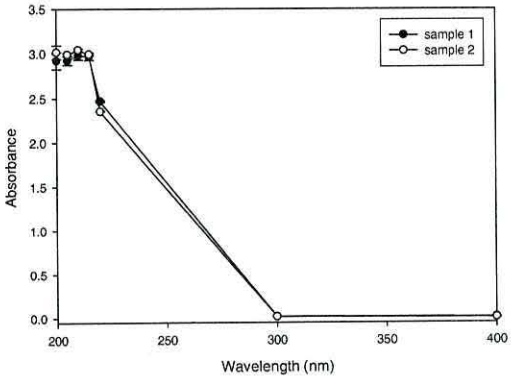


Figure 9.21: The absorbance of the Bottom Barn borehole samples from 200 to 400 nm. All values represent means ± SEM (n = 3).

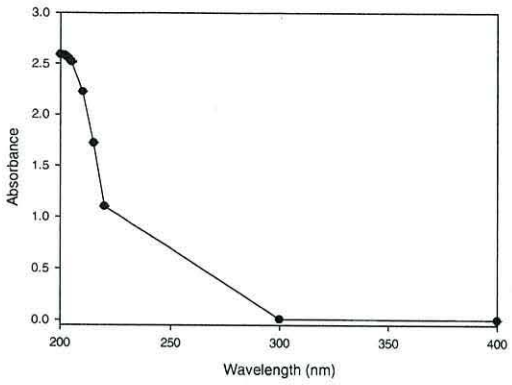


Figure 9.22: The absorbance of the Melling borehole sample from 200 to 400 nm. All values represent means ± SEM (n = 3).

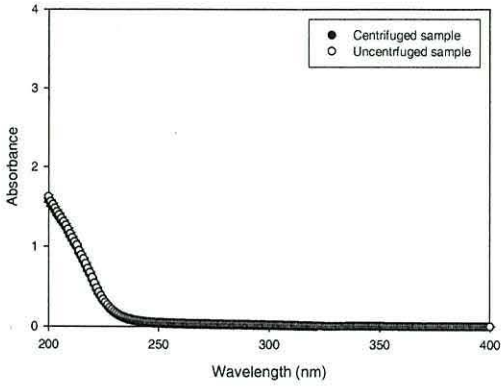


Figure 9.23: The absorbance of the Caldy Golf course samples from 200 to 400 nm. All values represent means ± SEM (n = 3).

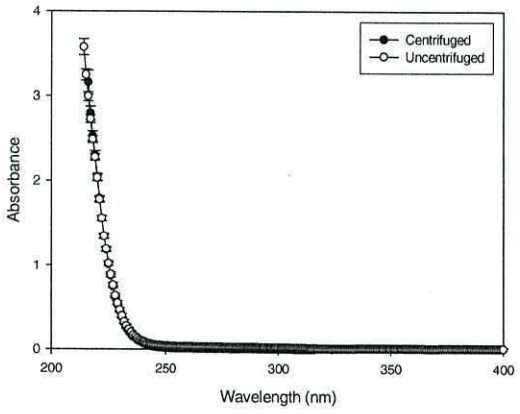


Figure 9.24: The absorbance of the Chester Zoo samples from 200 to 400 nm. All values represent means ± SEM (n = 3).

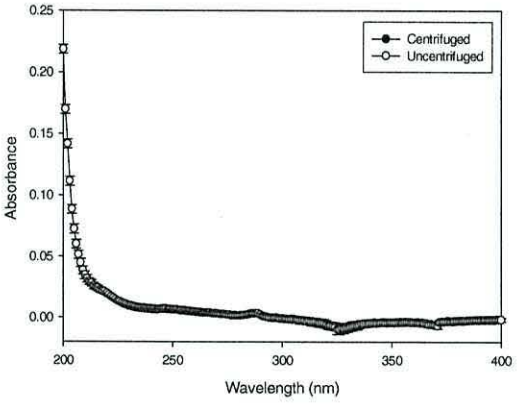


Figure 9.25: The absorbance of the Decoy Farm samples from 200 to 400 nm. All values represent means ± SEM (n = 3).

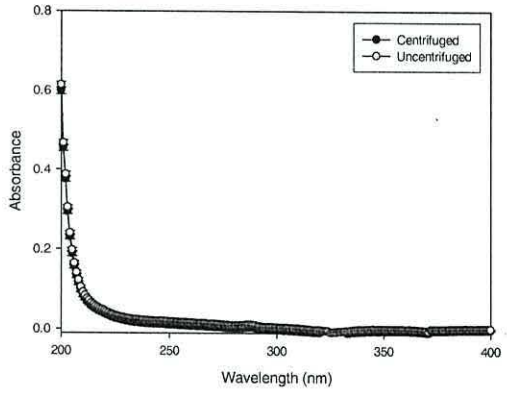


Figure 9.26: The absorbance of the Decoy Farm samples from 200 to 400 nm. All values represent means ± SEM (n = 3).

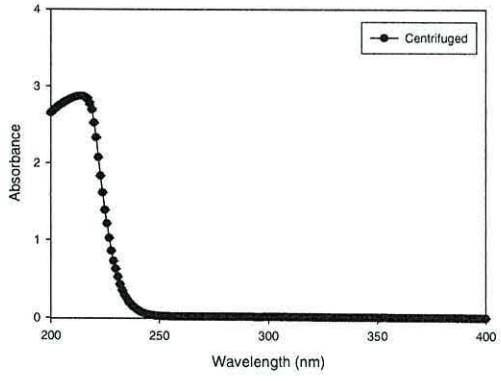


Figure 9.27: The absorbance of the Heswall Golf course samples from 200 to 400 nm. All values represent means ± SEM (n = 3).

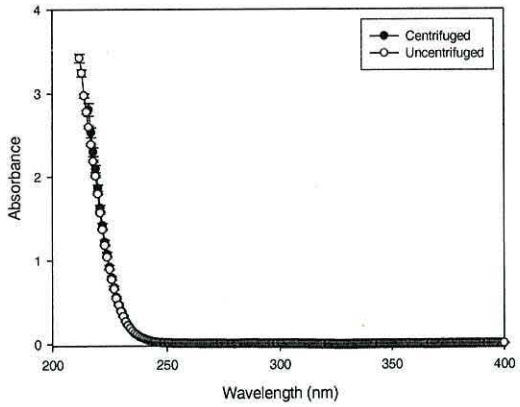


Figure 9.28: The absorbance of the Hill Farm samples from 200 to 400 nm. All values represent means ± SEM (n = 3).

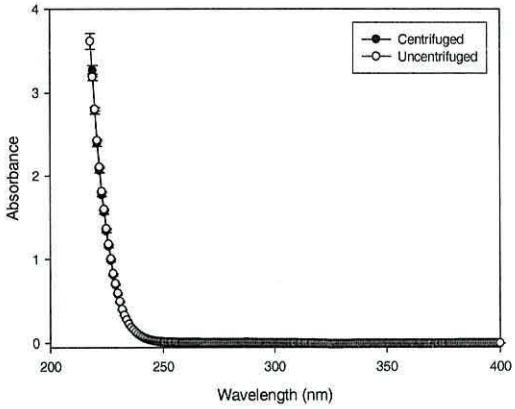


Figure 9.29: The absorbance of the Mostyn House School samples from 200 to 400 nm. All values represent means ± SEM (n = 3)

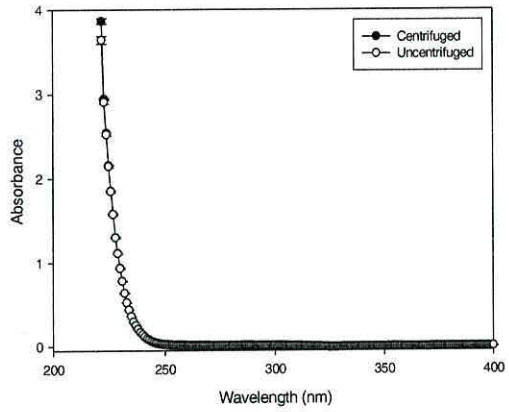


Figure 9.30: The absorbance of the Ness Garden samples from 200 to 400 nm. All values represent means ± SEM (n = 3).

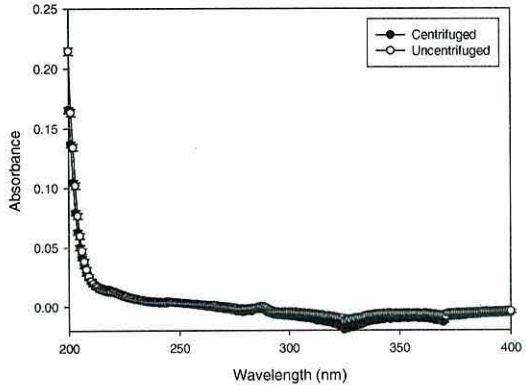


Figure 9.31: The absorbance of the Ness Garden samples from 200 to 400 nm. All values represent means ± SEM (n = 3).

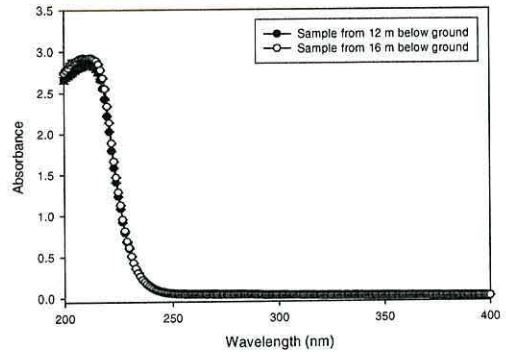


Figure 9.32: The absorbance of the Langwathby borehole samples from depths of 12 m and 16 m from wavelengths 200 to 400 nm. All values represent means ± SEM (n = 3).

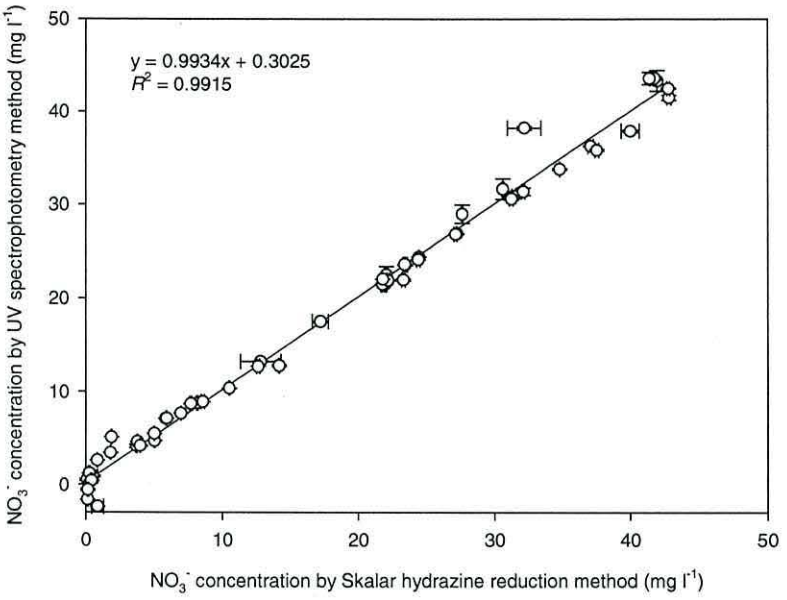


Figure 9.33: Comparison of nitrate concentrations in groundwater samples measured using the Skalar colorimetric and UV spectrophotometry methods. All values represent means ± SEM (n = 3).

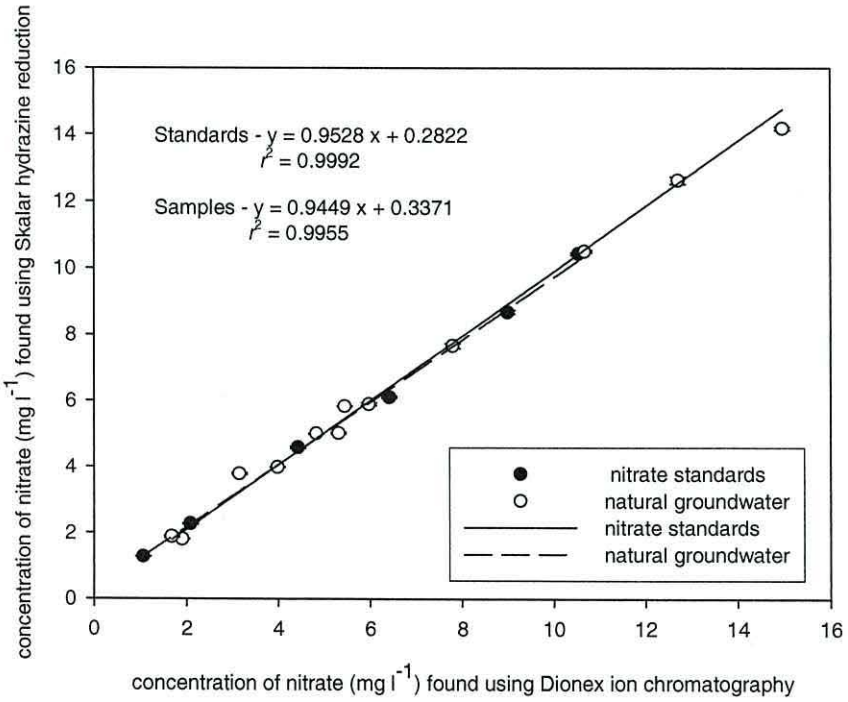
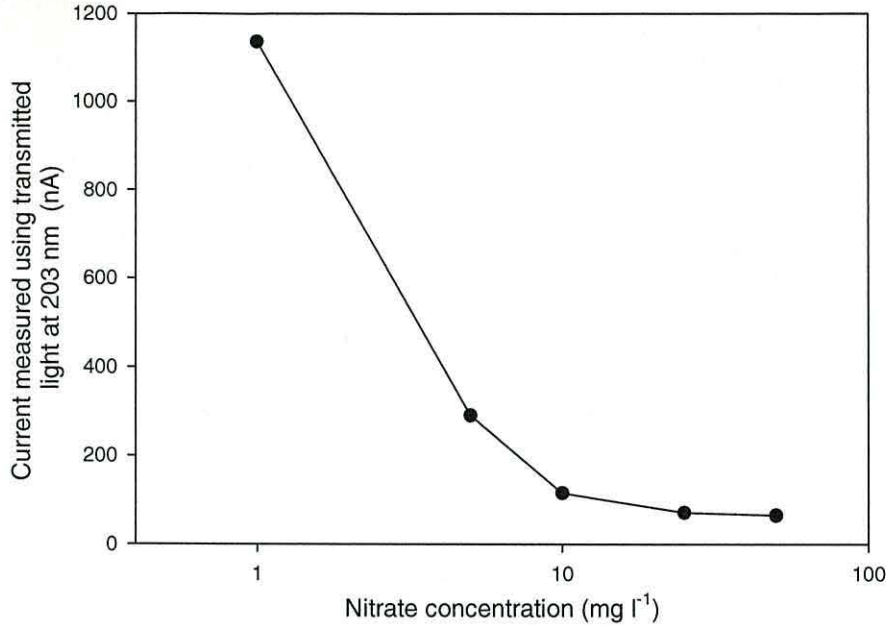


Figure 9.34: Comparison of nitrate concentrations found in groundwater samples and $NaNO_3$ standards using the Skalar hydrazine reduction and Dionex ion chromatography (IC) methods.

Figure 9.33 shows the comparison of the results from the natural groundwater samples when they were analysed using the UV spectrophotometric method and the Skalar hydrazine reduction method. The results show a good correlation with an r^2 value of 0.992 and equation of y = 0.9934 x + 0.3025. The y-intercept value indicates, however, that the UV spectrophotometric method tends to be unreliable at very low nitrate concentrations (i.e. <0.5 mg NO_3^- l^{-1}). Figure 9.34 shows the comparison of the results for the same standards and samples determined by the ion chromatography and Skalar methods. Again, the results showed a good correlation between the two methods (y = 0.9528 x + 0.2822, r^2 = 0.999) giving confidence that the results obtained by the UV spectrophotometry can be effectively compared with the results from the Skalar.

Figures 9.35a and b show the results from the sonde when using two different wavelengths (203 and 228 nm) to perform the nitrate measurement. As expected, the 228 nm filter allowed the determination of much higher concentrations of nitrate.

a)



b)

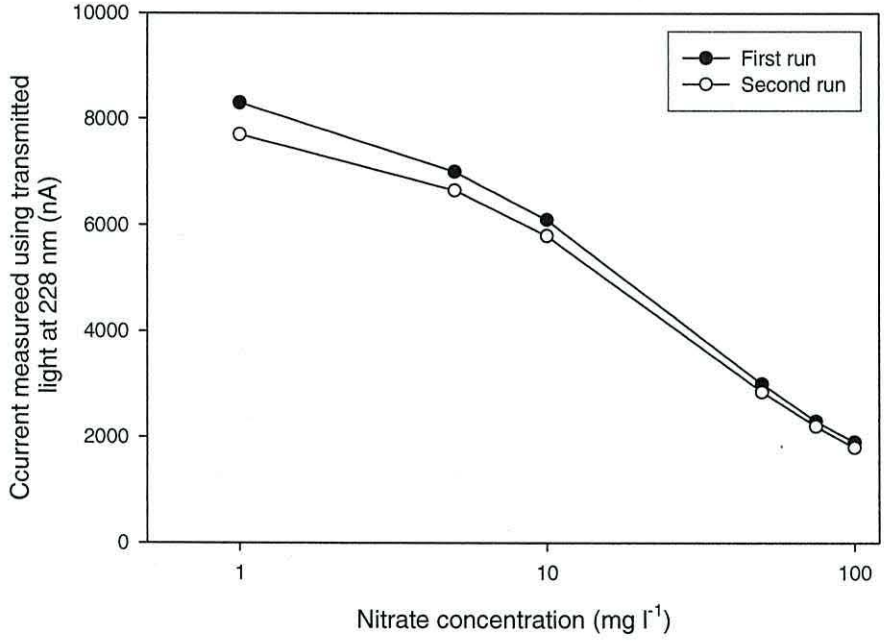


Figure 9.35: The absorbance of nitrate using the sonde with a filter at 203 nm (plate a) and 228 nm (plate b). Plate b also shows the results from two separate measurements to assess the drift that occurred between two discrete runs. All values represent means ± SEM ($n = 3$).

9.4. Discussion

It is a requirement that the commercial sonde be capable of measuring NO_3^- up to at least 50 mg l^{-1} as this is the area of greatest interest to the water industry. To allow higher concentrations to be measured another wavelength was chosen, in this case 228 nm. This reduces the sensitivity and so extends the linear range. Also this additional measurement could potentially be used to provide a secondary check on the results at 203 nm. To allow for measurement at higher concentrations (above 50 mg l^{-1}) would therefore require cuvettes with a shorter pathlength or to use a longer wavelength. This is something to consider when designing the commercial sonde, as it may be possible to vary the pathlength or wavelength to enable different concentration range measurements to be made as required by the customer.

As shown in Figure 9.35, the sonde was initially made with the absorbance measurement occurring at 203 nm, however, this was changed to allow for measurement at higher concentrations as requested by the customer. Figure 9.35b shows a successful application of this up to nitrate concentrations of 100 mg l^{-1}. Figure 9.35b also shows that a small amount of signal drift occurred over time. This was found to be due to temperature and investigated further by cooling the sonde by placing it in a freezer and then allowing it to warm up again. It was found that this caused a very small error (drift of only a few percent in the reading)

Tests using different humic acids to provide the correction factor for actual groundwater samples showed that it was best to have either no DOC correction at all or use the equation derived from the Elliot Soil humic acid. This correlates with communication with the British Geological Survey (not yet published) who stated that the levels of DOC in groundwater boreholes was usually very low and between 1 and 3 mg C l^{-1}. This has important implications for the development of the sonde, as ultimately it will need to be decided if it is actually necessary for the DOC correction at all. The target end-users are also most interested in nitrate concentrations >50 mg l^{-1} and where interference from DOC would be trivial unless heavily polluted with organic C (e.g. landfill leachate where DOC typically ranges from 500 to 10,000 mg C l^{-1}; Jones et al., 2006). Certainly, further tests are required to test its suitability for these potentially extreme environments. Positively, however, the results of the quality control check showed that there was good correlation between the different analytical nitrate methods and statistical analysis (paired t-test) revealed no significant difference ($P > 0.05$) between them. This gives confidence in the new sonde-based approach.

Although not investigated explicitly here, turbidity may also affect the nitrate measurement due to interference in the absorbance by light scattering. This could be corrected for using the turbidity sonde developed by GeoVista and a correction factor applied as for DOC measurement.

9.5. Conclusions

The UV method shows very good potential to be used *in situ* in a sonde. There is potential to combine the sonde with other sondes in a stack to allow for many parameters to enable simultaneous measurements. Further work now needs to be carried out using the sonde in boreholes before it can be commercially released.

9.6 References

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CHAPTER 10

Development of a Groundwater Turbidity Sonde

10.1 Introduction

During the development of the groundwater nitrate sonde it was apparent that some field-collected groundwater samples contained a significant amount of suspended material and consequently appeared turbid to the naked eye. Measurements indicated that this turbidity was significantly affecting the absorbance readings by blocking light transfer through the sample leading to an overestimation of solution NO_3^- concentration. This led to a correction factor being developed for the nitrate measurement and subsequently led to the interest in the development of a stand-alone turbidity sonde. The measurement of turbidity is of importance because it provides an easily measurable indicator of water quality and is of particular relevance when the borehole-derived water is to be used for human consumption (Egorov et al., 2003; Crump et al., 2005). It may also be important in the environmental measurement of groundwater plumes derived form industrial effluents or wastewaters (Vijayaraghavan et al., 2006). Turbidity also represents a long established measure of surface water quality (Wass and Leeks, 1999; Ginting and Mamo, 2006).

The measurement of turbidity undertaken here was carried out following the European Standard Water Quality approved method entitled the Determination of Turbidity (EN ISO 7027:1999; Hongve and Akesson, 1998). In this approved European Standard, two different turbidity measurement methods are specified, the individual use of which depends on the level of turbidity present in the water sample. For high turbidity samples (40 to 4000 NTU) the direct attenuation of a beam of light is measured. The wavelength of the incident radiation should be 860 nm and the measuring angle 0°. In samples containing lower levels of turbidity (0 to 40 NTU) the diffuse radiation at 90° is measured. As most of the natural water samples that the sonde is likely to encounter are expected to have turbidity values in the low range the latter assay (termed the nephelometric method) was used for the development of a new turbidity sonde.

For calibrating the turbidity response, formazine is typically used as the primary standard (Ebie et al., 2006) although the use of secondary standards (e.g. a polystyrene bead suspension) is permitted if verified by formazine standards every 6 months. The range of turbidity that is likely to be of interest in groundwater is from 0 to 50 NTU (Neku and Tandukar, 2003; Templeton et al., 2006). It will be unlikely that natural groundwater will be more turbid than this except during storm events or in areas of high contamination (Norton et

al., 1998; Schafer and Richards, 2005). For example, in a long term monitoring program carried out by United Utilities at Melling,z there were no samples higher than 3.7 NTU from 87 samples taken between 1983 and 2004. The mean turbidity readings from these groundwater samples was 0.50 ± 0.06 NTU lending support to the case that only a very low turbidity measurement range is largely required in a UK context. Other studies have demonstrated a wider variability in water turbidities depending upon geographical location. For example, Lawler et al. (2006) looked at the effect of storms on levels of turbidity in surface waters in the River Tame near Birmingham. A turbidity "rush" was found after the storm event where the turbidity increased from the baseline level of 50 or less NTU to a peak of 500 NTU at its peak. However, this was in a surface water setting and it is likely that boreholes will be subject to less variation due to particle removal by filtration during passage through the soil/aquifer. However, the temporal response to turbidity in response to storm events can be expected to be very context specific. This is exemplified by a study in a Swiss karst groundwater system where the baseline line turbidity level of *ca.* 0.5 NTU rose to 35 NTU in one of the springs during a storm event (Pronk et al., 2006). Amraoui et al. (2003) also studied the turbidity dynamics in a karstic system in the Atlas Mountains of Morocco over a two year period. In normal periods, the turbidity of the spring waters generally ranged between 1 and 2 NTU. After heavy rainfall, however, the turbidity reached a maximum of 250 NTU. In contrast, another study in the Atlas Mountains found that the mean turbidity in the monitored spring was high (*ca.* 24 NTU) but also rose during high rainfall events to peak at *ca.* 200 NTU (Bouchaou et al., 2002).

Prior to undertaking this study, the sponsoring company, GeoVista, had received a number of expressions of interest pertaining to the availability of a turbidity sonde that could be used independently or stacked with other sondes. The latter would allow greater flexibility in assessing water quality. The primary aim of this thesis chapter is to explain the development of an independent turbidity sonde that could be used alone or in a stacked configuration with other sondes. The effect of turbidity on nitrate measurement is shown in chapters 5, 6, 7, 8 and 9.

10.2 Materials and Methods

10.2.1 Formazine turbidity standard

Hexamethylenetetramine ($C_6H_{12}N_4$; 5 g) and hydrazine sulphate ($N_2H_6SO_4$; 0.5 g) were dissolved in separate aliquots of distilled water (*ca.* 40 ml). The two solutions were subsequently combined and diluted to a total volume of 100 ml in a volumetric flask. The solution was then stirred and left to stand for 24 h at 25 °C ± 3 °C. This produced a turbid suspension of 4000 NTU, which was subsequently diluted to yield a range of standard concentrations. A commercial turbidity standard (Hach Stablcal Turbidity Standard Solution, Hach Lange Ltd, Manchester, UK) was also used as it contained stabilised formazine and possessed a longer shelf life (> 1 y).

10.2.2 Initial investigations

The light absorbance of formazine in solution was investigated using UV-visible spectrophotometry so that in the future the nitrate and turbidity measurements could potentially be made simultaneously using the same analytical sonde. For comparison to the formazine standard solution, the absorbance of different concentrations of two clay mineral suspensions (kaolinite and chlorite) were also measured.

A prototype turbidity meter was initially constructed so that the electronics could be tested and validated in isolation prior to its incorporation into a sonde. This was achieved using a black plastic ring and a glass tube (Fig. 10.1). Briefly, holes were drilled in the plastic ring and two detectors and two emitters were inserted into the ring so that they were shining into the centre. The circuit board and electronics were connected to the prototype meter by a long wire so that it would not come into direct contact with water. The glass tube was then slid into the middle of the tube as shown in Figure 10.1. The emitters were turned on alternately with a dark period in between and the direct and diffuse light levels were measured. The electronics allowed different drives to be applied to the emitters, which in turn gave seven different light emission levels. In these initial investigations, outputs from all seven light emission levels were measured so that the best light level for the required measurement (i.e. signal) could be found.

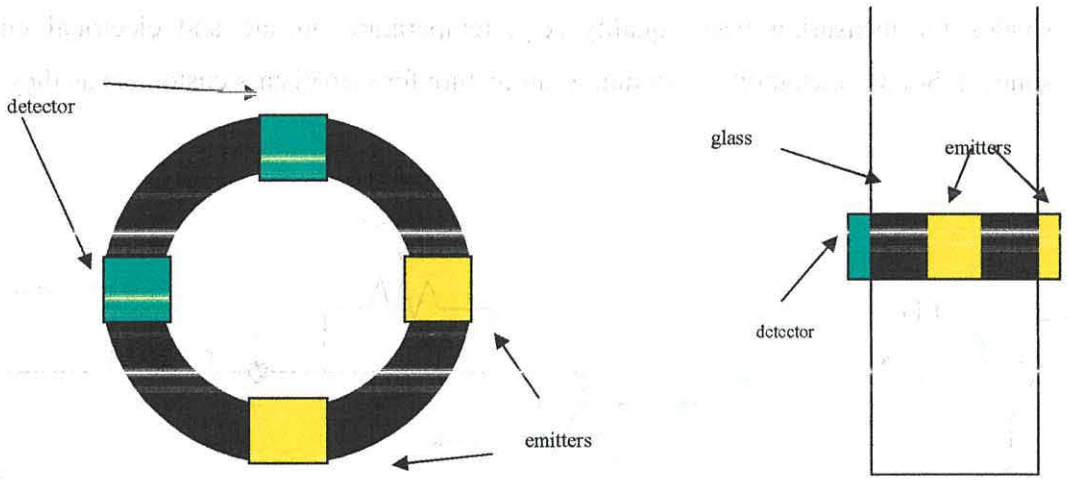

Figure 10.1: Schematic representation of the design of the prototype turbidity meter showing the location of the detectors and emitters.

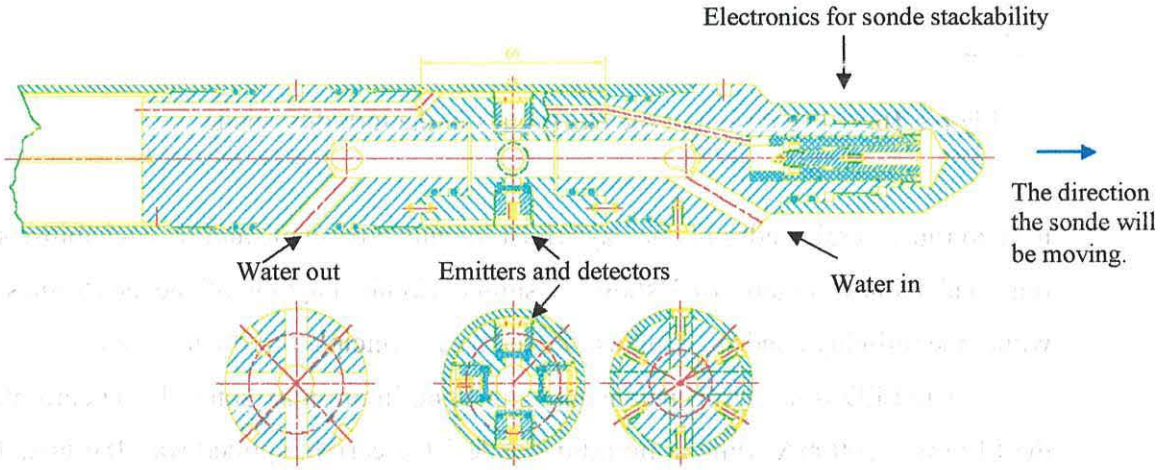

Figure 10.2: The design of the turbidity sonde showing the entrance and exit for the water and the positioning of the emitters and detectors.

The final design for the turbidity sonde is shown in Figure 10.2 and pictures a side view and a cross sectional view of the sonde. Ultimately, the design of the sonde was formulated to allow the continuous logging of water turbidity while being lowered down the borehole in conjunction with a range of other sondes. The turbidity sonde will be the first analytical device in series so that particles in the water are not disturbed by the passage of the other sondes present. It was envisaged that the turbidity sonde would be stacked with other

sondes for measuring water quality (e.g. temperature, nitrate and electrical conductivity sondes). Sonde stackability is an important feature for GeoVista's customers as they often

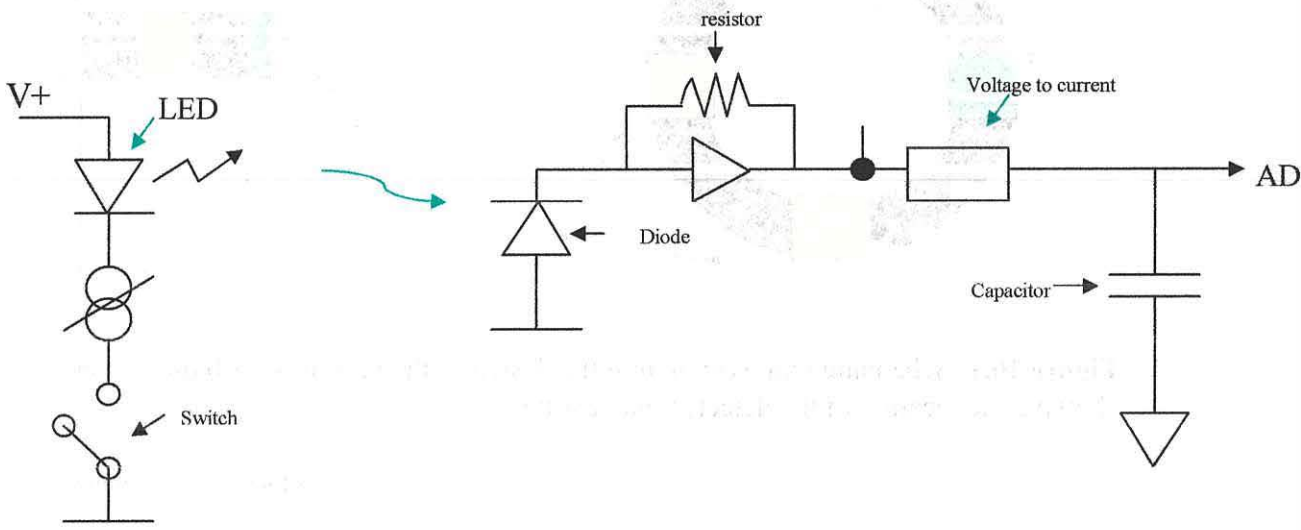

Figure 10.3: The design of the electronics in the turbidity sonde

have to run several sondes in one day whilst logging a borehole and so stackability saves them time and money. Figure 10.3 shows a simple circuit diagram of the electronics contained within the turbidity sonde to demonstrate the basic principle of how it works.

The LED is used to generate and emit light. In turbidity sonde 1, the current applied to the LED was 200 mA while in turbidity sonde 2 the current applied was 480 mA. The switch was turned on for 10 milliseconds at a time. The light generated by the LED is subsequently shone through the liquid sample with light scattered depending on the turbidity present. The detector measures the amount of light scattering. At this point the voltage is proportional to the amount of light received. It goes through a voltage to current converter and then the current goes to the capacitor where the amount collected over a 10 millisecond emission period is measured. This reduces the error on the measurement.

10.2.3 Testing turbidity sonde 1

The prototype sonde was tested in the laboratory to see if it worked efficiently and to produce a turbidity calibration curve to be used in the GeoVista software for the final sonde. The experiments were initially carried out in non-flowing water, however, due to particle sedimentation and unstable readings, a flowing water-circulating system was used. Consequently therefore, the sonde was placed in an upright position to mirror its position in a borehole and a peristaltic pump was used to keep the water in the tube moving as shown in Figure 10.4. The results from the GeoVista turbidity sonde were compared with results from a commercially available turbidity meter (T-100 Portable Turbidimeter, Oakton Instruments, Vernon Hills, IL, USA). The sonde was calibrated using formazine and then was tested using kaolinite solutions to see if the results were consistent with the commercial turbidimeter. During the experiment, three different currents were applied to the diffracted measurement at 90°, these were 100 mA, 180 mA and 250 mA. These were used to decide what current would provide the most accurate and useable result in the finished sonde.

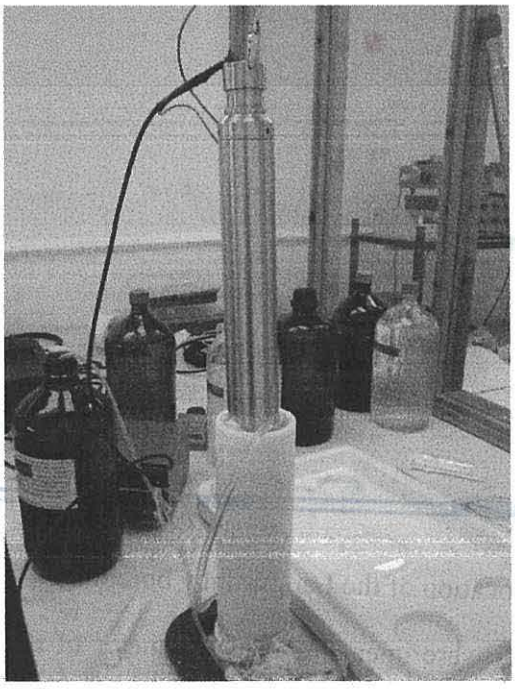

Figure 10.4: Testing the turbidity sonde with the water flowing through the peristaltic pump to simulate the sonde moving down a borehole.

10.2.4 Field-testing turbidity sonde 1

The sonde was taken to Langwathby borehole near Penrith in Cumbria (Figure 10.5) for field-testing. This was carried out with the assistance of the British Geological Survey who were monitoring the boreholes in the area to look at the changes in the hydrology over time and to see if it was possible to abstract more water from the area. The borehole had been drilled during the summer of 2005 and had been filled with sand and bentonite over the winter in an attempt to seal it over the winter. The borehole had then been cleaned out using a drill and had been actively pumped before we arrived. There was, however, a lot of bentonite still remaining in the borehole being stuck to the sides of the wall. To log the borehole, several GeoVista sondes were used. Firstly, a sonde containing a 2-arm caliper, temperature and conductivity sensor was used. This sonde was lowered to the bottom of the borehole (88 m)

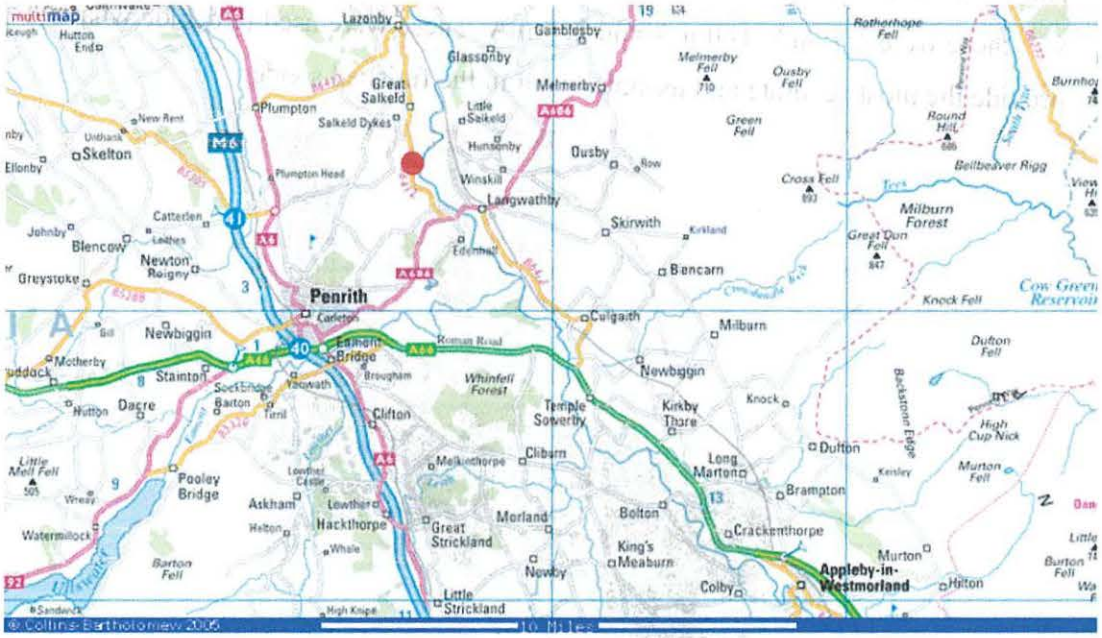

Figure 10.5: The location of the Langwathby borehole.

and then the two arms were opened and the diameter of the borehole logged upwards. The temperature sonde recorded the temperature of the water in the borehole and provided evidence on the possible inflows and outflows of the water into the borehole. All of the results were analysed using GeoVista software and stored on a computerised datalogger.

A heat-pulsed flowmeter sonde was next used to log the borehole. The sonde was lowered into the borehole and stopped at the required depth. The sonde measures the water flow in a borehole by sending out a pulse of heat. The heated water is then detected as it either travels up or down the borehole, depending on the rate and direction of flow, by two thermocouples that are positioned above and below the heat source at 5 cm distances away from the heat source. When the heat is detected, a peak is produced on the computer software with the interpretation following the premise that the faster the peak occurs, the faster the flow of water within the borehole is. If a peak occurs after 40 seconds this is assigned to convection current and the borehole is described as having no flow at that depth.

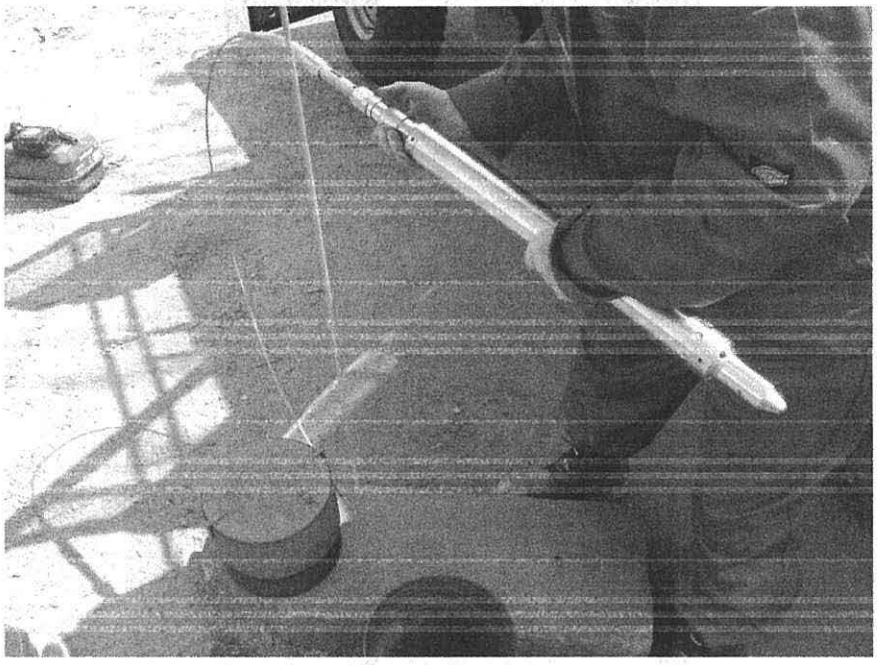

Figure 10.6: Preparing the turbidity sonde to be lowered down the borehole.

The next sonde was a camera that was used to examine the structure of the borehole and see the condition of the walls and possible inflows and outflows of water. The camera had a lens that showed the view down the hole and a side lens for looking more closely at the walls of the borehole. This was passed down the borehole at a slow speed to look at the features on

the walls of the hole and was then run up the holes at a higher speed to check any features that were missed on the way down. The results were saved on a DVD to be viewed at a later time. The final sonde that went down the borehole was the turbidity sonde (Figure 10.6). It was decided that it would be run slowly down to 80 m as the sonde had not been in a boreholes before and so it was not known what would happen to it. The sonde had a centraliser fitted to prevent damage to the sonde from the borehole wall. The sonde was then lowered down the borehole at a speed of 2 m min^{-1}. The sonde was also tested before going down the borehole using commercially available standard calibration solutions with a turbidity of 20 NTU (Figure 10.7).

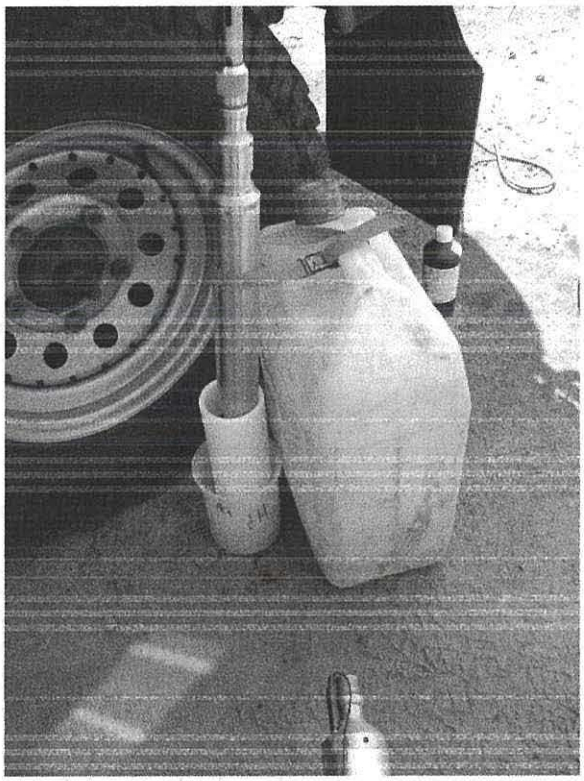

Figure 10.7: Testing the sonde in a 20 NTU commercially available turbidity standard solution

10.2.4 Testing turbidity sonde 2

Turbidity sonde 2 had a modified design in comparison to the original sonde. To make it stronger only one emitter and one detector were used and incorporated into a cut-away portion on the outside of the sonde. The inside of the sonde is shown in Figure 10.8 and the arrangement of the transmitter LED and the detector are shown in Figure 10.9. The new design shows the transmitter shining out from the sonde with the detector at 90° to it in the top of the water inlet area. To test the sonde a holder was made to contain the turbidity solutions. This also allowed the solutions to be changed easily. To test the sonde, formazine solutions were made containing 1, 5, 10 and 50 NTU alongside commercial standards containing <0.1, 20 and 200 NTU.

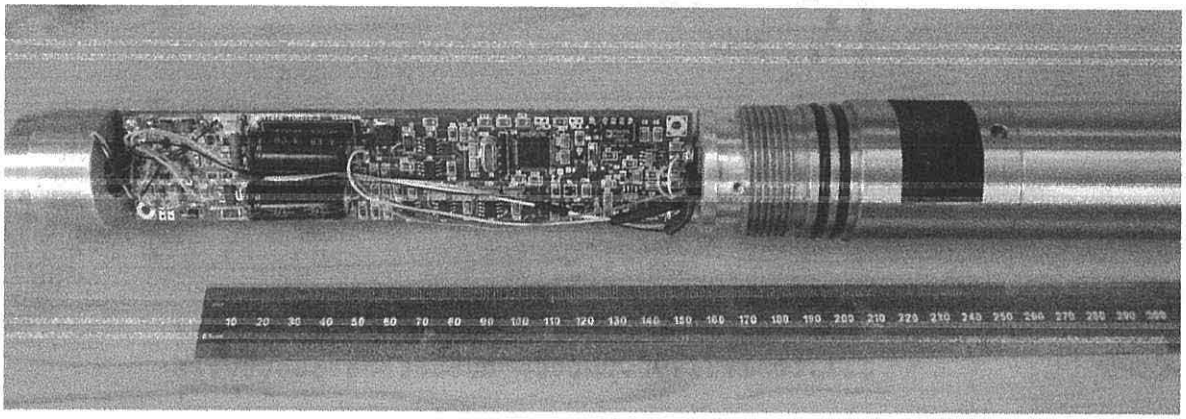

Figure 10.8: The circuit board inside Turbidity Sonde 2

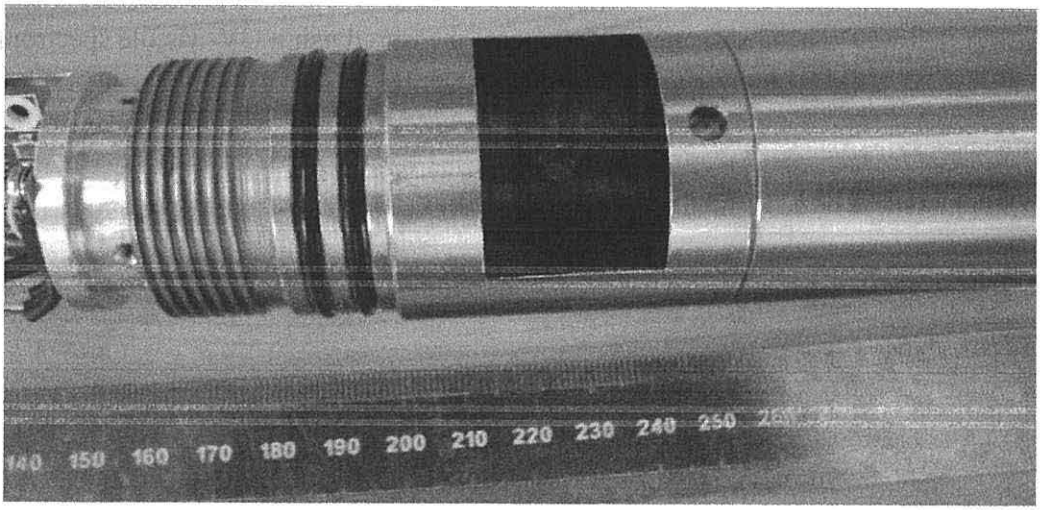

Figure 10.9: The arrangement of the transmitter and the detector at 90° to the transmitter on the left.

10.3 Results

10.3.1 Initial investigations

Figure 10.10 shows the absorbance of formazine when it was measured at a range of wavelengths using a UV-visible spectrophotometer. The graph shows that the absorbance responds well to changes in solution turbidity. Above 400 NTU the absorbance appears to be non-linear (Fig. 10.11). In the range 0-400 NTU, however, there was a good positive linear correlation between absorbance at 880 nm and turbidity ($r^2 = 0.998$).

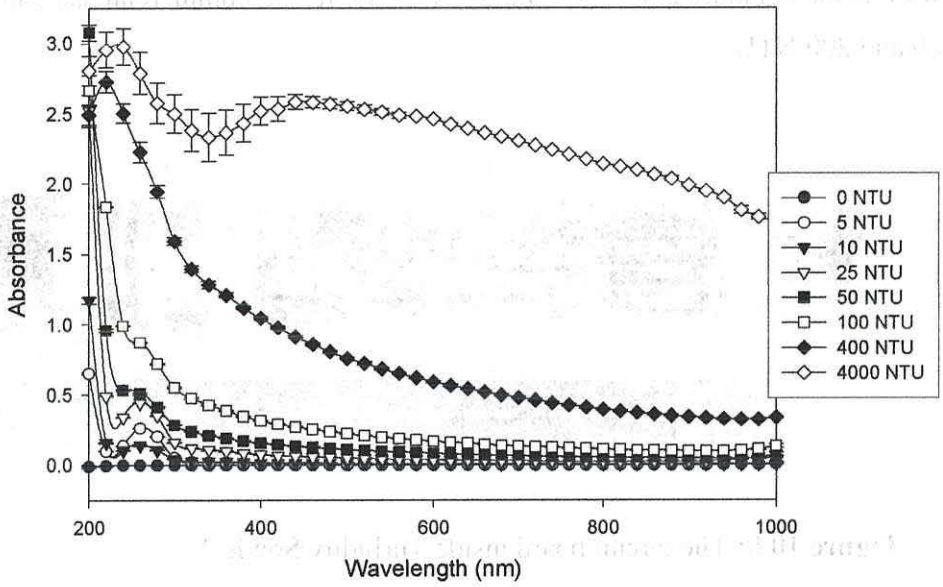

Figure 10.10: The absorbance of formazine measured using UV- visible spectrophotometry from 200 to 1000 nm. All values represent means ± SEM (n =3).

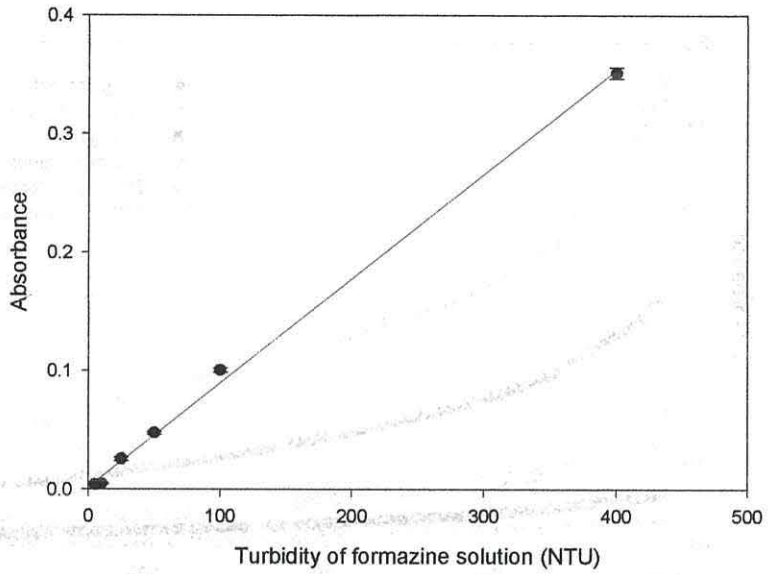

Figure 10.11: The absorbance of formazine solutions with different turbidities at 880 nm. All values represent means ± SEM (n =3).

Figures 10.12 and 10.13 show the relative absorbance of kaolinite and chlorite suspensions to assess whether there were any differences between the formazine turbidity standard and the natural suspensions that might reflect natural causes of turbidity. For the three different suspensions tested the absorbance pattern was almost identical with the highest absorbance observed at the 200 nm end of the spectrum and progressively declining towards 1000 nm.

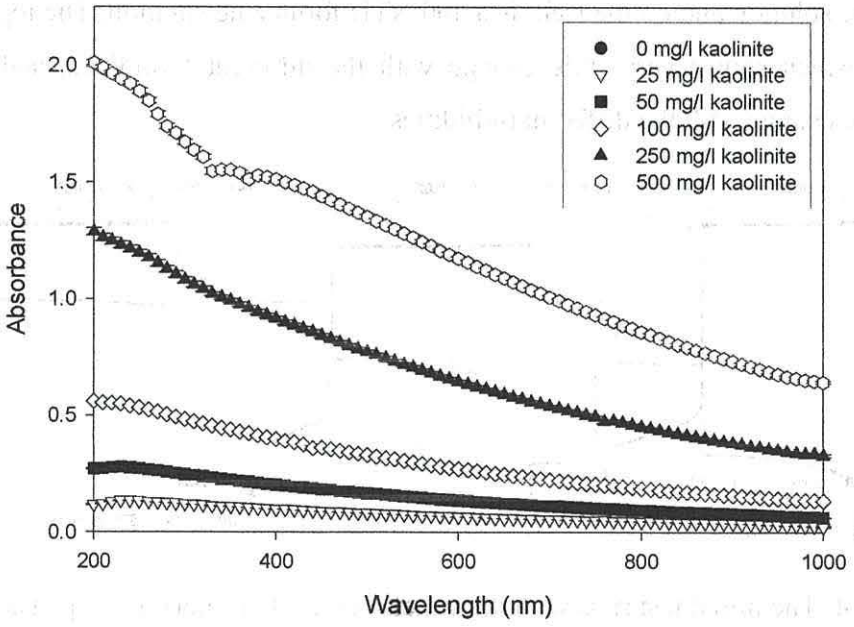

Figure 10.12: The absorbance of kaolinite suspensions using UV spectrophotometry at different concentrations. All values represent means ± SEM (n =3).

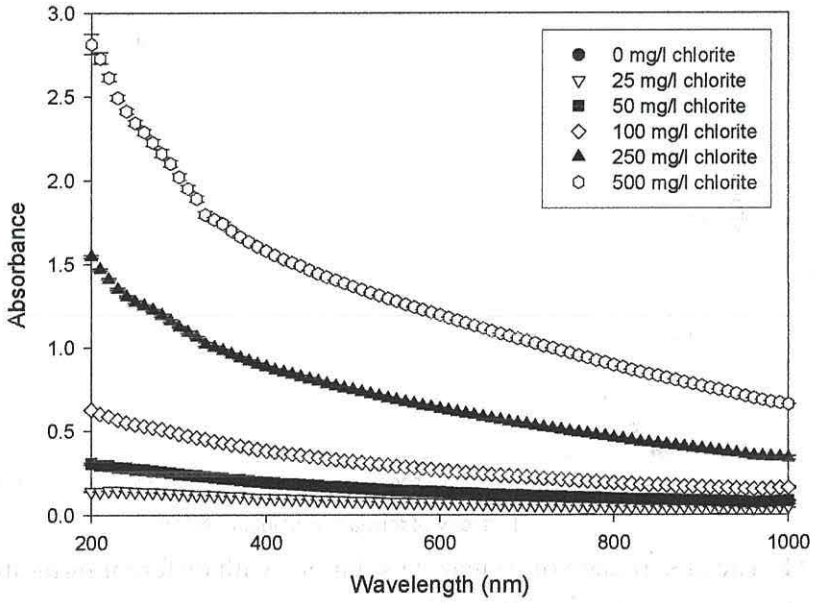

Figure 10.13: The absorbance of chlorite suspensions using UV spectrophotometry at different concentrations. All values represent means ± SEM ($n = 3$).

Figure 10.14 shows the first results that were obtained from the test sonde. The diagram captures a view of the oscilloscope and shows the change in the current when exposed to solutions of different turbidities. On the left is water, in the middle is a 100 NTU formazine solution and on the right is a 400 NTU formazine solution. The top line is the direct current, which shows very little change with the different turbidities, and the bottom line shows the change with the different turbidities

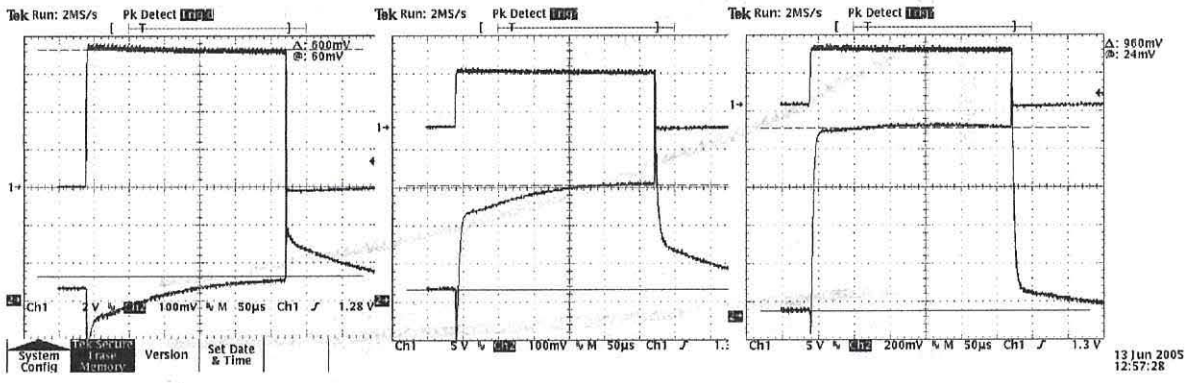

Figure 10.14: The initial test runs with the water, 100 NTU formazine suspension and 400 NTU formazine suspension.

Following on from this, a range of solutions were used to test the different levels of the electronics with level 7 having the most current applied to it, level 1 having the least and level 0 having no current applied to it at all. This is shown by the bit response being higher for level 7 than for level 0 (Fig. 10.15). Figure 10.16 shows the results for level 7 from a second run after the test sonde had been cleaned. This showed a good correlation between turbidity and bit response for the nephelometric measurement ($r^2 = 0.982$).

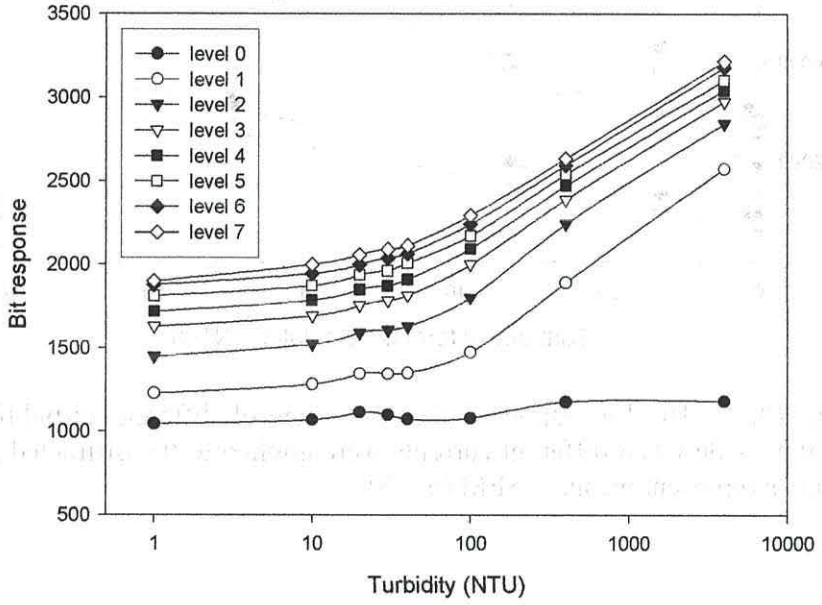

Figure 10.15: The results from the test sonde showing the different levels that the sonde was run at. All values represent means ± SEM (n =3).

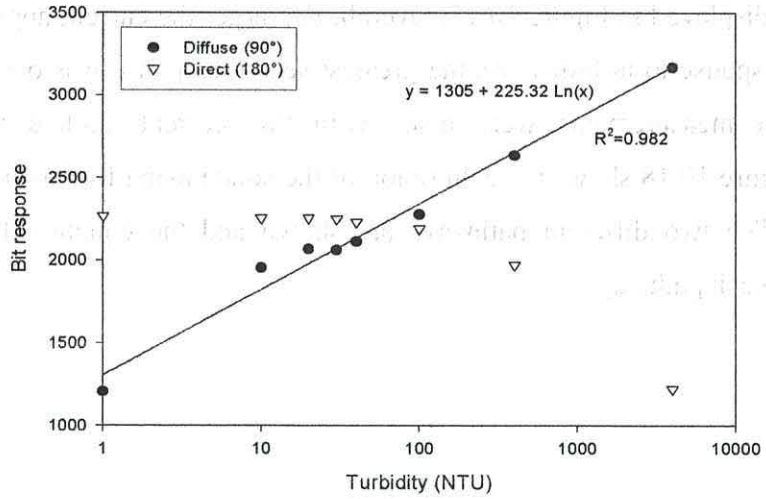

Figure 10.16: The response from the second run showing the diffuse and direct currents and the correlation of the diffuse results with turbidity for the level 7 results. All values represent means ± SEM (n = 3).

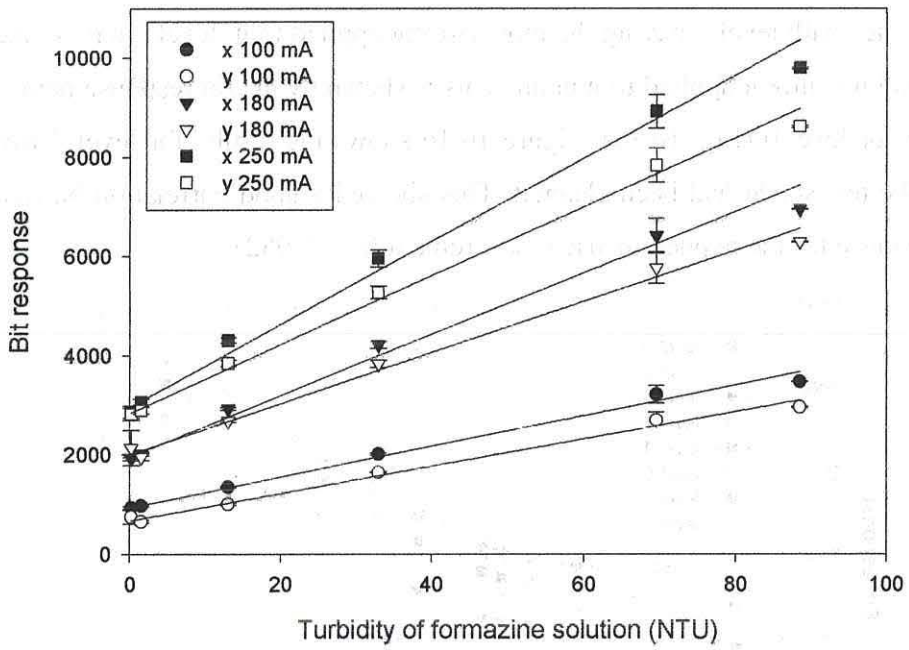

Figure 10.17: The bit response when solutions of different turbidities were measured using the sonde when different currents were applied to the diffracted path measurement. All values represent means ± SEM ($n = 3$).

10.3.2 Testing turbidity sonde-1

The results showing the influence of applying different currents on the turbidity response of the sonde are displayed in Figure 10.17. Overall, the larger the current applied, the greater the range in bit response to turbidity. As the greatest sensor response was observed for 250 mA, all subsequent measurements were made with this current applied to permit greatest sensitivity. Figure 10.18 shows the calibration of the sonde using formazine at turbidities of 0 to 60 NTU. The two different pathways are shown and the equation for calibration was calculated for each pathway.

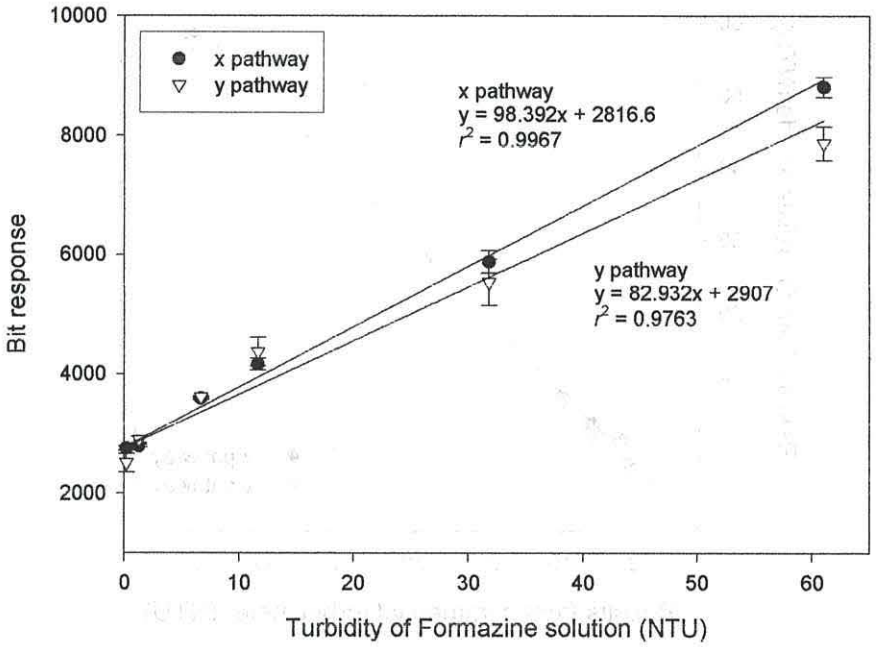

Figure 10.18: The calibration for the turbidity sonde using formazine at turbidities ranging from 0 to 60 NTU. The graph shows the results for the x and y pathway. All values represent means ± SEM ($n = 3$).

The two equations from Figure 10.18 were used to convert the bit response to turbidity in NTU. These were

Turbidity (NTU) using 'x' pathway = (bit response – 2816.6)/98.392

Turbidity (NTU) using 'y' pathway = (bit response – 2907)/82.932

Figure 10.19 shows the results of a test when kaolinite solutions of different concentrations were made and the turbidity was measured using the sonde and the commercial turbidimeter.

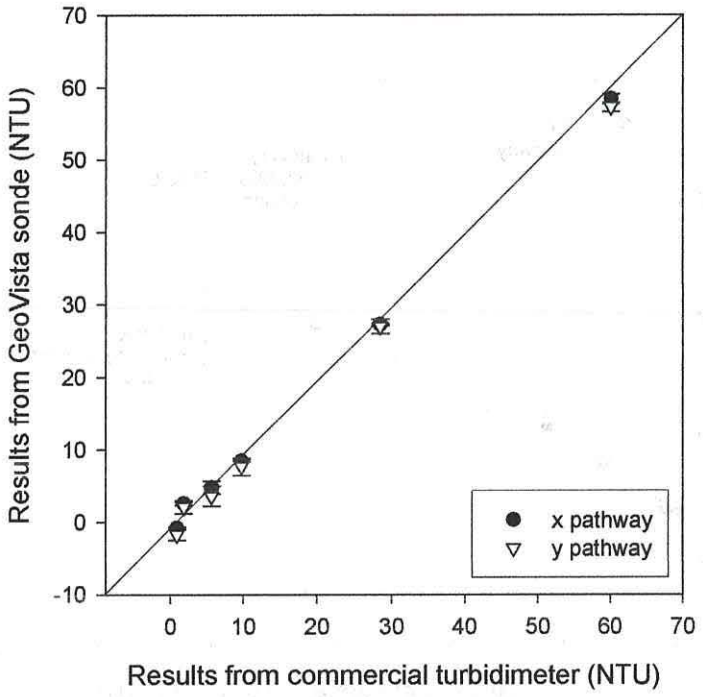

Figure 10.19: The comparison of the results from the commercial turbidimeter and the GeoVista sonde in the measurement of kaolinite suspensions and different formazine standards. Values represent means ± SEM (n = 6). A 1:1 line is drawn to show where the values should be.

10.3.3 Field-testing turbidity sonde-1

Figure 10.20 shows the downhole log of the turbidity sonde. The sonde appeared to be working reasonably well initially, measuring turbidities below 100 NTU. These were slightly higher than expected but it was decided to run another calibration afterwards to check that the sonde was running correctly and to recalibrate the sonde. Unfortunately, this could not be performed as the sonde broke during the logging. As can be seen in Figure 10.20 the y pathway stopped working at almost 12 m and the x pathway stopped at just below 16 m. This is apparent where the turbidity signal becomes saturated yielding a constant but unnaturally high turbidity. The dark signal displayed on the GeoVista software was also saturated at this point . The sonde was brought out of the hole and tested in a commercial 20 NTU solution but there was still no viable reading. When the sonde was taken back to GeoVista it was apparent that a housing leak had occurred and that approximately 100 ml of water had got into the

electronics. Consequently, the sonde was redesigned to prevent water leaking into the electronics at higher water pressures.

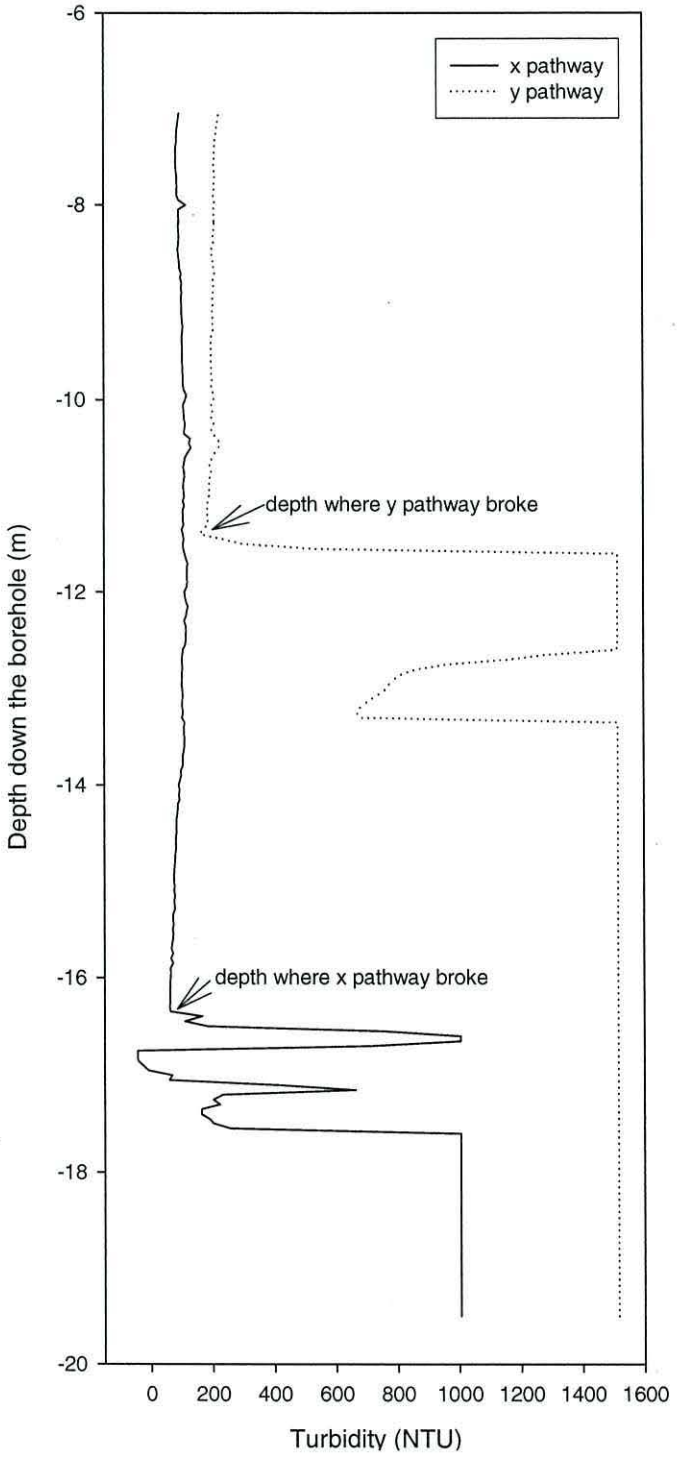

Figure 10.20: The downhole log of the turbidity sonde in the Langwathby borehole.

10.3.4 Testing turbidity sonde-2

Turbidity sonde 2 worked well as shown in Figure 10.21 however the bit response for the zero turbidity solution was lower than expected. The sonde was then taken by the British Geological Survey to a borehole with a water level at a depth of 18 m where it was tested and the results shown in Figure 10.22 obtained. There was a strong correlation between turbidity, temperature and conductivity at a depth of 36 m.

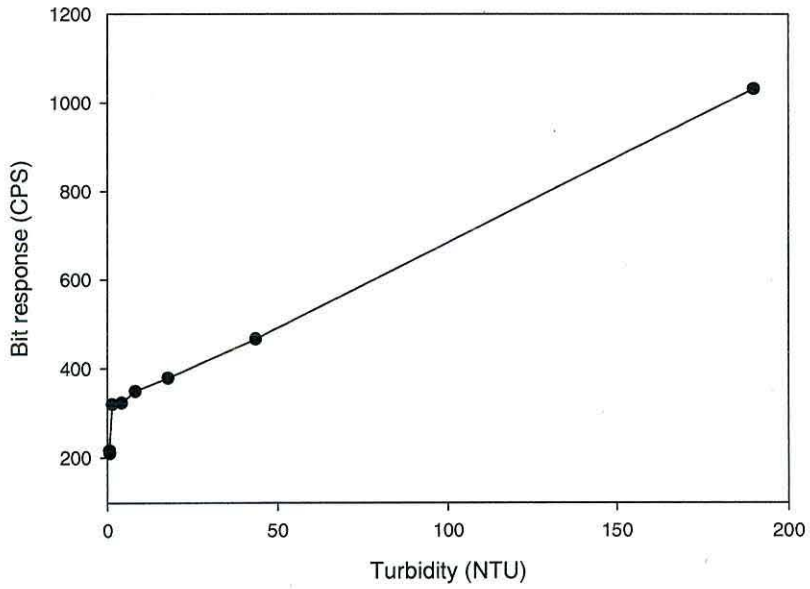

Figure 10.21: The results when formazine solutions and stabilised formazine solutions were measured using turbidity sonde 2 (all values were averaged over 2 minutes of measurement).

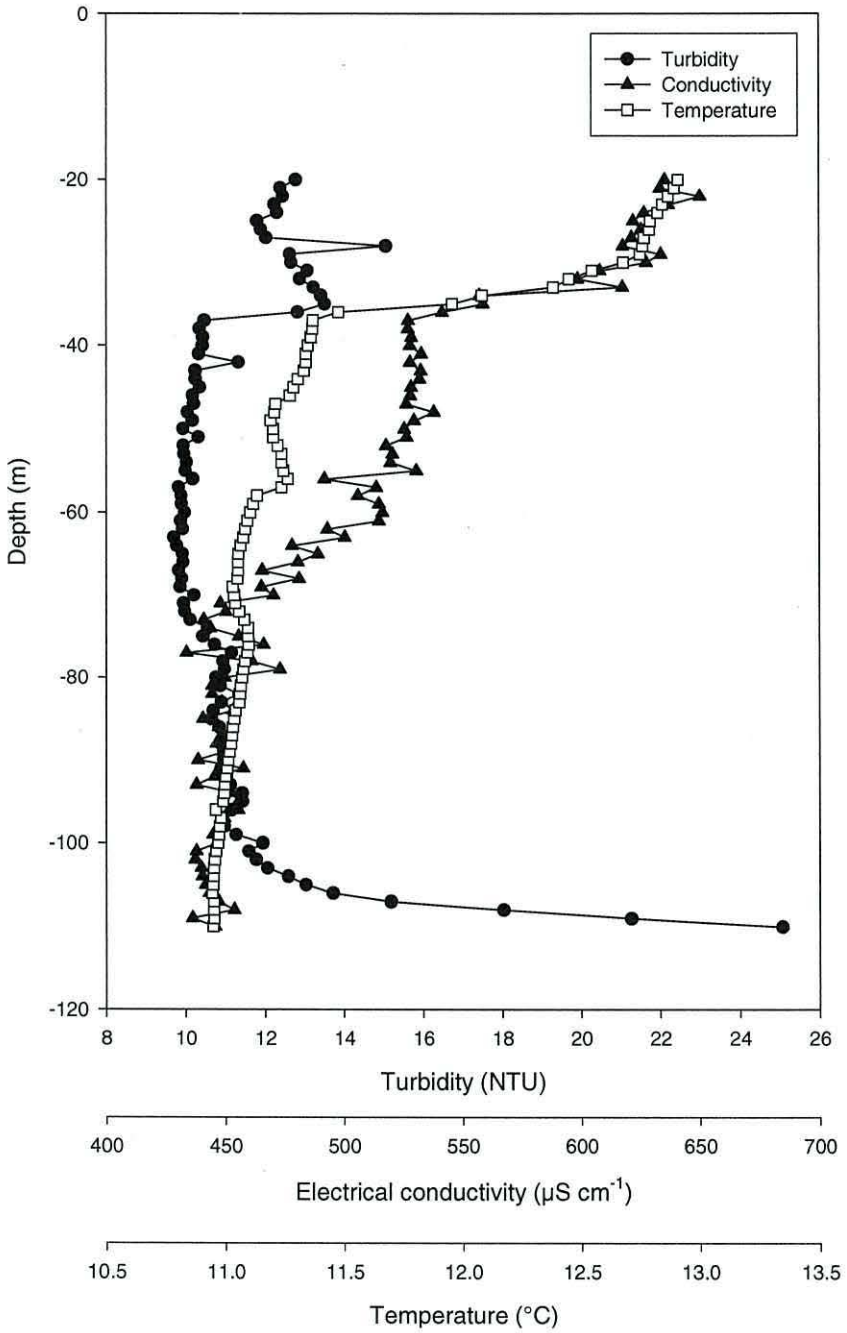

Figure 10.22: The downhole log showing the relationships between turbidity, temperature and electrical conductivity down to a depth of 110m.

10.4 Discussion

Here we show that using the international standard suspension for turbidity measurement, formazine, we can successfully develop a groundwater sonde that yields a linear response up to 400 NTU when tuned to an absorbance of 880 nm. The wavelength-absorbance scans, however, shows that this relationship does not hold if a lower wavelength is used. This verifies the importance of following the internationally recognized standard method. The absorbance of the clay minerals, kaolinite and chlorite, also followed a similar pattern to formazine with absorbance decreasing from 200 nm onwards. This is in agreement with Berho et al. (2004) who studied calcium carbonate and kaolin suspensions and also found that the absorbance values decreased with increasing wavelength. The similar behaviour of the clay minerals with formazine also indicates that formazine provides a realistic analogue for clays in suspension. However, future work is required to critically assess the linear response at 880 nm with suspensions recovered from actual boreholes or from vadose zone soil/sediment suspensions.

Based on the initial measurements made using the prototype sonde, several conclusions were reached. Firstly, the attenuation of the direct beam was negligible over the range of the turbidities of interest (< 50 NTU) as shown in Figure 10.16. This type of direct measurement would be more useful if higher turbidities were encountered (e.g. in very turbid freshwaters descending into a karst environment during storm events). The signal strength of the direct path was several orders of magnitude greater than the diffracted path. In practice, this means that the useful range of diode currents to achieve the diffracted measurement is in the range of 200 milliamperes to 800 milliamperes while the range of diode currents that are useful to make the direct measurement is in the order of 0.5 milliamperes.

The initial experiments were carried out with different levels of drive applied to the emitters. As can be seen from Figure 10.15 for all of the turbidities, the highest bit response was for level 7, which was to be expected as this was the highest drive applied. The software was designed to work logarithmically and so this is how the graphs are displayed. Consequently, this gave a linear response to turbidity using level 7 and thus level 7 was chosen as it also had the greatest difference between the 1 and 4000 NTU and hence the greatest sensitivity. The direct measurement with the detector at 180° to the emitter showed a decline in response at very high turbidities (> 100 NTU) but at turbidities of interest (< 50

NTU) there was no difference in the response. This was to be expected from previous work and is why the European Standard (EN ISO 7027:1999) specifies that the direct method should only be used in higher turbidity measurements and the nephelometric method for the lower turbidity measurements.

The optimization of the prototypes led to a final working design of the turbidity sonde with electronics for sonde stackability included to make the sonde more appealing to customers. The design of the sonde now allows it to be run down the borehole prior to the other sondes thus ensuring minimal disturbance of the water column. This frontline position largely prevents displacement of particulate material induced by the mechanical action of sonde-induced water displacement.

The laboratory testing of the turbidity sonde was carried out to: (1) determine the optimal electronic settings for the sonde, (2) to determine the amount of current that needed to be applied to the emitters and detectors, and (3) to see if the x and y pathways yielded similar results. Figure 10.19 shows the bit response that occurred with different turbidities when different currents were applied to the nephelometric measurement. As expected, the higher the current applied, the higher the bit response. However, there were differences between the x and y pathways which was a concern as they should have yielded near identical results. When the dark signal was, measured with both emitters turned off a difference in signal was also observed and consequently we conclude that the electronics may be responsible for the large difference between x and y pathways. Further, it was apparent that the analytical glass windows inside the sonde were difficult to keep clean. Therefore, any particles stuck to the window surface (e.g. oils, grease, mineral particles, microorganisms) may also significantly affect the light pathway and ultimately the observed reading. However, if this was biasing the result to any significant extent it would still be expected that the dark signal would be the same whilst in this study they were actually different.

For the calibration of the sonde, the two equations found in Figure 10.20 could be used for the conversion of bit response to turbidity. The GeoVista software also has a calibration feature that allows a calibration to be saved or to change the calibration each time the sonde is used depending on the conditions present. During the testing of the sonde the range that it could measure was investigated. Turbidities up to 900 NTU were used to determine the maximum. It was found that the maximum depended on the current that was applied to the

sonde. It had been decided previously that 250 mA would be used as this had the greatest sensitivity to the low turbidities that were most likely to be measured in groundwater boreholes and would be able to measure up to 100 NTU. By reducing the current to 150 mA, it was possible to measure up to 250 NTU and by reducing the current to 90 mA it was possible to measure up to 1000 NTU.

The field-testing of the sonde did not go as expected due to the water leak. We hypothesise that this was due to the glass windows inside the sonde not being able to cope with the pressure that was experienced with depth. The sonde had already been tested for pressures down to those experienced at 250 m but this was in a laboratory situation not in a real borehole. There is also the possibility that the sonde was damaged during transportation, however the sonde was transported securely and it is a vital requirement to GeoVista's customers that it is easy to transport and will not become broken.

Turbidity sonde 2 was more successful when it was tested in the field by the British Geological Survey. As shown in Figure 10.22 there was a correlation between turbidity, temperature and conductivity which all reduced at this point possibility indicating an inflow of water. A camera sonde was also run down the borehole when the recording was viewed it was possible to observe the reduction in turbidity at –36 m and also the increase in turbidity at the bottom of the borehole.

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CHAPTER 11

General Discussion

The examination of pollutants in groundwater is not expected to abate in the next few decades especially in view of recent advances in research and the increased drivers to mitigate environmental degradation (Lipton, 2007; Bunnell et al., 2007). While groundwater quantity will probably remain the biggest environmental issue globally (Wheida and Verhoeven, 2006), the issue of groundwater quality will also remain a major issue, particularly in response to the continuing in-depth analysis of the chemical, physical and biological contaminants in this hidden resource (Kouras et al., 2007). In a global media context, the major contaminant of groundwater may be viewed as being arsenic (Nickson et al., 1998), however, from a more scientific standpoint salinisation and contamination by agriculture is probably more important. This thesis has focussed on two aspects of environmental pollution, namely nitrate and hormones. Although indirectly linked, the two parts of the thesis were unfortunately not directly linked due to the nature of the PhD funding scheme. If this was repeated it would be ideal to study hormone persistence in groundwater, thus allowing the surface-to-groundwater flux pathways and chemical transformations to be studied. In addition, it would be useful to investigate the influence of groundwater chemistry on these processes (e.g. NO_3^- concentration). A summary of the major findings and avenues for further work is provided below.

11.1 Estrogen behaviour in the environment

As identified in Chapter 1, the work in this thesis had several aims. In the estrogens section of the thesis the aim was to investigate the degradation and mobility of naturally occurring estrogens.

As shown in Chapter 2 there had already been research into the fate of many different endocrine-disrupting chemicals in the environment and also their potential effects on organisms. However, a review published by Hanselman et al. (2003) during the beginning of the experiments in this thesis confirmed that there was a need to look at the behaviour of estrogenic hormones in soil when manure was present, as no studies had been carried out with additions of manure to soil. Since the start of these experiments there have been several publications which have examined the behaviour of estrogens in the natural environment or

laboratory settings with additions of manure (Khanal et al., 2006; Kolodziej and Sedlak, 2007).

Kolodziej and Sedlak (2007) concluded that in general there was little risk from grazing cattle that were allowed direct access to surface waters, however, in 10–20 % of samples the estrogens concentrations were over the predicted 'no effect' concentration for fish. The authors also identified that there may be increased risk immediately after heavy rainfall when accumulated wastes were flushed through the system as identified in Chapter 4. There are also complications arising from soil type, stocking density and species of livestock and season. It is also important to consider the route of animal wastes to the field. If the animals are housed indoors, the wastes are normally collected and composted. The findings in chapter 3 indicate that as the half-life of estrogens is generally only a few days in soil it is likely that the risk will be greatly reduced by the time the composted manure is spread onto land. However, if sheep and cows are grazing in the fields their wastes will reach the land immediately and so the potential for pollution is greater. The findings in chapters 2 and 3 show that mineralisation is generally rapid and so if the waste is stored before application to land the risk to the environment is probably very small.

11.1.1 Further work on estrogen behaviour

There are many experiments that could lead on from the work presented in Chapters 3 and 4. All of the soils used in this thesis had been disturbed and the animal wastes and manure were applied to the bare soil surface. If animals are grazing in a field then their faeces are more likely to be applied to the grass that they are grazing on. Therefore it would be interesting to repeat the experiments with grass swards and very fresh animal wastes and see if any differences occur. There is also the potential to replicate the experiments in the field in different locations, which could investigate further the effects of soil type, weather and season.

There is also a requirement to assess the concentration of estrogen that is actually being spread on fields. Radiolabelled hormone could be applied to individual faeces in fields under different weather conditions and the half-life found. This could then be compared with results from composting the manure from indoor animals to assess the optimum time to leave before spreading the manure and the best weather conditions.

On a more global scale, the soils used in Chapter 2 and 3 showed a lot of disparity between their estrogen mineralisation and sorption profiles despite the fact that they were geographically located very close to each other. This implies that nationally the variation in hormone behaviour could be extremely large and complicated by the different regional farming practices and livestock species. Although this thesis demonstrated hormone persistence, albeit for a short time, there also needs to be more work on the actual effects that estrogens and other endocrine disrupting chemicals are having on aquatic life and if these effects are then felt further up the food chain. Large concentrations of estrogens are released by sewage treatment works and so investigation of methods to reduce this is important, especially as the release is usually directly into water courses with much less chance for degradation before coming into contact with fish than the application of animal wastes to land. A comparison of the behaviour of man-made and natural potential endocrine disrupting chemicals in the environment is certainly warranted. Hydrological models describing hormone flow in complex landscapes are also required to help predict risk of pollution to fresh and groundwater supplies.

11.2 Measurement of nitrate and turbidity in groundwater boreholes

There are some similarities between the behaviour of nitrate and estrogens in the environment. One of their main sources in the environment is from their application to agricultural land as a manure and fertiliser and then subsequent leaching into water courses.

Overall the project was a success with two sondes produced which are suitable to be sold commercially. There is potential to alter the wavelengths of the nitrate measurement to allow for different concentration ranges to be measured. The sondes can also be stacked to allow for different combinations of sonde to be used to measure different aspects of water quality at the same time.

The investigations into the effect of different types of DOC on the measurement would become very important if the method was modified to be used in a region with high DOC such as upland streams and lakes.

The whole process has shown the frustration and hard work involved in developing a new product from the initial decision on the method to be used to the testing of the final product and the achievement felt when a working sonde was produced. It would also be

desirable to test the newly developed sondes in a wide range of groundwater contexts for further validation. Looking to the future, the next challenge will be the development of a sonde capable of measuring phosphorus at the very low concentrations typically found in groundwater (i.e. <1 mg P l^{-1}).

11.3 References

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APPENDIX

Photos of experimental set up in the ^{14}C experiments

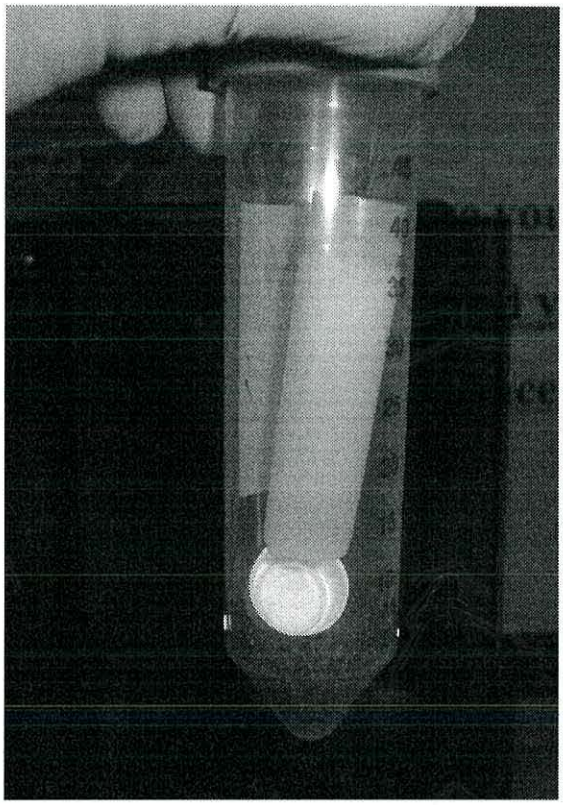

Figure I: The experimental set up for the mineralization experiments (Chapter 3) showing the soil, the spacer and the scintillation vial with 1 ml of NaOH for trapping the CO_2 evolved.

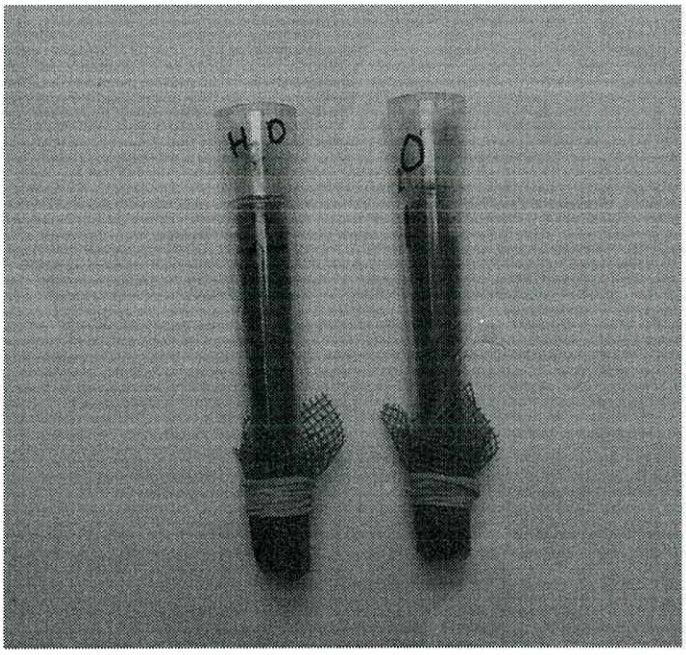

Figure II: The design of the leaching tubes used in Chapter 4.

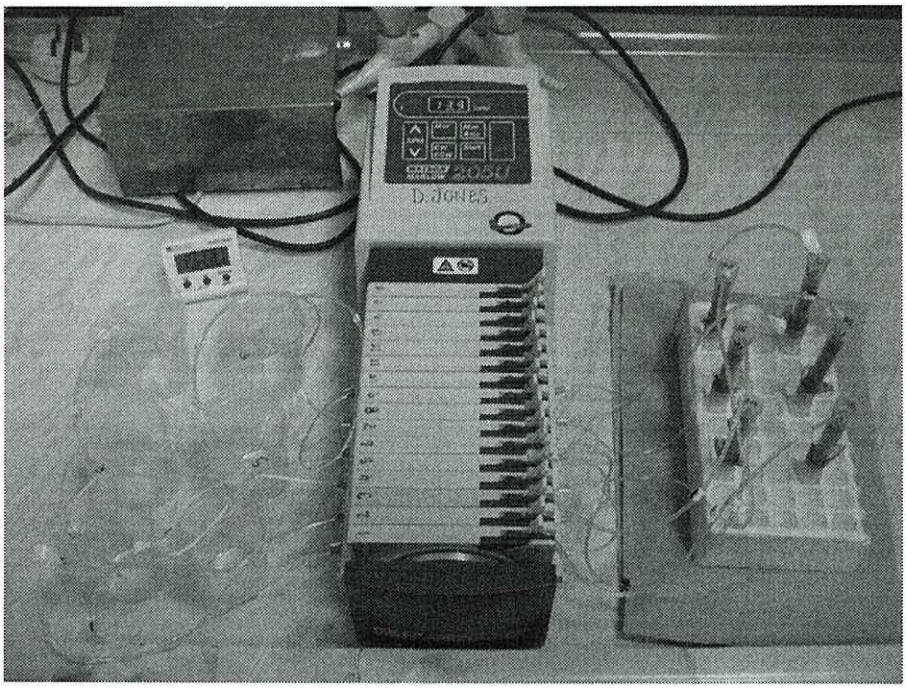

Figure III: The experimental set up of the leaching experiments (Chapter 4) showing the peristaltic pump used to apply the artificial rainwater to the leaching columns.